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THE CHEMICAL COMPOSITION AND VARIOUS SAMPLES PREPARATION METHODS FOR *In Vitro* GAS TEST OF TWO TROPICAL FEEDS

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ABSTRAK

Rancangan Acak Lengkap pola Faktorial (3x2) digunakan untuk mengevaluasi komposisi kimia dan pengaruh preparasi sampel serta penambahan polyethylene glycol (PEG) terhadap kecernaan daun *Sesbania grandiflora* (SG) dan daun *Manihot esculenta Crantz* (MEC) yang menghasilkan gas secara *in vitro*. Daun SG dan MEC sebagai sampel masing-masing dibagi menjadi tiga kelompok metode preparasi sampel yaitu sampel segar (F), sampel freeze drying -40°C (FD) dan sampel oven 55°C (OD). Hasil penelitian menunjukkan bahwa rata-rata produksi gas dari sampel segar pada sampel daun SG dan MEC nyata lebih tinggi (P<0,05) dibandingkan sampel FD dan OD. Pada kedua jenis daun, produksi gas sampel FD tidak berbeda nyata dengan sampel OD. Produksi gas dengan PEG nyata lebih tinggi (P<0,05) dibandingkan dengan tanpa PEG. Disimpulkan bahwa metode persiapan dan pengeringan sampel pakan mempengaruhi volume produksi gas. Penambahan PEG menghasilkan volume produksi gas yang lebih tinggi pada daun SG dan MEC.

Kata kunci: Freeze dried, Oven, Produksi gas, PEG, Segar

ABSTRACT

A 3x2 factorial experimental design was conducted to evaluate the chemical composition of Sesbania grandiflora (SG) and Manihot esculenta Crantz (MEC) leaves and to measure the effects of preparation and drying methods on the *in vitro* gas production in the presence and absence of PEG. The collected samples were divided into three groups: One group was fresh samples (F). The second group was oven-dried at 55°C for 48h (OD) and the last group was freeze-dried at –40°C for 72h (FD). Results showed that the mean value of gas production from fresh SG and MEC samples were not significantly higher (P<0.05) than from FD and OD samples. In SG and MEC, the mean value of gas production of FD was not significant compared to OD samples (P>0.05). Gas production from samples added with PEG were higher (P<0.05) than without PEG. In conclusion, the preparation and drying methods of feed samples could affect the volume of gas production. The addition of PEG in SG and MEC resulted in higher gas production volumes.

Keywords: Fresh, Freeze dried, Gas production, Oven, PEG

INTRODUCTION

Tree and shrub leaves can be an important component of small ruminant feed, especially during the dry season when the quality and quantity of green herbages is limited. The use of fodder shrubs and trees has been more widely practiced in Indonesia where population pressure on land makes it imperative that every available feed resource is fully utilized. Tree fodder is generally rich in protein and minerals and is used as a dry season supplement to poor quality natural pasture and/or fibrous crop residues (Tolera *et al.*, 1997). Cassava and Sesbania trees offer a good supplementary feed to those low quality grasses.

The nutritive value of a ruminant feed is determined by the concentrations of its chemical components, as well as their rate and extent of digestion, but this does not provide enough information on the feeds nutritive value (Hamid *et al*., 2007; Akinfemi *et al.*, 2009). The *in vitro* gas production method is widely used to evaluate the nutritive value of different classes of feeds (Getachew *et al*., 1998). The *in vitro* method of feed evaluation is less expensive and less time consuming compared with *in vivo* methods.

Evaluation of forage can be affected by several factors such as how the samples are prepared (fresh or dried) or thsually either dried (oven or freeze-dried) or fresh before being analyzed. The procedure used for preparation of fresh or dried forage samples before *in vitro* (or *in situ*) evaluation can affect the results. Parissi *et al*. (2004) have reported that leguminous species with a high content of protein should be dried at low temperature in order to avoid denaturation of protein and prevent depression of fermentation. The drying technique could affect the chemical composition and fermentation of animal feeds (Parissi *et al*., 2004). Furthermore, the nutritive value of some tanniniferous fodders can also be greatly influenced by drying method (Ahn *et al*., 1989; Palmer *et al*., 2000; Parissi *et al*., 2004).

The objective of this study was to measure the effects of preparation and drying methods on the *in vitro* gas production in the presence and absence of PEG. Comparisons have been made between fresh, freeze dried (FD) and oven dried samples (OD).

MATERIALS AND METHODS

Samples Preparation and Chemical Analysis

Leaves samples from two tropical feed species *Manihot esculenta* Crantz (MEC) and *Sesbania grandiflora* (SG) were collected from Yogyakarta region in Indonesia. The collected samples were divided into three groups: One group was fresh samples (F). The second group was freeze-dried at -40°C for 72 h (FD) and the last group was oven-dried at 55°C for 48h (OD). Fresh samples were blended for 2 minutes, FD samples and OD samples were ground in a Wiley mill through a 1 mm screen. Sub samples were taken for dry matter determination. Organic Matter (OM), Crude Fibre (CF) and Crude Protein (CP) were determined according to AOAC (2005). Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) of samples were assayed using the method proposed by Van Soest *et al.* (1991).

In Vitro Gas Production

Rumen fluid for the *in vitro* was obtained from cattle in the morning before feeding and gas production was measured as described by Menke and Steingass (1988). The gas production *in vitro* method was used to evaluate gas production in the absence or presence of polyethylene glycol (PEG). Gas production was recorded after 3, 6, 12, 24, 48, 72 and 96h. The mean gas volume readings were adjusted to the exponential equation $p = a + b\ (1 - e^{-ct})$ (Ørskov and McDonald, 1979), where p is the gas production at time t, a + b is the potential gas production and c is the rate of gas production.

Statistical Analyses

The data obtained were analyzed using analysis of variance (ANOVA) and if among the treatments showed significant differences (P<0.05), it then will be followed by LSD test at the 0.05 significance level (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Chemical Composition

The chemical composition of the *Manihot esculenta* Crantz (MEC) and *Sesbania grandiflora* (SG) feed samples (OM, CP, CF, NDF and ADF) are presented in the Table 1. The OM content of feeds ranged from 88.12% in MEC to 92.42% in SG. The CP content of SG was higher than that of MEC (Table 1). The CF content was higher in MEC (18.42%). The NDF content of feeds were ranged from 21.22% in SG leaf to 24.62% in MEC. This study indicated that MEC leaves were the higher in CF, NDF and ADF compare to SG leaves.

Volume of Gas Production

Table 2 and Table 3 show the *in vitro* gas production of the MEC and SG feed samples. The volume of gases produced from samples were ranged from 30.23 to 49.97 ml in the absence of

Table 1. Chemical Composition of the Two Feed Samples (% DM)

Forage	% (in DM)				
	OM	CP	CF	NDF	ADF
Manihot esculenta Crantz (MEC)	88.12	20.03	18.42	24.62	15.87
Sesbania grandiflora (SG)	92.42	24.66	14.27	21.22	10.81

OM : Organic Matter; CP : Crude Protein; CF : Crude Fibre; NDF : Neutral Detergent Fibre; ADF : Acid Detergent Fibre

Table 2. The Mean Value of The Volume of Gas Production (a+b) of MEC (ml/200 mg DM)

	Feed Sample Preparation Methods			Average
	F	FD	OD	
Without PEG	36.42	30.93	30.23	32.95[a]
With PEG	47.81	37.29	36.97	42.70[b]
Average	42.11[a]	34.11[b]	33.60[b]	

F : Fresh sample: FD : Freeze-dried; OD : Oven-dried
[a,b] Different superscripts in the same row indicate a significant difference (P<0.05)

Table 3. The Mean Value of The Volume of Gas Production (a+b) of SG (ml/200 mg DM)

	Feed Sample Preparation Methods			Average
	F	FD	OD	
Without PEG	49.97	38.35	38.89	44.31[a]
With PEG	50.25	45.37	43.16	47.38[b]
Average	50.11[a]	41.86[b]	41.02[b]	

[a,b] Different superscripts in the same row indicate a significant difference (P<0.05)

PEG, and 36.97 to 50.25 ml in the presence of PEG.

The volume of gases produced (*a*+*b*) from fresh samples was higher (P<0.05) than from FD and OD samples. This result was supported by Berhane *et al*. (2006) that gas production was higher in fresh cut vetch (*Vicia sativa*) than in the vetch hay. Calabrò *et al*. (2005) reported that gas production was higher (P<0.01) in fresh versus dried silage and time of accumulation of half the potential gas produced was similar (i.e., P=0.31).

The FD samples produced volume of gas similar to OD (Table 2). Parissi *et al*. (2004) suggested that leguminous species with a high content of protein should be dried at low temperature in order to avoid denaturation of protein and to prevent depression of fermentation.

The lower digestibility with aerobic drying than with freeze drying method is well established using nylon bag techniques (Palmer *et al*., 2000).

Palmer *et al*. (2000) conclude that in the absence of freeze drying facilities, samples should be dried at 45^oC to minimize the adverse effects of drying on measures of the fibre fractions and of *in vitro* digestibility. At this temperature, there was little difference between aerobic (oven) and anaerobic drying (freeze dry method) for the various characteristics measured. Nastis and Malechek (1988) working with *Quercus gambelli* have reported that oven-drying at 55, 65 or 100°C reduced digestibility of foliage samples more than freeze-drying. Parissi *et al.* (2004) suggested that leguminous species with a high content of protein should be dried at low temperature in order to avoid denaturation of protein and prevent depression of fermentation.

Generally, the potential of gas production for non forage high fibrous tropical feed was high, because of the high carbohydrate fraction (particularly NDF). It is well known that gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate. Whereas, protein fermentation does not lead to much gas production (Chumpawadee *et al.*, 2005).

The volume of gases produced (*a+b*) from *Sesbania grandiflora* (SG) sample was higher than *Manihot esculenta Crantz* (MEC) sample, this may be because NDF content of MEC sample was higher (24.62%) than that of SG sample (21.22%). Total gas and CH_4 productions were negatively correlated with neutral detergent fibre (NDF) content (Santoso and Hariadi, 2008).

The addition of PEG increased gas production. The mean value of gas production from samples added with PEG was higher (P<0.05) than those without PEG. The increase in the gas production in the presence of PEG can be resulted due to an increase in the available nutrients to rumen microorganisms, especially N (Bakhshizadeh and Taghizadeh, 2013). The potential digestion of fibre and protein can be influenced by anti-nutritional factors in the diet. Tannins are the most common anti-nutritional factor in plants and can be found in herbs, shrubs and leaves. Due to the ability to form complexes with proteins, tannins can bind feed proteins and also inhibit endogenous and microbial enzymes. The addition of polyethylene glycol (PEG) to the diet can markedly reduce the negative effect of tannins on digestion (Silanikove *et al.*, 1996; Kustantinah *et al*., 2004). The phenolic compounds depress *in vitro* gas production, while PEG has a potential for binding the phenolic compound and improves gas production (Tolera *et al.*, 1997). Rubanza *et al.* (2005) reported that the highest response on *in vitro* gas production due to addition of PEG, could be related to reversed tannin anti-nutritive activity.

The Rate of Gas Production

The rate of gas production from samples that without or added with PEG varied between 4.10 to 5.90% and 2.80 to 6.80%, respectively (Table 4 and Table 5). Higher rate of gas production was observed in fresh MEC with PEG, possibly it was influenced by the soluble carbohydrate fractions readily available to ruminal microbes. Deaville and Givens (2001) also reported that the carbohydrate fraction could affect the kinetics of gas production.

CONCLUSION

The preparation and drying methods of feed samples could affect the volume of gas production. An ideal system of substrate preparation requires further investigation, since ground and dried materials are not reach the rumen *in vivo*. The use of fresh samples of SG and

Table 4. The Mean Value of The Rate of Gas Production of MEC (%/hour)

	Feed Sample Preparation Methods			Average
	F	FD	OD	
Without PEG	5.60	5.30	4.90	5.43[a]
With PEG	6.80	6.20	5.00	6.25[b]
Average	6.20[a]	5.75[b]	4.93[c]	

[a,b,c] Different superscripts in the same row indicate a significant difference (P<0.05)

Table 5. The Mean Value of The Rate of Gas Production of SG (%/hour)

PEG	Feed Sample Preparation Methods			Average
	F	FD	OD	
Without PEG	5.90	5.80	4.10	5.55[a]
With PEG	6.10	4.90	2.80	5.00[b]
Average	6.00[a]	5.30[a]	3.50[b]	

[a,b] Different superscripts in the same row indicate a significant difference ($P<0.05$)

MEC leaves produces higher volumes of gas production than freeze-dried or oven samples, so to best simulate the fresh feed conditions *in vivo*, *in vitro* feed evaluation should be performed using fresh samples. Fresh forages should be used where possible, as drying have large effects on Gas Production Profile (GPP) of forages. The addition of PEG resulted in higher gas production volumes than without the addition of PEG, it may be due to antinutritive contents (possibly Tannin), that contained in SG and MEC leaves.

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