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# Feed Evaluation Based on in Vitro Gas Production of Tropical Forages with Addition of Different Polyethylene Glycol (Peg) Level

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**ABSTRACT**

This study aimed to determine the chemical composition and gas production based on fermentation in the rumen of nine types of forage tropical feed commonly used in Indonesia. The forages used in this study were: mahogany leaves (*Swietenia mahagoni L. Jacq.*), tayuman leaves (*Bauhinia purpurea*), bamboo leaves (*Bambusa arundinaceae*), canary leaves (*Canarium indicum L.*), tea leaves (*Camellia sinensis*), ketapang leaves (*Terminalia catapa L.*), lamtoro leaves (*Leucaena leucocephala*), tehtehan leaves (*Duranta repens*), and turi leaves (*Sesbania grandiflora*). Measurements of in vitro gas production were carried out at 10 observation points (2, 4, 6, 8, 12, 16, 24, 36, 48, 72 hour). Tannin activity was measured for each sample at observation points using measurements of gas production divided into three groups with modified levels of *polyethylene glycol* (PEG), ie samples without PEG (P1); sample + PEG in the amount of 200 mgDM (P2); and PEG + samples of tannins contained in each forage based on literature studies (P3). Data were analyzed for a variance by following the factorial completely randomized design (CRD) pattern. Statistical analyzes were performed on all data by following the general linear procedure in PROC GLM from SAS. The data obtained were analyzed for variance at the 5% significance level. The results of gas production calculations showed that crude protein (CP) from each forage ranges from 5.75 - 22.37% where the highest CP was owned by turi leaves (*S. grandiflora*). The content of crude fiber (CF) ranged from 5.30 - 20.93%. The most optimal measurement of gas production was in the sample given PEG in the amount of 200mg/kg with a significant difference ($P<0.05$). The higher of the tannin content contained in the forage, the lower of gas produced. Measurement of tannin content showed that condensed tannin content varied from turi leaves by 0.20% to the highest in mahogany leaves by 8.60%. The addition of 200 mg/100mgDM of PEG optimizes the rate of forage gas production, especially for grass plants (*gramineae*).

Keywords: Evaluation, Forage, Gas-production, Polyethylene glycol, Tannin, Tropical-feedstuffs

## Introduction

Although ruminants can digest low quality forages, not all nutrients consumed by them can be utilized by rumen microbes, especially if they were bind to secondary metabolite compounds such as tannins. Limitation of nutrient intake due to tannin binding can decrease protein solubility and $NH_3$-N concentration in the rumen, reduced rumen microbial population, thus decreased feed intake and digestibility which indirectly affect livestock productivity. Protein supplement is restricted by the presence of tannins in their foliage (Brown *et al*., 2017). At a level above 50 g/kg DM, condensed tannin (CT) restricts nutrient utilization and digestibility and may bind to digestive enzymes, thus reducing their activities (Christopher, 2012). However, forages containing low to moderate level of tannin also showed positive effects on the ruminants' performances. Some of the beneficial effects associated with feeding ruminants with forages containing tannin were the reduction of ruminal $CH_4$ production in the rumen, increase dietary rumen undegraded protein (RUP) fraction flow into abomasum and intestine, and inhibited the last step of the biohydrogenation that leading to the accumulation of trans vaccenic acids (TVA) at the cost of C18:0. These benefits can be gained when the tannin level in the forages can be maintained in the low to moderate levels. Thus, a proper method of tannin-binding agents, such as *polyethylene glycol* (PEG), can be applied so forages containing tannins still can be used as feed and more benefits can be gained from those.

*Polyethylene glycol* is a substance that is stable in acidic, basic, and relatively high temperatures (Chen *et al*., 2005). For several

years, it has been known that PEG can prevent the formation of complex tannin-protein formations because PEG's affinity is higher for tannins than for proteins. The PEG-tannin formation can deactivate tannins. The addition of PEG in plants containing tannins increases in vitro gas production, production of short-chain fatty acids (SCFA), and in vitro nitrogen degradation (Besharati and Taghizadeh, 2011). The molecular weight of PEG by 3500 to 4000, has been proven by researchers to reduce the effect of tannins (Makkar, 2003). PEG can be applied both on a small scale to farmers and industrial scale. Farmers can provide PEG directly to livestock through drinking water or mixed with concentrate, sprayed on tannin-rich feedstuffs, or even better as a nutrient block. On an industrial scale, it can be given in the form of pellet feed, which contains tannin-rich by-product feed ingredients.

This study aims to explore the optimal potential of tannin-contained forage as feed ingredients especially for ruminants, with the help of PEG compounds to optimize feed digestibility so that optimal levels of PEG administration are obtained in the application of feeding.

## Materials and Methods

This research was conducted at the Laboratory of Animal Feed Sciences, Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia from March 2017 to August 2018. This research includes determining the digestibility of forage using in vitro gas production methods (Menke and Steingass, 1979) with the modification of giving three PEG levels.

Table 1. Forages used in the study

| Local names | Latinness name |
|---|---|
| Mahogany (leaves) | *Swietenia mahagoni L. Jacq.* |
| Tayuman (leaves) | *Bauhinia purpurea* |
| Bamboo (leaves) | *Bambusa arundinacea* |
| Canary (leaves) | *Canarium indicum* L. |
| Tea (leaves) | *Camellia sinensis* |
| Ketapang (leaves) | *Terminalia catappa* L. |
| Lamtoro (leaves) | *Leucaena leucocephala* |
| Tehtehan (leaves) | *Duranta repens* |
| Turi (leaves) | *Sesbania grandiflora* |

Forage samples were collected of the edible portion (the part that can be consumed by ruminants). The chemical composition analysis of all forages were carried out refers to the proximate analysis method (AOAC, 2005), except for ether extract (EE) was followed the modified Soxhlet method of Kamal (1994). Tannin analysis was carried out according to the Abdulrazak (1995). Determination of tannin content includes several stages, including sample preparation, tannin extraction, total phenol content measurement, as well as determination of total tannin and condensed tannin. Condensed tannins (%DM) equivalent to leucocyanidin was calculated using the following formula:

$$\frac{\text{absorbance of 550 nm} \times 78.26 \times \text{dilution factor}}{\%\ \text{DM}}$$

*In vitro* gas production measurement was performed on forages samples were done in 3 treatments according to their PEG levels: P1 = forages samples without PEG addition, P2 = forages samples + PEG 200 mg DM (P2), and P3 = forages samples + PEG in adjusted amount of tannin content in each forages (based on literature study). Detailed of PEG levels of each treatments is presented in Table 2. *In vitro* gas production analysis (Menke and Steingass, 1988) used rumen liquid collected from 2 fistulated cows fed king grass (Pennisetum hybrid) and wheat pollard. Rumen fluid was collected in the morning before feeding (06.30 AM). Observation of gas production was carried out at 2, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hour. Samples were taken using 4 replications. The calculation of gas production is formed using the Neway (Excel) program (Chen, 1994). The calculation of gas production gets the equation Y = a + b (1-e-ct), which is where Y = the volume of gas produced at time t, a = fraction of soluble, b = fraction of potential degraded or gas production potential, c = rate of degradation fraction b (Chen, 1994; Kustantinah, 2012). Data were analyzed for a variance by following a unidirectional completely randomized design (CRD). Statistical analyzes were performed on all data by following the general linear procedure in PROC GLM from SAS. The data obtained were analyzed for variance at the 5% significance level. If the data shows a significant difference as an effect of the treatment, then continue with Duncan's new multiple range test (DMRT).

## Results and Discussion

**Gas production of tropical forages**

The rate of gas production from the nine forages experienced an increasing in gas production at the beginning (hours 2 to 24) of the observation point (Table 3), meaning that there was a rapid nutrient degradation process. This then followed by a decline in feed degradation starting from hour 24 and continued until hour 72. The gas production rate of all types of forages added by PEG were improved compared to those without PEG addition, which implied an increase in nutrient degradation of forages. The addition of PEG in forage was useful to reduce the negative effects of tannins, as evidenced by the high gas production of forage samples that were added with PEG. The mechanism of feed and tannin bonds can be understood through the ability of tannins to form complex bonds with feed protein. Tannins can form complex bonds with feed proteins and also inhibit the performance of endogenous proteins such as digestive enzymes. Tannins, especially CT not only bind to protein feed, but they also bind to carbohydrates to form complex tannin-carbohydrate bonds, which a large amount of PEG is needed in addition to binding the tannins (Reed, 1995). McManus *et al*. (1985) *cit*. Le

Bourvellec *et al*. (2004) reported that a bond between plant cell wall polysaccharides and tannins will be formed because plant cell wall polysaccharides also contain hydroxyl groups and oxygen atoms that can form hydrogen bonds and hydrophobic interactions with tannins.

The gas production of forages that added by PEG were different among forage, depend on the type of forage and the level of PEG addition.

However, most of the forage samples showed positive responses on the rate of gas production when receiving additional PEG (Table 3). Among nine types of forage used, only two types of forage gave different responses, which gas production as well as the rate of gas production were more optimal when no PEG were added. *B. purpurea* and *D. repens* showed different responses to the gas production while

Table 2. The PEG levels applied on each tropical forage

| Samples | Tannin content (reference) (mg/100mg)DM | Treatment | Number of Sample (mgDM) | Total PEG (mg) in 200 mgDM sample |
|---|---|---|---|---|
| *Swietenia mahagoni L. Jacq.* (Mahogany leaves) | 11.9 | P1 | 200 | 0 |
| | | P2 | 200 | 200 |
| | | P3 | 200 | 23.9 |
| *Bauhinia purpurea* (Tayuman leaves) | 1.6 | P1 | 200 | 0 |
| | | P2 | 200 | 200 |
| | | P3 | 200 | 3.1 |
| *Bambusa arundinacea* (Bamboo leaves) | - | P1 | 200 | 0 |
| | | P2 | 200 | 200 |
| | | P3 | 200 | 0 |
| *Canarium indicum* L. (Canary leaves) | 11.8 | P1 | 200 | 0 |
| | | P2 | 200 | 200 |
| | | P3 | 200 | 23.5 |
| *Camellia sinensis* (Tea leaves) | 6.9 | P1 | 200 | 0 |
| | | P2 | 200 | 200 |
| | | P3 | 200 | 13.8 |
| *Terminalia catappa* L. (Ketapang leaves) | 1.1 | P1 | 200 | 0 |
| | | P2 | 200 | 200 |
| | | P3 | 200 | 2.3 |
| *Leucaena leucocephala* (Lamtoro leaves) | 6.6 | P1 | 200 | 0 |
| | | P2 | 200 | 200 |
| | | P3 | 200 | 13.1 |
| *Duranta repens* (Tehtehan leaves) | 6.6 | P1 | 200 | 0 |
| | | P2 | 200 | 200 |
| | | P3 | 200 | 13.2 |
| *Sesbania grandiflora* (Turi leaves) | 4.7 | P1 | 200 | 0 |
| | | P2 | 200 | 200 |
| | | P3 | 200 | 9.4 |

*Bauhinia purpurea*

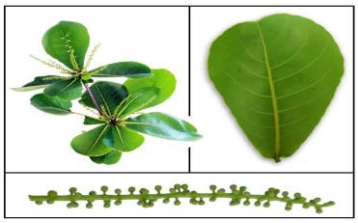
*Leucaena leucocephala*

*Sesbania grandiflora*

*Swietenia mahagoni L. Jacq.*

*Canarium indicum* L.

*Camellia sinensis*



*Terminalia catappa* L.

*Bambusa arundinacea*

*Duranta repens*

Figure 1. Forages used in the study.

treatments without PEG addition showed the most optimal gas production as well as the rate of gas production. Although *B. purpurea* and *D. repens* leaves contain similar amount of CT as *C. indicum* and *C. sinensis* (2.7 to 3.2 mg/100mgDM; Table 4), they showed different responses on gas production. This showed that the correlation between tannin content (CT) in forage was not always linear to gas production. This is due to tannin structure that very complex and also due to the different genetic response of each plant. Kustantinah *et al*. (2017) reported that the total concentration of phenols, total tannins, and condensed tannins did not always correlate with gas production. That was due to the complex tannin structure, just like the genetic variation between each forage.

**Total gas production of degraded fraction**

The optimal fraction value degraded (a + b) after incubation for 72 hours on nine forage leaves (Table 5) were indicated by forage without PEG that showed the lowest soluble fraction (fraction a) compared to those with PEG addition. The most optimal interaction between forage and PEG level on the content of a + b fractions were noticed in bamboo leaves with 200 mgDM PEG, while the a + b fraction has the lowest value on mahogany leaves with PEG. The high value of a + b fraction on bamboo leaves with 200 mgDM PEG due to the high potential of degraded fraction (fraction b) on bamboo leaves which contain high amount of CF (Table 6). Since bamboos are *gramineae* (grasses) that use structural carbohydrates (lignin, cellulose, hemicellulose) to support their bodies, they are in contrast to the other forages used in this study that were either legumes or non-legumes in the form of tree or shrub plants. Carbohydrates can also form complex bond with tannin. The formation mechanism of tannin complex bonds with carbohydrates (polysaccharides) plant cell walls is similar to the mechanism of complex bonds between proteins and tannins. This is due to the protein and polysaccharides of plant cell walls both have hydroxyl groups so that when they cross the aromatic components of tannins they form hydrogen bonds. McManus *et al*. (1985) reported that the aromatic component of tannins has a high affinity for dextran gels. The affinity is increased due to the addition of hydroxyl groups into the aromatic structure of tannins, which can occur with the mechanism of absorption of tannins by dextran gels through the pores of gels due to

Table 3. Production of forage gas 2, 4, 6, 8, 12, 16, 24, 36, 48, and 72 (mL/200mg DM)

| No | Sample | Treatment | 2 | 4 | 6 | 8 | 12 | 16 | 24 | 36 | 48 | 72 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Swietenia* | P1 | 5.1$^{t}$ | 10.0$^{t}$ | 14.1$^{s}$ | 16.9$^{u}$ | 20.7$^{v}$ | 24.2$^{t}$ | 35.5$^{v}$ | 33.4$^{x}$ | 35.2$^{w}$ | 37.5$^{w}$ |
| | *Mahagony L. Jacq.* | P2 | 6.5$^{o}$ | 13.5$^{o}$ | 19.5$^{l}$ | 24.0$^{l}$ | 28.7$^{n}$ | 32.1$^{o}$ | 43.4$^{n}$ | 38.9$^{r}$ | 40.6$^{s}$ | 43.2$^{t}$ |
| | | P3 | 7.5$^{m}$ | 13.9$^{m}$ | 20.0$^{k}$ | 23.7$^{n}$ | 24.8$^{r}$ | 20.1$^{w}$ | 31.5$^{x}$ | 24.9$^{z}$ | 25.6$^{z}$ | 27.1$^{z}$ |
| 2 | *Terminallia* | P1 | 5.2$^{s}$ | 9.4$^{v}$ | 12.3$^{v}$ | 14.3$^{y}$ | 19.2$^{y}$ | 24.1$^{u}$ | 36.2$^{t}$ | 35.9$^{w}$ | 32.8$^{y}$ | 34.8$^{y}$ |
| | *Catappa L.* | P2 | 5.1$^{t}$ | 8.1$^{w}$ | 10.5$^{x}$ | 13.1$^{z}$ | 18.7$^{z}$ | 23.1$^{v}$ | 35.1$^{w}$ | 23.7$^{aa}$ | 24.5$^{aa}$ | 26.1$^{aa}$ |
| | | P3 | 6.1$^{p}$ | 10.2$^{s}$ | 13.3$^{u}$ | 15.9$^{v}$ | 21.7$^{u}$ | 27.1$^{s}$ | 39.1$^{s}$ | 38.8$^{s}$ | 41.2$^{r}$ | 43.5$^{s}$ |
| 3 | *Camellia* | P1 | 8.6$^{i}$ | 14.2$^{k}$ | 18.9$^{n}$ | 22.5$^{p}$ | 29.5$^{l}$ | 35.1$^{l}$ | 51.6$^{j}$ | 46.5$^{n}$ | 47.8$^{n}$ | 49.5$^{o}$ |
| | *sinensis* | P2 | 8.2$^{k}$ | 17.8$^{e}$ | 24.9$^{c}$ | 30.6$^{b}$ | 37.6$^{b}$ | 42.0$^{c}$ | 58.5$^{c}$ | 50.8$^{h}$ | 52.4$^{i}$ | 54.7$^{j}$ |
| | | P3 | 10.3$^{h}$ | 17.1$^{g}$ | 23.1$^{e}$ | 27.9$^{g}$ | 35.5$^{e}$ | 40.9$^{e}$ | 57.3$^{d}$ | 48.5$^{k}$ | 49.8$^{m}$ | 51.6$^{m}$ |
| 4 | *Bauhinia* | P1 | 11.0$^{g}$ | 19.2$^{c}$ | 25.2$^{b}$ | 27.7$^{h}$ | 35.6$^{d}$ | 39.9$^{f}$ | 55.4$^{f}$ | 50.5$^{i}$ | 53.7$^{h}$ | 56.5$^{h}$ |
| | *purpurea* | P2 | 8.5$^{j}$ | 16.0$^{i}$ | 21.3$^{h}$ | 28.1$^{f}$ | 33.5$^{h}$ | 37.6$^{i}$ | 53.1$^{i}$ | 47.1$^{m}$ | 50.5$^{l}$ | 53.6$^{l}$ |
| | | P3 | 5.1$^{t}$ | 10.9$^{q}$ | 15.6$^{r}$ | 20.9$^{q}$ | 26.5$^{p}$ | 29.4$^{r}$ | 44.8$^{m}$ | 36.7$^{u}$ | 39.6$^{u}$ | 42.0$^{u}$ |
| 5 | *Canarium.* | P1 | 12.0$^{d}$ | 16.2$^{h}$ | 19.4$^{m}$ | 22.5$^{p}$ | 27.2$^{o}$ | 30.4$^{q}$ | 46.0$^{l}$ | 37.2$^{t}$ | 40.3$^{t}$ | 47.6$^{q}$ |
| | *indicum L* | P2 | 16.1$^{a}$ | 24.4$^{a}$ | 29.9$^{a}$ | 36.6$^{a}$ | 44.0$^{a}$ | 49.9$^{a}$ | 65.6$^{a}$ | 64.1$^{c}$ | 69.6$^{b}$ | 75.9$^{b}$ |
| | | P3 | 11.8$^{e}$ | 18.1$^{d}$ | 21.6$^{g}$ | 26.3$^{j}$ | 32.6$^{i}$ | 37.7$^{h}$ | 53.4$^{h}$ | 51.5$^{f}$ | 56.2$^{f}$ | 61.8$^{f}$ |
| 6 | *Bambusa* | P1 | 11.2$^{f}$ | 15.5$^{j}$ | 18.6$^{o}$ | 23.0$^{o}$ | 29.5$^{l}$ | 36.6$^{k}$ | 54.2$^{g}$ | 55.2$^{e}$ | 60.1$^{e}$ | 63.2$^{e}$ |
| | *arrundinaceae* | P2 | 14.8$^{b}$ | 20.7$^{b}$ | 23.9$^{d}$ | 28.9$^{d}$ | 36.1$^{c}$ | 43.7$^{b}$ | 61.3$^{b}$ | 66.3$^{a}$ | 71.6$^{a}$ | 76.3$^{a}$ |
| | | P3 | 12.8$^{c}$ | 17.4$^{f}$ | 20.8$^{j}$ | 25.4$^{i}$ | 31.8$^{j}$ | 38.6$^{g}$ | 56.2$^{e}$ | 57.6$^{d}$ | 62.2$^{d}$ | 66.7$^{d}$ |
| 7 | *Duranta* | P1 | 3.5$^{v}$ | 7.7$^{x}$ | 12.3$^{v}$ | 14.8$^{x}$ | 20.0$^{x}$ | 29.4$^{r}$ | 30.7$^{y}$ | 40.7$^{q}$ | 43.3$^{q}$ | 45.9$^{r}$ |
| | *repens* | P2 | 5.2$^{s}$ | 9.7$^{u}$ | 13.9$^{t}$ | 17.6$^{t}$ | 22.8$^{t}$ | 29.4$^{r}$ | 30.6$^{z}$ | 36.6$^{v}$ | 39.0$^{v}$ | 41.4$^{v}$ |
| | | P3 | 3.5$^{v}$ | 7.0$^{y}$ | 11.2$^{w}$ | 15.1$^{w}$ | 20.3$^{w}$ | 27.1$^{s}$ | 28.4$^{aa}$ | 31.5$^{y}$ | 34.2$^{x}$ | 36.8$^{x}$ |
| 8 | *Sesbania* | P1 | 4.1$^{u}$ | 9.4$^{v}$ | 15.8$^{q}$ | 19.0$^{s}$ | 24.2$^{s}$ | 30.4$^{q}$ | 40.3$^{q}$ | 43.8$^{p}$ | 46.0$^{o}$ | 48.3$^{p}$ |
| | *grandiflora* | P2 | 6.7$^{n}$ | 13.9$^{m}$ | 22.3$^{f}$ | 29.0$^{c}$ | 34.2$^{f}$ | 32.5$^{n}$ | 42.4$^{o}$ | 49.9$^{j}$ | 51.7$^{j}$ | 53.9$^{k}$ |
| | | P3 | 5.8$^{q}$ | 12.7$^{p}$ | 21.2$^{i}$ | 28.4$^{e}$ | 33.6$^{g}$ | 41.6$^{d}$ | 51.5$^{k}$ | 64.3$^{b}$ | 66.8$^{c}$ | 70.2$^{c}$ |
| 9 | *Leucaena* | P1 | 5.3$^{r}$ | 10.8$^{r}$ | 16.3$^{p}$ | 20.1$^{r}$ | 25.3$^{q}$ | 31.1$^{p}$ | 35.7$^{u}$ | 43.9$^{o}$ | 45.5$^{p}$ | 51.4$^{n}$ |
| | *leucocephala* | P2 | 7.6$^{l}$ | 14.0$^{l}$ | 20.8$^{j}$ | 24.8$^{k}$ | 30.0$^{k}$ | 36.9$^{j}$ | 41.6$^{p}$ | 51.4$^{g}$ | 54.7$^{g}$ | 57.4$^{g}$ |
| | | P3 | 7.6$^{l}$ | 13.6$^{n}$ | 19.5$^{l}$ | 23.9$^{m}$ | 29.1$^{m}$ | 34.9$^{m}$ | 39.5$^{r}$ | 48.4$^{l}$ | 51.0$^{k}$ | 55.8$^{i}$ |

$^{a-aa}$differences in superscripts in the same column show significantly different results (P<0.05).

Table 4. Content of phenolic and tannin components in nine types of feed plants

| Samples | Concentration (mg/100mgDM) | | | | |
|---|---|---|---|---|---|
| | Total phenol | Total non-tannin phenol | Total tannin | Condensed tannin | Hydrolisable tannin |
| *S. mahagoni (L.) Jacq* | 20.7$^{c}$ | 6.90$^{c}$ | 13.8$^{a}$ | 8.60$^{a}$ | 5.20$^{b}$ |
| *B. purpurea* | 29.4$^{a}$ | 10.4$^{a}$ | 7.10$^{c}$ | 3.21$^{c}$ | 3.74$^{d}$ |
| *C. sinensis* | 13.4$^{d}$ | 4.73$^{d}$ | 6.56$^{e}$ | 3.16$^{d}$ | 3.40$^{e}$ |
| *T. catappa L.* | 21.1$^{b}$ | 7.45$^{b}$ | 10.3$^{b}$ | 4.98$^{b}$ | 5.35$^{a}$ |
| *B. arrundinaceae* | 1.43$^{i}$ | 0.51$^{i}$ | 0.70$^{h}$ | 0.34$^{h}$ | 0.36$^{h}$ |
| *C. indicum (L.)* | 11.9$^{e}$ | 4.20$^{e}$ | 5.82$^{f}$ | 2.80$^{e}$ | 3.01$^{f}$ |
| *S. grandiflora* | 1.90$^{h}$ | 1.70$^{h}$ | 0.20$^{i}$ | 0.20$^{i}$ | - |
| *L. leucocephala* | 9.60$^{g}$ | 2.90$^{g}$ | 6.70$^{d}$ | 1.80$^{g}$ | 4.90$^{c}$ |
| *D. repens* | 11.4$^{f}$ | 4.03$^{f}$ | 5.59$^{g}$ | 2.69$^{f}$ | 2.90$^{g}$ |

$^{a-i}$differences in superscripts in the same column show significantly different results (P<0.05).

Table 5. Value of fractions a, b and a + b of nine kinds of forage

| No | Samples | Treatment | a | b | a+b |
|---|---|---|---|---|---|
| 1 | | P1 | 8.21±0.00$^{f}$ | 49.13±1.68$^{p}$ | 57.35±1.68$^{p}$ |
| 2 | *T. catappa L.* | P2 | 5.89±2.09$^{n}$ | 36.14±0.00$^{x}$ | 42.03±0.00$^{u}$ |
| 3 | | P3 | 7.36±2.09$^{k}$ | 54.40±1.68$^{m}$ | 61.76±1.68$^{o}$ |
| 4 | | P1 | 8.05±0.00$^{g}$ | 63.44±0.00$^{j}$ | 71.48±3.35$^{j}$ |
| 5 | *C. sinensis* | P2 | 10.26±4.19$^{d}$ | 71.26±3.35$^{e}$ | 81.52±3.35$^{c}$ |
| 6 | | P3 | 7.78±2.09$^{j}$ | 65.96±3.35$^{h}$ | 73.73±3.35$^{g}$ |
| 7 | | P1 | 5.96±2.09$^{m}$ | 46.29±1.68$^{s}$ | 52.25±0.00$^{r}$ |
| 8 | *S. mahagoni (L.) Jacq* | P2 | 7.90±4.19$^{h}$ | 55.52±1.68$^{k}$ | 63.42±3.35$^{n}$ |
| 9 | | P3 | 2.38±0.00$^{v}$ | 32.27±1.68$^{a}$ | 34.65±1.68$^{a}$ |
| 10 | | P1 | 5.54±2.09$^{o}$ | 67.05±3.35$^{g}$ | 72.59±0.00$^{i}$ |
| 11 | *B. purpurea* | P2 | 7.88±2.09$^{j}$ | 65.40±0.00$^{i}$ | 73.28±0.00$^{h}$ |
| 12 | | P3 | 9.30±0.00$^{e}$ | 55.18±1.68$^{l}$ | 64.47±0.00$^{m}$ |
| 13 | | P1 | -0.67±2.62$^{a}$ | 46.65±1.68$^{q}$ | 45.98±1.68$^{t}$ |
| 14 | *C. indicum (L.)* | P2 | 3.16±0.00$^{t}$ | 81.63±0.00$^{b}$ | 84.79±3.35$^{b}$ |
| 15 | | P3 | 3.67±0.00$^{s}$ | 67.31±0.00$^{f}$ | 70.97±0.00$^{k}$ |
| 16 | | P1 | 6.28±2.09$^{l}$ | 73.49±3.35$^{d}$ | 79.77±3.35$^{e}$ |
| 17 | *B. arrundinaceae* | P2 | 5.04±2.09$^{p}$ | 85.54±3.35$^{a}$ | 90.58±3.35$^{a}$ |
| 18 | | P3 | 4.71±2.09$^{q}$ | 74.72±0.00$^{c}$ | 79.43±0.00$^{f}$ |
| 19 | | P1 | 3.04±2.09$^{u}$ | 49.27±1.68$^{o}$ | 52.31±0.00$^{q}$ |
| 20 | *D. repens* | P2 | 0.20±1.31$^{z}$ | 40.80±0.00$^{t}$ | 41.00±0.00$^{w}$ |
| 21 | | P3 | 1.69±1.05$^{x}$ | 37.21±1.68$^{v}$ | 38.90±1.68$^{y}$ |
| 22 | | P1 | 16.02±8.38$^{c}$ | 33.64±0.00$^{z}$ | 49.66±0.00$^{s}$ |
| 23 | *S. grandiflora* | P2 | 29.32±1.67$^{a}$ | 51.44±0.00$^{n}$ | 80.76±3.35$^{d}$ |
| 24 | | P3 | 18.85±0.00$^{b}$ | 46.48±1.68$^{r}$ | 65.33±3.35$^{j}$ |
| 25 | | P1 | 4.37±2.09$^{r}$ | 34.44±0.00$^{y}$ | 38.81±0.00$^{z}$ |
| 26 | *L. leucocephala* | P2 | 1.22±5.24$^{y}$ | 39.97±0.00$^{u}$ | 41.19±0.00$^{v}$ |
| 27 | | P3 | 1.89±5.24$^{w}$ | 37.12±1.68$^{w}$ | 39.02±1.68$^{x}$ |

$^{a-z}$differences in superscripts in the same column show significantly different results (P<0.05).

Table 6. Results of analysis of forage chemical composition

| Samples | DM (%) | DM (%) | | | | |
|---|---|---|---|---|---|---|
| | | OM | EE | CP | CF | Non-nitrogen extract |
| *S. mahagoni (L.) Jacq* | 37.5$^{e}$ | 89.3$^{h}$ | 2.08$^{e}$ | 5.75$^{j}$ | 9.40$^{g}$ | 72.1$^{a}$ |
| *B. purpurea* | 50.4$^{a}$ | 89.5$^{g}$ | 2.48$^{d}$ | 14.7$^{e}$ | 20.9$^{a}$ | 51.4$^{i}$ |
| *C. sinensis* | 37.9$^{d}$ | 94.0$^{a}$ | 3.24$^{b}$ | 17.0$^{c}$ | 8.03$^{h}$ | 65.7$^{b}$ |
| *T. catappa* | 29.0$^{h}$ | 89.7$^{e}$ | 1.82$^{f}$ | 13.3$^{g}$ | 17.4$^{c}$ | 57.3$^{f}$ |
| *B. arrundinaceae* | 39.6$^{c}$ | 84.3$^{i}$ | 1.74$^{g}$ | 14.9$^{d}$ | 11.5$^{e}$ | 56.2$^{g}$ |
| *C. indicum (L.)* | 44.0$^{b}$ | 89.6$^{f}$ | 0.52$^{i}$ | 9.01$^{h}$ | 17.9$^{b}$ | 62.2$^{d}$ |
| *S. grandiflora* | 36.2$^{f}$ | 91.7$^{c}$ | 4.21$^{a}$ | 22.4$^{a}$ | 5.30$^{i}$ | 59.8$^{e}$ |
| *L. leucocephala* | 33.1$^{g}$ | 92.6$^{b}$ | 3.03$^{c}$ | 22.2$^{b}$ | 13.7$^{d}$ | 53.7$^{h}$ |
| *D. repens* | 27.3$^{i}$ | 91.3$^{d}$ | 1.01$^{h}$ | 14.4$^{f}$ | 10.3$^{f}$ | 65.6$^{c}$ |

$^{a,b,c,d,e,f,g,h,i}$differences in superscripts in the same column show significantly different results (P<0.05).

interactions between the tannin hydroxyl groups and the involvement of oxygen atoms in the glycosidic bonds that make up the branch chain of polysaccharides belonging to the gel the dextran gel. The nutrient content of bamboo leaves, especially CF, was high (Table 6), but bamboo leaves has low CT levels, so tannin-carbohydrate complex bonds will not be formed much.

Most of the forages given an additional 200 mgDM PEG resulted with the highest a + b fraction gas production, although tehtehan leaves showed different results, which the most optimal a + b fraction gas production was shown by those without PEG addition. That is because when a given PEG exceeds the optimal threshold, it cause a decrease in a + b gas production. Improper PEG level will have a negative effect. Silanikove *et al*. (1996) found that goats fed high-tannin Pistacia lentiscus with PEG (10 g/day) had negative CP digestibility. On the contrary, other authors have reported significant improvement in the apparent digestibility of nutrients (OM and CP) with PEG supplementation (Barry *et al.,* 1986; Ben Salem *et al.,* 2005; Yisehak *et al.,* 2013).

The effect of supplementation level for optimal productivity depend on the production parameters of interest. This has implications on supplementation recommendations. The very complex structure of tannins can cause the PEG-tannin bond not formed optimally so tannin in the digestive tract will interfere with microbial activity in degrading feed nutrients. It is possible that PEG 4000 was not enough to have effect on the a + b gas production fractions in ketapang.

## Conclusions

The addition of 200 mg/100mgDM of PEG optimizes the rate of forage gas production, especially for grass plants (*gramineae*). *Sesbania grandiflora* leaves which were added with 200 mgDM of PEG had the greatest nutrient value.

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