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

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ABSTRACT

The \textit{in vitro} study was designed to evaluate total gas production, dry matter degradability (DMD), and VFA profile; while \textit{in vivo} study was designed to evaluate nutrient intakes, blood metabolites, and performance of sheep fed native grass mixed with \textit{Calliandra calothyrrus (CC)}, \textit{Leucaena leucochepala (LL)}, \textit{Moringa oleifera (MO)}, \textit{Gliricidea sepium (GS)}, and \textit{Artocarpus heterophyllus (AH)}. The best three from the \textit{in vitro} results were used to formulate diets in \textit{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 \textit{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 \textit{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 heterogeneous 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 reduces rumen fermentation, 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.

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 were averaged.

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= Gliricida 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% G. sepium; 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 randomize 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 have 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 (Astiti 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)

| Parameters                  | GS         | LL         | CC         | MO         | AH         |
|-----------------------------|------------|------------|------------|------------|------------|
| Gas production (ml/500 mg)  |            |            |            |            |            |
| Single feed                 | 65.02±9.590 | 51.30±6.580 | 44.39±7.820 | 90.24±5.990 | 58.95±2.660 |
| Plus grass                  | 70.73±6.150 | 54.81±10.40 | 59.20±0.970 | 76.41±2.370 | 66.80±5.660 |
| As ration                   | 77.19±3.120 | 65.30±10.50 | 66.67±4.750 | 85.65±1.860 | 70.40±3.350 |
| DMD (%):                    |            |            |            |            |            |
| Single feed                 | 71.53±2.850 | 55.51±1.840 | 48.21±7.210 | 89.01±7.150 | 58.78±3.660 |
| Plus grass                  | 47.42±2.820 | 45.49±5.020 | 37.61±3.900 | 53.75±2.350 | 50.08±0.960 |
| As ration                   | 48.35±4.340 | 58.45±7.780 | 47.34±4.430 | 60.24±10.72 | 45.97±6.95  |

Note: Means in the same row with different superscript differ significantly (P<0.05). GS= Gliricidia sepium; LL= Leucaena leucochepala; CC= Calliandra calothyrrus; MO= Moringa oleifera; AH= Atriplex 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 Tomaszewska 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

| Parameters | GS      | LL      | CC      | MO      | AH      |
|------------|---------|---------|---------|---------|---------|
| Acetic acid| 81.02   | 82.32   | 76.43   | 101.01  | 82.40   |
| Propionic acid| 22.14 | 20.68   | 15.79   | 27.50   | 17.15   |
| Butyric 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  |

| Parameters | GS      | LL      | CC      | MO      | AH      |
|------------|---------|---------|---------|---------|---------|
| Acetic acid| 66.15   | 75.79   | 73.94   | 62.64   | 72.93   |
| Propionic acid| 15.45 | 16.47   | 15.52   | 13.67   | 16.16   |
| Butyric 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   |

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

| Parameters | GS | LL | CC | MO | AH |
|------------|----|----|----|----|----|
| Intakes (g/h/d) | | | | | |
| Dry matter  | 506±28* | 610±17* | 501±0.64* | 604±24* |
| Crude protein | 41±2* | 77±2* | 54±0.15* | 66±3* |
| Crude fiber  | 160±9* | 158±4* | 137±0.3* | 161±6* |
| DM digestibility (%) | 61±7* | 62±2* | 69±0.43* | 63±2* |
| ADG (g/d)   | 29±0.29* | 48±0.48* | 44±0.47* | 47±0.51* |
| Feed efficiency | 0.05±0.01* | 0.08±0.01* | 0.08±0.01* | 0.07±0.01* |

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

| Parameters | Control | GS      | MO      | AH      |
|------------|---------|---------|---------|---------|
| Glucose    | 37.50±1.30* | 50.19±3.01* | 46.54±4.24* | 59.49±3.87* |
| Triglycerides | 70±2* | 62±6* | 79±1.5* | 80±3* |
| Cholesterol | 60.86±4.10* | 46.71±7.10* | 38.39±2.51* | 56.91±4.40* |
| Total protein | 7±0.02* | 6.2±0.20* | 7.0±0.56* | 7.27±0.17* |
| Albumin    | 42.8±2.5  | 44.45±0.90 | 48.92±3.39 | 46.87±3.15 |
| Globulin   | 50.40±1.11 | 49.91±0.76 | 52.01±2.70 | 49.57±1.67 |
| IgG        | 807±14*   | 890±19*  | 923±6*  | 881±19* |

Note: Means in the same row with different superscript differ significantly (P<0.05). GS= *Gliricidia 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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