The Relationship between Ruminal Macro Mineral Solubility and Fermentability of Selected Tropical Legumes Tree with Mineral Absorption on Local Sheep

I.G. Permana, N.P. Haryanti and Suharlina
Department of Nutrition and Feed Technology, Faculty of Animal Science,
Bogor Agricultural University
email: permana@ipb.ac.id

ABSTRACT

A research to study relationship between macromineral solubility, fermentability of some tree legumes with *in vivo* digestibility using local sheep has been conducted. In the first experiment, five tropical legume trees such as *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliricidia sepium* (GS), *Callyandra callotyrsus* (CC) and *Leucaena leucocephala* (LL) were used. A modified *in vitro* technique was used to determine degradation, fermentation and macromineral bioavailability of selected legume trees. The gas production was measured using Hohenheim method. The ruminal DM degradation and the cumulative gas productions were calculated using a model of $Y = a + b (1\text{-}e^{-ct})$ following method of Ørskov and McDonald (1979). The second experiment aimed to evaluate macro mineral abroption of the legumes. The *in vivo* digestibility was measured using eighteen male local sheep. The animals were divided into 6 groups with 3 replications (A = native grass as a control, B = Ration A + 20% PI, C = Ration A + 20% SG, D = Ration A + 20% GS, E = Ration A + 20% LL and F = Ration A + 20% CC). The results showed that biodegradation and cummulative gas production of selected legume trees were not significantly different. However, the gas production rate of SG and GS were significantly higher. No difference was observed on VFA production of the legumes, while the NH_3 production was different. Legume SG produced more NH_3 than other tree legumes. In general, the ruminal Ca solubility was higher than P and Mg. The solubility of Ca and Mg of legume LL were higher than other legumes, while the solubility of P from legume LL was the highest. The *in vivo* experiment showed that digestibility of the ration containing 20% of tropical legume trees was no difference. However, the DM and OM consumption of ration were significantly different. The DM and OM consumption of ration D was higher (398 and 347 kg head $^{-1}$day^{-1}) than other rations. The absorption of mineral Ca and P from ration E was higher than other legumes.

Key words: *tropical tree legumes, macromineral, solubility, fermentability, in vivo digestibility*

INTRODUCTION

In tropical countries, legume is normaly used as protein sources to increase the quality of grasses in ruminant ration. Legume is adaptable in a wide range climate and soil condition, even under heavy grazing (Khamseekhiew, 2001). Legume tree is more adapted in tropical climate. The mineral content of legume was higher than grass (Underwood and Suttle, 1999). Sutardi *et al*. (1994) reported that legume trees had macro mineral especially calcium and therefore the legumes tree can be used as mineral supplement.

In ruminant, minerals are required not only for the animal it's self but also for the activity of rumen microbes. The mineral are used for cellulolytic microbe activities, osmotic pressure, buffering capacity, and reduction potential in the rumen (Duran and Kawashima, 1990). Therefore, in assessing mineral requirements of ruminant, both the quantity of minerals in the feeds and their bioavailability need to be considered. A method that can be used to evaluate the mineral availability in the rumen is *in vitro* technique. A modified *in vitro* technique can measure the extent and rate of release of macrominerals (especially Ca, P. Mg and S) in the rumen where most of organic matter digested.

The aim of this research was to study DM degradability, fermentability, gas production and solubility of macro mineral (Ca, P. Mg and S) of selected legumes tree using *in vitro* and *in vivo* method.

MATERIALS AND METHODS

Materials

Five tree legumes namely *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliricidia sepium* (GS), *Callyandra callotyrsus* (CC) and *Leucaena leucocephala* (LL) were used in this experiment. The samples were cut about 10-30 cm from growing point and dried at 60^{o}C and then ground through 2 mm screen sieve.

Dry Matter Degradation

Ruminal DM degradation was determined according to a modified method of Tilley and Terry (1963). Rumen liquid was collected from a sheep using vacuum pump. The samples (1.0 g) were placed in 50 ml fermentor tubes and suspended anaerobically with 8 ml of rumen fluid and 12 ml of phosphatecarbonate buffer. All samples were prepared in duplicates. The suspension was incubated at 39°C for 3, 6, 12, 24, 48 hours in shakerbath. Every 12 hours the samples were gassed with CO_2. After the specified incubation incubation, the samples were centrifuged at 3,000 rpm for 15 min. The supernatant was collected and the residues were filtered with filter paper and washed with boiled water. After that, the residues were dried at 60^{o}C for 48 hours. Samples in tubes without fermentation (0 h) were washed and dried in similar manner as the above samples as a control.

Gas Production

Gas production was measured using Hohenheim Gas Method (Close and Menke, 1986). Samples (230 mg) were put in syring glasses and then added 30 ml suspension of rumen fluid mix with buffer. The samples were incubated in water bath at 39^{o}C for 3, 6, 12, 24 and 48 hours. The cumulative gas productions were measured. The kinetic of gas productions were calculated following method of Ørskov and McDonald (1979).

In vivo Experiment

In vivo experiment digestibility was measured using 18 male local sheeps with average body weight was 15 kg. The animals were divided into 6 groups with 3 replications. The animals were kept in individual metabolic cages for 4 weeks (2 week for adaptaion period, followed by data collection period for 2 weeks). The rations used in this experiment were

Ration A = 100% Native grass as a control
Ration B = 80% Native grass + 20% *Pterocarpus indicus* (PI)
Ration C = 80% Native grass + 20% *Sesbania gradiflora* (SG)
Ration D = 80% Native grass+ 20% *Gliricidia sepium* (GS)
Ration E = 80% Native grass + 20% *Leucaena leucocephala* (LL)
Ration F = 80% Native grass + 20% *Callyandra callotyrsus* (CC)

During data collection period, diet and fecal were totally collected. The diet and fecal samples were dried under the sun and in oven (60^{o}C) for 24 hours, then analysed for dry matter, Ca and P content. The paramenters in this *in vivo* experiment were feed consumption, DM digestibility and mineral absoption

Chemical Analysis

The nutrient compositions of legumes trees were determined by the AOAC (1984) procedures. The DM content of all tree legumes and residue collected in the *in vitro* experiment were also determined. Then, they were digested with nitric and percloric acids using wet ashing method for determination of Ca and P. The prepare solution were analyzed for Ca, Mg and S using Atomic Absorption Spectrophotometric, and for P using a UV-Visible Spectrophotometer.

Total VFAs of supernatan were analyzed using steam distilation method, while NH_3 were analysed using Conway's micro diffusion method.

Statistical Analysis

Cumulative gas production and ruminal DM degradation rate were evaluated mathematically as a function of incubation time according to the method of Ørskov and McDonald (1979). The equation was $P = A + B (1 - e^{-ct})$, where: P is actual degradation at time t, A is water soluble fraction, B is the insoluble but potentially degradable fraction in time t, and c is degradation rate of B (% h^{-1}) and t is incubation time (h).

The data were subjected to analysis of variances using the general linear model procedure of the SPSS package program. The differences between means were tested using the contrast analysis.

RESULTS AND DISCUSSION

Chemical composition of legume trees

The chemical composition of legume tree was shown in Table 1. The CP content of tree legumes varied from 19.97 – 24.09%, where the CP of LL was relative the highest concentration of Ca contents ranged from 1.02% (CC) to 1.84% (LL). In the tropic, the Ca content of legumes tree was relatively higher than grasses (Serra *et al*, 1996). The P content of the legumes tree ranged from 0.27% (CC) to 0.41% (SG). The P content of legumes trees were also much close to that reported by Serra *et al*. (1996).

In Vitro

Ruminal Dry Matter Degradation

The ruminal DM degradation of selected tree legumes were shown in Table 2. The data showed that at 3 hours all legumes were degraded in the same level, while from 6 to 24 hours the DM degradation of GS and SG were significantly higher (P<0.05). However, after 48 hours the DM degradation of all legumes was not significantly different

The water soluble fraction (A), the insoluble fraction (B), the potential degradation and the degradation rate of all legumes were not significantly different. The potentially degradability of the legumes as source of CP and macro minerals were the same.

Gas Production

Gas (carbondioxide and methane) is a waste product of ruminal fermentation. In this experiment, the cumulative gas production was measured using Hohenheim Method. The cumulative gas productions of selected legumes tree were shown in Table 3. The cumulative gas production for 24 hours varied from 12.71 ml (CC) to 23.7 ml (SG). For 48 hours, legum GS produced gas relatively higher (28.2 ml) compare to CC (17.6 ml).

Table 1. Chemical composition (% based on DM basis) of *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliricidia sepium* (GS), *Leucaena leucocephala* (LL) and *Callyandra callotyrsus* (CC).

Parameter	Legumes				
	PI	SG	GS	LL	CC
Organic matter (OM)	92.54	90.52	91.20	91.89	94.88
Crude protein (CP)	23.95	26.18	21.46	25.50	21.50
Ether Extract (EE)	1.58	1.65	2.39	3.39	1.66
Crude fibre (CF)	27.64	27.07	22.81	20.69	22.37
NFE	39.38	35.62	44.53	42.31	49.35
Calsium (Ca)	2.01	1.14	1.68	1.50	1.18
Phosphorus (P)	0.40	0.30	0.31	0.43	0.36
Magnesium (Mg)	0.45	0.46	0.47	0.50	0.45

Table 2. Ruminal DM degradation (%) of *Pterocarpus indicus (PI), Sesbania gradiflora (SG), Gliricidia sepium (GS), Leucaena leucocephala (LL)* and *Callyandra callotyrsus (CC).*

Parameter	Legumes				
	PI	SG	GS	LL	CC
Ruminal DM degradability					
3 hours	16.28	17.00	17.72	18.04	17.37
6 hours	17.36^{b}	19.72^{a}	22.23^{a}	21.05^{a}	17.67^{b}
12 hours	25.70^{b}	28.36^{a}	29.01^{a}	26.50^{b}	23.85^{b}
24 hours	33.63^{a}	34.63^{a}	33.12^{a}	29.98^{b}	29.66^{b}
48 hours	39.39	42.04	41.50	40.12	37.67
DM degradability parameter (%)					
A	10.58	11.86	14.42	16.44	14.03
B	31.59	32.55	29.34	35.05	31.87
(A+B)	42.17	44.31	44.76	51.49	45.90
C (% h^{-1})	0.052	0.053	0.049	0.023	0.028

Means in the same row with different superscripts are significantly different (P<0.05)

The gas production rate (C) of SG and GS were significantly higher than PI, LL and CC (P<0.05). The gas production rate of LL and CC was low due to content of tannin and mimmosin (Keir, *et al*. 1997). However, the potential gas production (A+B) for all legumes was not significant (P>0.05). The gas productions correspond to ruminal DM degradation as described above.

VFAs and NH_3 production

Total VFA production at 12 hours incubation varied from 39.98 to 96.82 mM (Table 4). According to Hungate (1966) the total VFA concentration was lower than the optimum level (11m mM). At 12 hours GS and SG produced total VFA was relatively lower than another legume.

At 12 hours LL and CC produced total VFA relatively lower than another legume, however after 24 hours incubation, LL and CC produced more total VFA. The data showed that rumen microbes need more time to degraded of LL and CC.

Ammonia (NH_3) is a product of protein degradation with rumen microbe. The CP content of SG was higher than another legume. The CP content correlated with the NH_3 concentration. As shown in Table 5, the ammonia concentration after 12 hours incubation varied from 2.51 mM (CC) to 28.83 mM (SG). After incubation for 24 hours the concentration increased. The NH_3 production of SG after incubation for 12 and 24 hours was significantly highest (P<0.05). The indication correspond with Orskov (1982), that the NH_3 production depend on protein solubility, protein content, time incubation and rumen pH. The optimum concentration for microbe growing was 6-21 mM (McDonald *et al*., 1995).

Table 3. In vitro gas production (ml) of *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliricidia sepium* (GS), *Leucaena leucocephala* (LL) *and Callyandra callotyrsus* (CC).

Parameters	Legumes				
	PI	SG	GS	LL	CC
Cumulative Gas production (ml)					
3 hours	3.8	4.0	6.0	4.5	3.8
6 hours	7.6	9.5	10.2	6.9	5.5
12 hours	13.7	16.9	16.6	11.1	8.4
24 hours	21.4	23.7	23.6	16.7	12.71
48 hours	27.9	27.0	28.2	22.2	17.6
Gas production parameter (ml)					
A	0.45	0.00	1.46	1.53	1.60
B	26.4	27.9	28.3	24.9	34.3
(A+B)	36.9	27.9	29.8	26.4	35.9
C (% h^{-1})	0.05^b	0.10^a	0.07^a	0.04^b	0.04^b

Means in the same row with different superscripts are significantly different (P<0.05)

Table 4. The production of total VFA and NH_3 of *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliricidia sepium* (GS), *Leucaena leucocephala* (LL) and *Callyandra callotyrsus* (CC) after 12 and 24 hours incubation.

Parameter	Legum				
	PI	SG	GS	LL	CC
Total Volatile Fatty Acids (mM)					
12 hours	60.94	86.51	96.82	43.88	39.98
24 hours	52.75	52.94	69.89	83.65	72.20
NH_3 production (mM)					
12 hours	12.28^b	28.83^a	8.30^c	6.30^d	2.51^d
24 hours	19.91^b	39.55^a	13.33^c	11.30^d	3.49^d

Means in the same row with different superscripts are significantly different P(<0.05)

The correlation between degradation rate of selected legumes tree with production of VFAs and NH_3 were calcullated. The result showed that the degradation rate correlated negatively with VFAs production (r = -0.831). However, the degradation rate correlated closly with NH_3 production (r = 0.931). More ammonia was produced when the degradation rate more high.

In Vivo

Macro Mineral Solubility

The procentage of macro mineral solubilization (Ca, P, Mg and S) were shown in Table 5. Generally the solubility of Ca was higher than P, Mg and S. The data showed that the solubilities of Ca and P at 6 and 24 hours incubation time were siginificantly different (P<0.05). The Ca solubility of LL was higher than CC, GS and PI. However, in contrast the P solubility of PI was the highest. The solubility of Mg of selected legumes tree was not significantly different. However, the solubilities of CC and LL tended to be higher than PI and GS. The data of S presented the ruminal insoluble S. CC had tendency less insoluble S compare to another legumes.

Feed Consumption and Macro Mineral Absorption

The dry matter and organic matter consumption of ration B, D and E significantly higher than A and C ration, while the consuption of ration F containing 20% *G. sepium* was the lowest The Ration D which containing 20% *G. sepium* has the highest palatability. However the digestibility of the rations containing 20% of legumes tree was no significant different compare to control.

Table 5. The solubility of Ca, P, Mg and S of *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliricidia sepium* (GS), *Leucaena leucocephala* (LL) and *Callyandra callotyrsus* (CC) after 6 and 24 hours incubation.

Parameters	Legumes				
	PI	SG	GS	LL	CC
Ca (%)					
6 hours	80.44^{c}	ND	94.87^{b}	96.91^{a}	95.49^{b}
12 hours	85.08^{d}	ND	96.12^{c}	97.85^{a}	97.32^{b}
P (%)					
6 hours	98.44^{a}	ND	76.59^{b}	74.97^{b}	76.55^{b}
12 hours	93.93^{a}	ND	81.83^{b}	83.37^{b}	83.45^{b}
Mg (%)					
6 hours	61.36^{b}	ND	57.62^{b}	67.36^{a}	62.48^{b}
12 hours	77.61	ND	74.98	79.36	79.60
S (mg residu /g sample)*					
6 hours	1.400	ND	1.610	1.093	0.890
12 hours	1.295	ND	0.755	1.011	0.587

Means in the same row with different superscripts are significantly different (P<0.05); ND is not determined

Table 6. Dry matter and organic matter consumption of ration containing tree legumes

Ration*	Feed Consumption (g animal-1 day-1)	
	Dry Matter	Organic Matter
A	220.9^{b} ± 106	190.4^{b} ± 91.6
B	276.1^{a} ± 29.1	242.0^{a} ± 23.9
C	213.2^{b} ± 72.4	184.7^{b} ± 63.6
D	398.5^{a} ± 19.9	347.4^{a} ± 17.3
E	358.4^{a} ± 62.5	313.7^{a} ± 54.7
F	123.0^{c} ± 58.7	107.7^{c} ± 51.5

Note: A = native grass as a control, B = Ration A + 20% PI, C = Ration A + 20% SG, D = Ration A + 20% GS, E = Ration A + 20% LL and F = Ration A + 20% CC. Means in the same row with different superscripts are significantly different (P<0.05)

Table 7. The consumption, excretion and absoprtion of calcium and phosphor of ration containing tree legumes

Parameters	Ration					
	A	B	C	D	E	F
Calcium (g animal-1 day-1)						
consumption	1.44 ± 0.69	1.81 ± 0.19	1.39 ± 0.47	1.90 ± 1.23	2.34 ± 0.41	0.93 ± 0.38
excretion	0.98 ± 0.18	1.33 ± 0.30	0.92 ± 0.16	1.98 ± 1.42	1.61 ± 0.24	0.81 ± 0.43
absorption	0.46 ± 0.33	0.38 ± 0.65	0.47 ± 0.32	-0.08 ± 0.32	0.73 ± 0.27	0.12 ± 0.05
Phosphor (g animal-1 day-1)						
consumption	0.38 ± 0.18	0.54 ± 0.11	0.42 ± 0.16	0.64 ± 0.41	0.76 ± 0.13	0.24 + 0.11
excretion	0.38 ± 0.11	0.41 ± 0.12	0.32 ± 0.08	0.56 ± 0.29	0.55 ± 0.12	0.35 + 0.22
absorption	0.00 ± 0.17	0.13 ± 0.07	0.10 ± 0.06	0.08 ± 0.16	0.21 ± 0.11	-0.11 + 0.11

Note: A = native grass as a control, B = Ration A + 20% PI, C = Ration A + 20% SG, D = Ration A + 20% GS, E = Ration A + 20% LL and F = Ration A + 20% CC

Calcium and Phosphor Absorption

The consumption, excretion and absorption of Ca and P of ration containing 20% of legumes tree is shown in Table 7. The result showed that ration E containing 20% of *L. leucocephala* had the highest Ca and P absorption, while ration D (20% *G. sepium*) and F (*C. callotyrsus*) had low Ca and P absorption.

CONCLUSIONS

Ruminal DM degradation and cummulative gas production of selected legumes tree were not significantly different. The legumes tree had significatly different on macro mineral solubility. However, the highest Ca and P absorption was in ration containing 20% *Leucaena leucocephala.*

REFERENCES

AOAC, 1984. Official Methods of Analysis. Association of Official Agricultural Chemists. Washington DC.

Close, W. and K. H. Menke. 1986. Selected Topics in Animal Nutrition. The Institute of Animal Nutrition, University of Hohenheim. Germany.

Durand, M. and R. Kawashima. 1990. Influence of mineral in rumen microbial digestion. In: Y. Ruckebusch and P. Thivend (Eds.). Digestive Physiology and Metabolism in Ruminants. MTP Ltd. Lancaster. 275–408.

Hungate, R. D. 1966. The Rumen and Its Microbes. Academic Press. New York.

Keir, B., N. V. Lai, T.R. Preston, E. R.Orskov. 1997. Nutritive value of leaves from tropical trees and shrubs: 1. *in vitro* gas production and *in sacco* rumen degradability. Livestock Research for Rurar Development. 9:4.

Khamseekhiew, B., J.B. Liang., C.C. Wong and Z.A. Jalan. 2001. Ruminal and intestinal digestibility of some tropical legume forages. AJAS. 14:321-325.

McDonald, P., R.A. Edwards, J.F.D. Greenhalg, and C.A. Morgan. 1995. Animal Nutrition. 5th Ed. Lingman Scientific and Technical. New York.

Orskov, E. R. 1982. Protein Nutrition in Ruminants. Academic Press. London.

Orskov, E. R. and P. Mc Donald. 1979. The estimation of protein degradability in rumen from incubation measurements weighed according to rate of passage. J. Agri. Sci. 1979:499-503.

Serra, S. D., A.B. Serra, T. Ichinohe and T. Fujihara. 1996. Ruminal solubilization of macromineral in selected Philiphine forage. AJAS. 6:75-81.

Sutardi, T.. D. Sastradipradja, T. Toharmat, A. Sardiana dan I.G. Permana. 1994. Peningkatan produksi ternak ruminansia melalui amoniasi pakan serat bermutu rendah, defaunasi dan suplementasi protein tahan degradasi dalam rumen. Fakultas Peternakan, Institut Pertanian Bogor. Bogor.

Tilley J M A & Terry R A 1963 A two-stage technique for the in vitro digestion of forage crops. J. of the British Grassland Society 18:104-111

Underwood, E. J. and N. F. Suttle. 1999. The Mineral Nutrition of Livestock. 3rd edition. CABI Publishing. London.