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In vitro gas production of legume *Bauhinia purpurea*, *Cassia alata* and *Macroptilium atropurpureum*

A Ikhwanti^{1*}, I G Permana², Y Retnani², A A Samsudin³ and A Jayanegara²

¹ Graduate School of Nutrition and Feed Science, Bogor Agricultural University, Bogor 16680, Indonesia

² Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Bogor 16680, Indonesia

³ Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang Selangor Dahrul Ehsan 43400 Malaysia

*ikhwanti@gmail.com

Abstract. Legume is feed commodity for livestock which is widely available in tropical country. The availability of tree legumes does not depend on seasons. However, the quality of legumes is varied, requiring information on nutrient composition. This study aimed to evaluate total gas production in several legume types (tree, clump, creep) *in vitro*. This study was conducted from January 2018 until April 2018 at Nutrition Laboratory, Department of Animal Science, Faculty of Agriculture, University Putra Malaysia. All samples were determined for proximate analysis, Van Soest fiber fractions, i.e. Neutral detergent fiber (NDF), Acid detergent fiber (ADF), Acid detergent lignin (ADL) and total gas production *in vitro*. There were three tropical legumes tested in this study, i.e. (1) *Bauhinia purpurea* (BP), (2) *Cassia alata* (CA), (3) *Macroptilium atropurpureum* (MA). Randomized Block Design (3 treatments x 3 replicates) was used to analyse data. Data were tested using analysis of variance (ANOVA) and continued with Duncan Test if there was a significant difference. The data showed that *Bauhinia purpurea* produced the highest total gas production 27.83 mL ($P < 0.05$) at 24 h of incubation time. Furthermore, there were no significant differences between total gas production of three legumes at 48 h of incubation time ($P > 0.05$).

1. Introduction

Legume is easily found in tropical forest, especially Indonesia. The productivity of feed crops is high during the rainy season in the tropics [1]. For tree legume, its availability does not depend on wet or dry season, they are available every time. Tree legume has the ability to grow on poor soil nutrients and dry soil for several months. Legume is used by farmers for feeding ruminant because of its nutrient composition [2]. Legume plants are known as green concentrate because they contain high protein and digestibility comparing grass [3]. Tropical plants contain much higher starch and are stored in leaves [4]. Nowadays, society are exploring and evaluating nutrient composition of legumes. There are several common legumes which is often used by farmers. However, there are still many legume plants that have not been explored. The information consisting of nutrient composition, digestibility as well as *in vitro* parameters from those legumes are very important to increase effectiveness and efficiency in feeding trial.



Provision feed for ruminant is certainly important to pay attention to the adequacy of nutrients needed, especially from forage. The quality of forage (grass) is still far from expectation. In the end, by giving additional reinforcement feed to meet the nutritional needs, which needs high cost. Meanwhile, the potential forage of protein sources (leguminous) that are around us quite available, but unfortunately many of them are not fully utilized optimally for animal feed.

Gas production is one of *in vitro* parameter that is very useful to predict digestibility value. Furthermore, *in vitro* gas production system can predict methane gas production *in vivo* [5]. However, *in vivo* digestibility studies are expensive as compared to *in vitro* studies. In addition to reducing cost, *in vitro* studies also have a lower risk effect for animals [6]. The use *in vitro* studies can support data and information collection of legume in large amounts. This study aims to evaluate total gas production in several legume types (tree, clump, creep) *in vitro*.

2. Materials and methods

This research were conducted from January 2018 until April 2018 at Agrostology Laboratory and Food Science Laboratory Bogor Agricultural University and University Putra Malaysia, Department of Animal Science University Putra Malaysia, and Livestock Research Institute (Balitnak) Ciawi, Bogor.

2.1. Samples collecting

Samples from 3 legume species were collected in Dramaga, Bogor. The treatments consisted of *Bauhinia purpurea* (BP), *Cassia alata* (CA) and *Macroptilium atropurpureum* (MA). Each sample is tree legume, creep legume and clump legume. Approximately, 2 kg weight of each legume were collected by chopping the edible part of each plant, then those samples were dried directly under sunlight in 3 days and followed by oven drying at 60°C. After that, the samples were grinded by grinder to pass sieve 1 mm.

2.2. Analysis of chemical composition

Legumes were analyzed for discovering nutrient composition by using proximate analysis [7] and cellulose, hemicellulose and lignin analysis [8]. Proximate analysis consisted of dry matter (DM), total ash, crude protein (CP), ether extract (EE) and crude fiber (CF). Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were analysed using Van Soest *et al.* (1991). After samples were grinded, each of samples was balanced approximately 3 grams-weight, then those samples were putting into crucible. Those crucible that filled by sample was entered into oven 105°C. Measurement of dry matter (DM) was done for 24 hours.

The crucible and sample that have been heated in oven 105°C, were entered into furnace 550°C for 3 hours. Ether extract (EE) was analysed by FOSS Tecator Soxtec Avanti 2050. Crude protein (CP) was analysed by FOSS Tecator 2400 Kjeltac Analyzer Unit. Crude fiber (CF) was analysed by electric heater with beaker filled-samples.

2.3. *In vitro* gas production

In vitro gas production was evaluated according to the described method by [9] with some modifications. Firstly, 200 mg weight of oven-dried inserted into well-lubricated glass syringe. Previously, the bottom end of syringe was closed by using a clip. Buffer solution was made by fusing several materials, consisted of rumen fluid with anaerobic condition at 39°C, micro mineral, macro mineral, buffer solution, resazurin solution, reducing solution (must be freshly prepared each time), and distilled water. Then, the buffer solution is mixed with rumen fluid with 2:1 ratio. That mixture is prepared and put into water bath 39°C, followed by continuous stirring in an anaerobic condition. After that, 30 mL buffer-rumen solution was put into each of glass syringes which were containing legume treatments. Each of treatments were analysed in four replicates and three round of incubation. The observed water bath that filling glass syringe was maintained in temperature 39°C. The observation of total gas production was conducted and recorded at 0, 3, 6, 9, 12, 24, 48 and 72 hour of incubation.

Table 1. Nutrient composition (DM Basis).

Plant species	Sample type	DM (%)	Ash (%)	CP (%)	EE (%)	CF (%)	NDF (%)	ADF (%)	ADL (%)
BP	Tree	24.74±0.05	11.18±0.07	28.95±0.61	2.45±0.06	23.09±0.25	57.57±0.02	29.29±0.18	0.27±0.02
CA	Clump	21.45±0.02	8.71±0.03	25.86±0.03	1.74±0.03	18.44±0.15	49.20±1.82	23.94±0.36	0.63±0.03
MA	Creep	21.04±0.03	6.81±0.01	17.87±0.34	1.22±0.04	31.44±0.68	80.62±0.59	49.54±0.39	0.51±0.02

Notes : BP : *Bauhinia purpurea*, CA : *Cassia alata*, MA : *Macroptilium atropurpureum*, DM: Dry matter, CP: Crude protein, EE : Ether extract, CF :Crude fiber, NDF : Neutral detergent fiber, ADF : Acid detergent fiber, ADL : Acid detergent lignin.

2.4. Statistical analysis

All the experimental data were analysed with General Linear Model procedure by SPSS 16.0 software. The significant differences among treatment means were compared by using Duncan Multiple Range Test.

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij} \quad (1)$$

- Y_{ij} : the observed value for the j-th replicate of the i-th treatment
 μ : the grand mean
 α_i : is the i-th treatment effect
 β_j : the j-th block effect
 ϵ_{ij} : the random error associated with the Y_{ij} experimental unit

3. Result and discussion

Total gas production is one of *in vitro* analysis parameter. The data showed (figure 1) that legume *Bauhinia purpurea* has the highest volume of total gas production. Based on nutrient composition (table 1), *Bauhinia purpurea* also has highest dry matter and crude protein percentage. Relationship between nutrient composition and total gas production has not declared widely yet. However, total gas production can be mentioned to predict feed or substrate digestibility. Basically, total gas production can be obtained by rumen microbe activity. Rumen microbe helps host animal to digest diet that they consume [10]. There are many microbes in the rumen, but hereinafter referred to as rumen microbes are microbes that aid for feed digestibility in the rumen.

Regarding to degradation by rumen microbe, to reach efficiency in feeding trial, it is needed both of quality and quantity of substrate. Both of them must be measurable. For example, urea has high protein content but can not be degraded by rumen microbes.

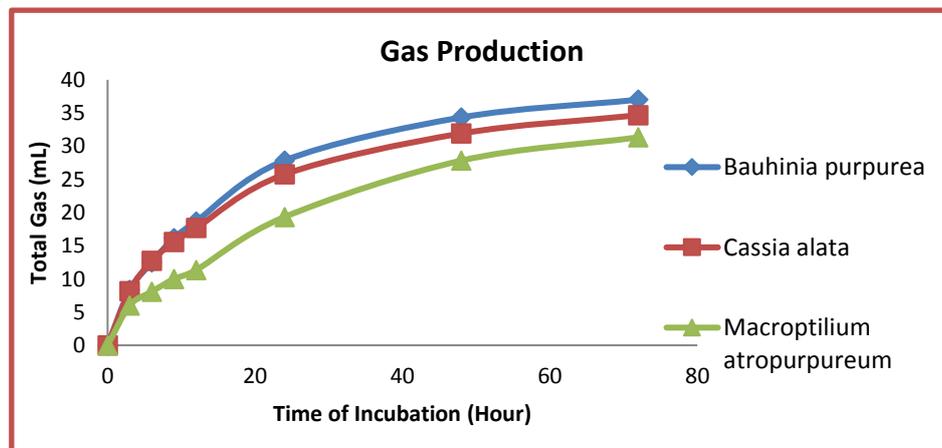


Figure 1. Kinetics curve of total gas production from *Bauhinia purpurea*, *Cassia alata* and *Macroptilium atropurpureum*.

As we know that urea is easily converted to ammonia which is mentioned as RDP (rumen degradable protein).

Rumen microbes ferment in degrading substrate. Meanwhile, the final product of fermentation is gas (CO₂ and CH₄). Moreover, rumen microbes need time to degrade and ferment more substrate. The result (figure 1) determined that total gas production rate at 24th hour is the highest than 48th and 72nd hour of incubation. This matter happens because of substrate availability. In the first hours, the incoming substrate will soon be degraded by rumen microbes. So that in the final hours of incubation, rumen microbes only degrade the rest of substrate. This is reflected in total gas produced. The longer incubation time will also decrease rate of total gas production. It is stated that the lower availability of digested substrate during longer incubation periods causes the rate of gas production to slow down in line with incubation time [11]. The presence of organic matter (OM) influence total gas production because OM presence contributed to support animal microbe activity in producing VFA [12].

Table 2. Comparison of total gas production by time.

Treatment	Time of Incubation		
	24	48	72
<i>Bauhinia purpurea</i>	27.83a	34.33	37
<i>Cassia alata</i>	25.75a	31.92	34.67
<i>Macroptilium atropurpureum</i>	19.33b	27.83	31.33
SEM	1.53	1.44	1.45
P Value	0.027	0.075	0.105

Notes : SEM (Standard Error Means)

According to statistical result (table 2), it showed that total gas production at 24th hour of time incubation has significant difference ($P < 0.05$). *Bauhinia purpurea* and *Cassia alata* showed the highest volume of total gas production. Furthermore, nutrient composition (DM and CP) of them sequentially showed the higher ones than *Macroptilium atropurpureum*. This is in line with statement constituent of the diets affects the digestibility and the total gas production is the protein and fibrous content [13]. Protein content in legumes are relatively high, but there is tannin as constrain factor. Besides reducing methane, on the other hand, tannin can bind to protein and decrease protein availability [14]. Tannins may inhibit growth and affect development activity of methanogens [15]. In the *in vitro* gas production method, the effects of tannin in the rumen fermentation are reflected in total gas production [16].

Total gas production is an *in vitro* digestibility indicator. There should be many studies that discuss whether *in vitro* total gas production can be used as an indicator of *in vivo* digestibility. Furthermore, it can be explained how much chance *in vitro* total gas production affects *in vivo* digestibility.

4. Conclusion

Each of legumes has specific characteristic, included nutrient composition *in vitro* total gas production. Total gas production can be influenced by nutrient composition and also its availability in rumen.

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References

- [1] G G P Carvalho, R A Reboucas, F S Campos, E M Santos, G G L Araujo, G C Gois, J S Oliveira, R L Oliveira, L M Rufino, J A G Azevedo and J G A Cirne 2017 Intake, digestibility, performance, and feeding behavior of lambs fed diets containing silages of different tropical forage species *J.ani. feed. Sci.* **228** 140 – 148
- [2] P Huhtanen and J Sveinbjornsson 2006 Evaluation of methods for estimating starch digestibility and digestion kinetics in ruminants *J. Anifeedsci* **130** 95–113
- [3] S N Heinritz, S D Martens, P Avilaa and S Hoedtke 2012 The effect of inoculant and sucrose addition on the silage quality of tropical forage legums with varying ensilability *J. anifeedsci* **174** 201 – 210
- [4] M B Hall 2014 Selection of an empirical detection method for determination of water-soluble carbohydrates in feedstuffs for application in ruminant nutrition *Anim. Feed Sci. Tech.* **198** 28 – 37
- [5] R Danielsson, M Ramin, J Bertilsson, P Lund and P Huhtanen 2017 Evaluation of a gas in vitro system for predicting methane production in vivo *J. Dairy. Sci.* **100** 8881–8894
- [6] L Eggera, O Ménard, C Baumann, D Duerr, P Schlegel, P Stoll, G Vergères, D Dupont and R Portmann Digestion of milk proteins: Comparing static and dynamic in vitro digestion systems with in vivo data *J. Foodres.*
- [7] [AOAC] Association of Analytical Communities 2005 *Official Methods of Analysis of AOAC International* (18th Ed.) (Arlington (US): Association of Official Analytical Chemical)
- [8] P J Van Soest, J B Robertson and B A Lewis 1991 Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition *J. Dairy Sci.* **74** 3583–3597
- [9] K H Menke and H Steingass 1988 Estimation of the energetic feed value obtained from chemical analysis and in-vitro gas production using rumen fluid *Anim. Res. Dev.* **28** 7–55
- [10] E B Laconi and A Jayanegara 2015 Improving nutritional quality of cocoa pod (*Theobroma cacao*) through chemical and biological treatments for ruminant feeding: *In vitro* and *in vivo* evaluation *Asian-Australas. J. Anim. Sci.* **28** 343–350
- [11] A Jayanegara, E Wina, C R Soliva, S Marquardt, M Kreuzera and F Leiber 2011 Dependence of forage quality and methanogenic potential of tropical plants on their phenolic fractions as determined by principal component analysis *J. anifeed. Sci.* **163** 231–243
- [12] A Sofyan, A A Sakti, H Herdian, G Khairulli, A E Suryani, P D M H Karti and A Jayanegara 2017 *In vitro* gas production kinetics and digestibility of king grass (*Pennisetum hybrid*) added by organic mineral and natural crude protein *Journal of applied animal research* **45** 122 – 125
- [13] R K O Morais, A M A Silva, L R Bezerra, H Carneiro, M N Moreira and F F Medeiros 2015 In vitro degradation and total gas production of byproducts generated in the biodiesel production chain. *Acta. anim. sci.* **37** 143 – 148
- [14] A Jayanegara and E Palupi 2010 Condensed tannin effects on nitrogen digestion in ruminants: A meta-analysis from *in vitro* and *in vivo* studies *Media Peternak* **33** 176–181
- [15] A Cieslak, M Szumacher-Strabel, A Stochmal and W Oleszek 2013 Plant components with specific activities against rumen methanogens *Animal* **7** 253 – 265
- [16] S Pashaei, V Razmazar and R Mirshekar 2010 Gas production : A proposed *in vitro* method to estimate the extent of digestion of a feedstuff in the rumen *Jbs.* **10** 573 – 580