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Rumen Fermentation, Blood Metabolites, and Performance of Sheep Fed Tropical Browse Plants

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ABSTRAK

Penelitian ini ditujukan untuk mengevaluasi produksi gas total, laju degradasi bahan kering (BK), dan profil VFA dari *Calliandra calothyrrus* (CC), *Leucaena leucochepala* (LL), *Moringa oleifera* (MO), *Gliricidea sepium* (GS), dan *Artocarpus heterophyllus* (AH) *in vitro*. Kajian *in vivo* merupakan lanjutan evaluasi *in vitro* konsumsi nutrien, gambaran metabolit darah, dan performa domba. Sejumlah 16 ekor domba garut jantan (rataan BB 20 kg) diberi ransum kontrol (100% rumput lapang); 70% rumput lapang + 30% GS; 70% rumput lapang + 30% MO; dan 70% rumput lapang + 30% AH dengan rancangan acak lengkap empat perlakuan dan empat ulangan. Hasil *in vitro* menunjukkan bahwa kandungan tanin dan saponin pada CC cukup tinggi dengan produksi VFA yang rendah. Total produksi gas dan laju degradasi BK pada CC dan LL paling rendah dibandingkan perlakuan lain (P<0,05). Total produksi gas dan laju degradasi BK tertinggi didapat pada GS, MO, dan AH. Konsumsi BK, protein, dan serat kasar pada GS dan AH tertinggi. Laju degradasi BK tertinggi pada MO (P<0,05). Rataan PBBH, serum albumin, dan globulin pada semua perlakuan sama, sedangkan serum protein, tri-gliserida dan glukosa pada perlakuan MO dan AH lebih tinggi daripada perlakuan lain. Perlakuan MO menghasilkan serum kolesterol terendah, sedangkan konsentrasi IgG tertinggi (P<0.05). Suplementasi 30% hijauan MO sangat direkomendasikan karena menghasilkan nilai cerna dengan performa dan status kesehatan yang lebih baik.

Kata kunci: hijauan tropika, rumput lapang, metabolit darah, fermentasi rumen dan laju degradasi

ABSTRACT

The *in vitro* study was designed to evaluate total gas production, dry matter degradability (DMD), and VFA profile; while *in vivo* study was designed to evaluate nutrient intakes, blood metabolites, and performance of sheep fed native grass mixed with *Calliandra calothyrrus* (CC), *Leucaena leucochepala* (LL), *Moringa oleifera* (MO), *Gliricidea sepium* (GS), and *Artocarpus heterophyllus* (AH). The best three from the *in vitro* results were used to formulate diets in *in vivo* study. Sixteen male growing sheep (average BW 20 kg) were fed 100% native grass (NG) as control; 70% NG + 30% GS; 70% NG + 30% MO; and 70% NG + 30% AH. Nutrient consumptions, DMD, blood metabolites, and sheep performances were analyzed by using Completely Randomized Design. The *in vitro* results showed that the total gas production and DMD of CC and LL were the lowest (P<0.05) while the highest was found in GS, MO, and AH treatments (P<0.05). Meanwhile, the *in vivo* results showed that nutrient intakes (DM, CP, and CF) of GS and AH rations were the highest. The ADG, concentration of albumin, and globulin in all treatments were similar, while total serum protein, triglycerides, and glucose concentration in MO and AH rations were higher than others. Serum cholesterol concentration in MO ration was the lowest, meanwhile the concentration of IgG was the highest (P<0.05). Supplementation of 30% MO was the best choice for optimum rumen fermentation and maintaining health status of local sheep.

Key words: tropical browse plants, native grass, blood metabolites, rumen fermentation

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INTRODUCTION

In most parts of humid tropic countries, browse may constitute an important fodder component to meet the maintenance requirements of ruminants, especially for farmers who practice extensive or semi-intensive farming. A number of browse plants (include legumes and herbs) may also contain chemical compounds such as protein, fiber, mineral, and vitamin concentrations adequate for the maintenance requirement of grazing animals (Fall-Touré *et al*., 1998; Aganga & Mosase, 2001). Problem with tropical browse plants is that they have high content of secondary compounds (tannins and saponin) which could reduce the nutritive values. Tannins (condensed, bound, or hydrolysable) are heterogenous compounds (Makkar *et al*., 1995) and because of the multitude of sizes and structures, their binding to protein and fibers varies tremendously. Tannins in browse plants can affect nutrient utilization in beneficial, prevent bloat, protect proteins, improve N utilization, and inhibit gastrointestinal parasitism (Min *et al*., 2003) as well as detrimental, such reduce forage quality and adversely affect herbivore nutrition (Singh *et al*., 2001). Animals gradually change their diets as a consequence of changes in rumen microbes that have higher efficiencies in degrading feedstuff high in tannin contents (Ephraim *et al*., 2003). The secondary compounds present in plants provide protection against predators, pathogens and invaders because of their antimicrobial activities. The antimicrobial activity of these compounds is highly specific and therefore may be used for the manipulation of rumen fermentation by selective inhibition of a microbial group of the ecosystem. In addition, saponin which has active compounds can stimulate immune response of the animals.

It is reported that some tropical legumes have different degradation rates in the rumen, as well as gas production. Offering these plants in regulated amounts as supplements to grass diet may yield better intake, utilization, improve blood metabolites and growth response. There is a need to evaluate objectively the potential of browse plants degradation under such circumstances. *In vitro* methods are viewed with high preference. However few data are available that document the effect of feeding tropical brows plants supplemented with native grass to animals on rumen fermentation and supporting to the animal health and performance. Saponin contents in *S. rarak* modify lipid metabolism, with final effects in decreasing serum cholesterol and improvement of cattle performance by improving daily gain and health status (Astuti *et al*., 2008). Hence *in vivo* studies that address digestibility, rumen microbial fermentation, and subsequently on growth and health, would be best used to evaluate the feeding values of plants rich in uncharacterized secondary compounds.

The objective of the study was to evaluate *in vitro* gas production and dry matter degradability (DMD) of tropical browse plants (*C. calothyrrus, L. leucochepala, Moringa oleifera, G. sepium,* and *A. heterophyllus*) as complete ration mixed with native grass. The best tropical browse plants from the *in vitro* study were used in *in vivo* study to measure performance production and the blood metabolite parameters of growing male sheep. The experiments was designed to obtain scientific bases on existing farmers practices in using tropical browse plants (legume and herbs) in improving small ruminant nutrition status.

MATERIALS AND METHODS

The first study was designed to evaluate *in vitro* total gas production, volatile fatty acid (VFA), and dry matter degradation (DMD) of tropical browse plants when the feed were presented as single, mixed with grass and as complete ration. Five tropical browse plants tested were *C. calothyrrus* (CC), *L. leucochepala* (LL), *M. oleifera* (MO), *G. sepium* (GS), and *Artocarpus heterophyllus* (AH). The animals donor of rumen fluid were two permanently fistulated male Indonesian thin tailed sheep. Gas production was measured serially in 0, 2, 4, 8, 12, and 24 hours incubation by using Hohenheim Gas Test (HGT; Menke & Close, 1986) and followed by measurement of DMD from material of gas test (Baba *et al*., 2002). Data of triplicate samples (except for VFA) in each ration

Table 1. Chemical composition of tropical browse plants (%)

Sample	GS	LL	CC	MO	AH
Dry matter	49.87	64.23	57.85	68.19	60.75
Ash	7.31	6.56	5.40	5.03	8.02
Crude protein	20.54	18.56	21.42	22.74	15.08
Extract ether	4.40	4.26	4.87	5.07	3.54
Crude fiber	15.86	16.77	13.42	8.55	19.64
NFE	51.89	53.81	54.89	58.61	53.72
NDF	52.24	52.74	57.74	42.70	70.17
ADF	29.39	47.72	49.92	27.58	58.02
Tannin	0.51	0.67	4.02	0.15	0.40
Saponin	4.91	2.80	8.61	4.65	5.97

Note: GS= *Gliricidea sepium*; LL= *Leucaena leucochepala*; CC= *Calliandra calothyrrus*; MO= *Moringa oleifera*; AH= *Artocarpus heterophyllus*; NFE= nitrogen free extract; NDF= neutral detergent fiber; ADF= acid detergent fiber.

were analyzed descriptively. Total and partial VFA were analyzed by Gas Chromatography. Chemical composition and secondary compounds of tropical browse plants were presented in Table 1.

The best rations and the lowest secondary compounds from *in vitro* experiment were used in *in vivo* feeding trial and growth studies to enable the assessment of nutrient utilization. Feed intakes (DM, protein and crude fiber), DMD, blood metabolite parameters (glucose, lipid and cholesterol, albumin, globulin, IgG,) and animal performance (ADG and feed efficiency) were evaluated. Sixteen growing male sheep (av. BW 20 kg) were divided into four treatments and maintained in individual cages. The treatments were 100% native grass as control group; 70% native grass plus 30% *M. oleifera* (MO); 70% native grass plus 30% *G. sepium* (GS) and 70% native grass plus 30% *A. heterophyllus* (AH). A one-week feed adaptation period was allowed before measurement of daily intakes and DMD during 2 months trial. Therefore nutrient intakes were measured for 7 weeks and were concluded by feces collection in the 8th week. The DMD was measured using Ash Insoluble Acid (AIA) method (Van Keulen & Young, 1977). At the end of the experiment, blood samples were drawn from the jugular vein to directly measure blood metabolites concentrations such as glucose, triglyceride, cholesterol, albumin, globulin, and Immunoglobulin G (IgG) using general procedure of KIT diagnosis. A set of *in vitro* blood test for immunity response was done at the end of this study. Performance was evaluated by dividing body weight gain to duration of the experiment and feed efficiency was calculated by dividing ADG to daily intake. Data of this research were analyzed using completely randomized design with four treatments and four replications, and mean of treatments were further analyzed using Duncan Multiple Range Test (Steel & Torrie, 2003).

RESULTS AND DISCUSSION

In Vitro Experiment

In vitro study showed that tannin and saponin contents of *C. calothyrrus* feedstuff were high, 4.02% and 8.61%, respectively while the lowest was in *M. oleifera*. *In vitro* fermentation showed the *C. calothyrrus* legume had low total VFA caused by the presence of anti nutrition. The total gas production for 24 hours fermentation and DMD of *C. calothyrrus* and *L. leucochepala* as a single substrate or mixed with native grass were the lowest ($P<0.05$) as compared to the other tested tropical browse plants (Table 2). On the other hand the highest values ($P<0.05$) of total gas production and DMD were found in ration containing *G. sepium*, *M. oleifera*, and *A. heterophyllus* mixed with native grass. *M. oleifera* in all status, either as a single forage, mix with native grass, or part of the ration resulted the best fermentation characteristics as compared to other treatments. Total and partial VFA productions (acetic acid, propionic acid, butyric acid, and valeric acid) in *M. oleifera* substrate as a single forage was higher than the other treatments, while *L. leucochepala* mixed with grass and as a ration was the lowest (Table 3).

Forage contained high antinutrition could affect rumen fermentation (Mc. Donald *et al.,* 2002). Saponin in some concentrations depressed protozoal population, allowing the bacteria grow well, while tannin has effect to bind protein of feedstuff, so that the quality of the forage will decrease. Reduced DMD with a stable gas production for *M. oleifera* treatment *is* an indication of improved nutrient partitioning this plant may have on rumen microbial metabolism. Baba *et al*. (2002) reported that the evaluation of ten tropical browse plants have different performances of gas production. The *M. oleifera* grows in the tropics climates has several industrial and medicinal uses (Becker & Makkar, 1999) and it has excellent nutritive value and low content of secondary compounds. The nutritional and energy content of *M. oleifera* leaves are 25.10%; 3.80%; 42.28%; 22.01%; and 18.70 MJ/kg for crude protein, crude lipid, neutral detergent fibre, acid detergent fibre, and gross energy, respectively (Astuti *et al*., 2011). The present study showed that *M. oleifera* has the highest potential to increase nutrient supply and to positively manipulate the rumen microbial function. Other forage such *G. sepium* has good crude protein value also, but they contained high secondary compounds likes saponin, tannin, cumarin and fenolic acid (Wood *et al.,* 1998). The leaves of *A. heterophyllus* has 15.9% of CP with 6% of tannin (Baba *et al.,* 2002). Suharti *et al.* (2011)

Table 2. Total gas production and DMD of tropical browse plants

Parameters	GS	LL	CC	MO	AH
Gas production (ml/500 mg):					
Single feed	65.02±9.59^{b}	51.30± 6.58^{c}	44.39± 7.82^{c}	90.24± 5.99^{a}	58.95±2.66^{b}
Plus grass	70.73±6.15^{a}	54.81±10.4^{b}	59.20± 0.97^{b}	76.41± 2.37^{a}	66.80±5.66^{a}
As ration	77.19±3.12^{a}	65.30±10.5^{b}	66.67± 4.75^{b}	85.65± 1.86^{a}	70.40±3.55^{b}
DMD (%):					
Single feed	71.53±2.85^{b}	55.51± 1.84^{c}	48.21± 7.21^{d}	89.01± 7.15^{a}	58.78±3.66^{c}
Plus grass	47.42±2.82^{a}	45.49± 5.02^{b}	37.61± 3.90^{c}	53.75± 2.35^{a}	50.08±0.96^{a}
As ration	48.55±4.34	58.45± 7.78	47.34±14.43	60.24±10.72	45.97±6.95

Note: Means in the same row with different superscript differ significantly ($P<0.05$). GS= *Gliricidea sepium*; LL= *Leucaena leucochepala*; CC= *Calliandra calothyrrus*; MO= *Moringa oleifera*; AH= *Artocarpus heterophyllus*; Single feed= 100% tropical browse plant (TBP); Plus grass= 30% TBP:70% native grass; As ration= 30% TBP : 20% concentrate : 50% native grass.

Table 3. Partial and total VFA of tropical browse plants (n=1)

Parameters	GS	LL	CC	MO	AH
Single feed (mM):					
Acetic acid	81.02	82.32	76.43	101.01	82.40
Propionic acid	22.14	20.68	15.79	27.50	17.15
Butytic acid	6.09	5.87	4.83	5.87	5.03
Valeric acid	0.92	1.51	0.62	1.90	0.45
Total VFA	110.17	110.38	97.67	138.04	105.03
Plus grass (mM) :					
Acetic acid	66.15	75.79	73.94	62.64	72.93
Propionic acid	15.45	16.47	15.52	13.67	16.16
Butytic acid	6.13	5.17	5.67	4.51	5.51
Valeric acid	0.91	0.85	0.90	0.96	1.02
Total VFA	88.64	98.28	96.03	81.78	95.62
As ration (mM) :					
Acetic acid	77.19	80.17	74.74	71.29	46.95
Propionic acid	18.57	17.65	16.68	16.22	10.11
Butytic acid	6.99	6.59	6.90	5.76	4.77
Valeric acid	1.02	0.73	0.84	1.01	0.91
Total VFA	103.77	105.14	99.16	94.28	62.74

Note: GS= *Gliricidea sepium*; LL= *Leucaena leucochepala*; CC= *Calliandra calothyrrus*; MO= *Moringa oleifera*; AH= *Artocarpus heterophyllus*; Single feed= 100% tropical browse plant (TBP); Plus grass= 30% TBP:70% native grass; As ration= 30% TBP : 20% concentrate : 50% native grass.

reported that secondary compounds likes saponin from *Sapindus rarak* De Candole could increase CMCase activity may be due to the increased *R. albus* population.

In Vivo Experiment

Based on the result of *in vitro* study, it was decided to use forage *G. sepium, M. oleifera,* and *A. heterophyllus* for the *in vivo* experiment. Result during two months feeding-trial showed that nutrient intakes (DM, CP, and CF) of rations containing *G. sepium* and *A. heterophyllus* were the highest (P<0.01). Meanwhile the highest DM digestibility (69.73%) was found in *M. oleifera* treatment (P<0.05) (Table 4). Data of ADG, concentrations of albumin and globulin were similar in all treatments, while the total protein, triglycerides and glucose concentrations in *M. oleifera* and *A. heterophyllus* mixed rations were higher (P<0.05) than in 100% grass and *G. sepium* treatments. Cholesterol status in *M. oleifera* treatment was the lowest (P<0.05), meanwhile the concentration of IgG was the highest (P<0.05) as compared to the other treatments (Table 5).

Supplementation with 30% *G. sepium* and *A. heterophyllus* leaves increased DM intake by 20% and 19%, respectively, in growing male sheep. According to Tomaszweska *et al.* (1993), maintenance requirement of DM intake for 10-20 kg BW sheep was 500-1000 g/h/d or 4%-5% of BW. The experimental sheep consumed

Table 4. Nutrient intake, digestibility and performance sheep fed with tropical browse plants

Parameters	Control	GS	MO	AH
Intakes (g/h/d):				
Dry matter	506±28^{b}	610±17^{a}	501±0.64^{b}	604±24^{a}
Crude protein	41±2^{d}	77±2^{a}	54±0.15^{c}	66±3^{b}
Crude fiber	160±9^{a}	158±4^{a}	137±0.1^{b}	161±6^{a}
DM digestibility (%)	61±7^{b}	62±2^{b}	69±0.43^{a}	63±2^{b}
ADG (g/d)	29±0.29^{b}	48±0.48^{a}	44±0.47^{a}	47±0.51^{a}
Feed efficiency	0.05±0.01^{b}	0.08±0.01^{a}	0.08±0.01^{a}	0.07±0.01^{a}

Note: Means in the same row with different superscript differ significantly (P<0.05). GS= *Gliricidea sepium*; MO= *Moringa oleifera*; AH= *Artocarpus heterophyllus*.

Table 5. Blood metabolites parameter of sheep fed with tropical browse plants (mg%)

Parameters	Control	GS	MO	AH
Glucose	37.50±1.30^{c}	50.19±3.01^{b}	46.54±4.24^{b}	59.49±3.87^{a}
Triglycerides	70±2^{b}	62±6^{b}	79±1.5^{a}	80±3^{a}
Cholesterol	60.86±4.10^{a}	46.71±7.10^{b}	38.39±2.51^{c}	56.91±4.40^{b}
Total protein	7.4±0.02^{a}	6.2±0.20^{b}	7.0±0.56^{a}	7.27±0.17^{a}
Albumin	42.8±2.5	44.45±0.90	48.92±3.89	46.87±3.15
Globulin	50.40±1.1	49.91±0.76	52.01±2.70	49.57±1.67
IgG	807±14^{c}	890±19^{b}	923±6^{a}	881±19^{b}

Note: Means in the same row with different superscript differ significantly (P<0.05). GS= *Gliricidea sepium*; MO= *Moringa oleifera*; AH= *Artocarpus heterophyllus*.

DM around 555±56 g/h/d (4.5% of BW), that meet for maintenance and growing requirements. Astuti & Sastradipradja (1999) reported that local sheep with 19 kg BW consumed 500 g/h/d of ration equal to 4% of BW. There was a significant increased in DM digestibility by AIA method of ration using *M. oleifera* treatment as compared to others. Van Keulen & Young (1977) reported that digestibility measurement using AIA and total collection methods had the same result. The advantage of AIA method is more practice and not invasive and cheaper than total collection method. The *M. oleifera* treatment with low feed intake has high percentage of digestibility. Low content of secondary compound and availability of nutrient in *M. oleifera* leaves caused high utilization of nutrient. Firdus *et al.* (2004) reported that sheep fed 30% *C. calothyrrus* had only 54.32% DM digestibility due to the high tannin content.

Concentrations of albumin and globulin in all treatments were similar, while total protein, triglycerides and glucose in *M. oleifera* and *A. heterophyllus* were higher than those of the other treatments. Cholesterol status in *M. oleifera* treatment was the lowest, meanwhile the concentration of IgG was the highest (P<0.05). Ration containing *M. oleifera* with a certain amount of saponin has good effect on the animal health as expressed in low serum cholesterol, normal essential fatty acid concentration and high IgG concentration. Saponin from lerak (*S. rarak* De Candole) could reduced cholesterol concentration in plasma ongole crossbred cattle (Astuti *et al*., 2009). Hosoda *et al*. (2006) reported that there is effect of three herbs as feed supplements on blood metabolites, hormones, antioxidant activity, IgG concentration, and ruminal fermentation in holstein steers. Astuti *et al*. (2008) reported that sheep raised under the tropical forest management had low serum glucose and triglyceride concentrations with normal concentration of total protein.

According to animal performance, there were significant differences (P<0.05) of ADG in sheep fed 30% of *G. sepium, M. oleifera,* and *A. heterophyllus* leaves, while the feed efficiency in all treatments were the same, except for the control. The ADG of sheep fed tropical browse plants increased around 33% as compared to control. Astuti & Sastradipradja (1999) reported that ADG of sheep reared in individual cage was 50 g/h/d compared to 45 g/h/d when reared in pasture.

CONCLUSION

M. oleifera, G. sepium, and *A. heterophyllus* as tropical browse plants have the highest potential to increase nutrient supply and to improve the rumen microbial fermentation. Supplementation of 30% *M. oleifera* was the best choice for optimum rumen fermentation with normal growing performance and improved health status of local sheep.

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