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Nutritive Value, Energy Metabolism, Organic Matter Digestibility of Some Fodder Tree Species and its Potential as Methane Inhibitor

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ABSTRACT

Background: A negative impact on livestock productivity and the environment due to accumulation of greenhouse gases, might be mitigated by using secondary metabolic compounds from plants in the form of tannins which potentially proven in reducing the methane production of ruminants. This study aims to explore the *Fodder Trees Species* (FTS) in terms of nutritional value, energy metabolism content and its potential as *an inhibitor* of methane gas produced in rumen fermentation.

Methods: This research was carried out in two stages. Phase I, evaluation of the nutritional value of the FTS which includes a proximate analysis and analysis of the NDF and ADF fiber fractions components. Phase II, the evaluation of FTS with *in vitro* gas methods. The study was carried out in a Completely Randomized Design (CRD) consisting of 6 species of FTS as treatment and 4 replications. Profil GP and metan production are measured at incubation time of 3, 6, 9, 12, 24, 48 and 72 h. DM digestibility, OM digestibility, N-amonia and energy metabolism were measured at the end of *in vitro* gas study.

Result: The Species of FTS has varied chemical composition, potential nutritional value and not only provides protein but also a source of energy for ruminant animal. At incubation times of 12 to 24 hours, the production of methane gas from *M. oleifera, S. grandiflora* and *L. leucocephala* is greater than that from *G. sepium* and *C. calothyrsus*. However, at incubation times exceeding 24 hours, *C. calothyrsus* produces more methane gas than *P. falcataria* and *G. sepium*. The highest levels of N-ammonia are produced by *M. oleifera*, *L. leucocephala*, *S. grandiflora* and *C. calothyrsus*, while the lowest levels are observed in *P. falcataria* and *G. sepium*. *P. falcataria* and *G. sepium* found as the potential low methane-producing feeds and have protein characteristic which are resistant to rumen microbe digestion.

Key words: Methane inhibitors, Nutritional value, Tree species.

INTRODUCTION

The main feed for ruminants in the tropics is dominated mostly from forage, even close to the value of 100%. As forage high of fibre content, the metabolism process tended to produce high portion of acetate and increase methanogenesis in rumen (Goel *et al*., 2005; Silivong *et al*., 2013). Thus, ruminant is claim as high contributor for greenhouse gasses (GHG) to atmosphere (Leng 2010). Methane produced from feed fermentation in the rumen contribute around 30-40% of methane gas production in agricultural sector (Moss *et al*., 2000). Therefore, the focus strategy in methane mitigation is how to get economic benefit as well as environmental (Thornton 2010; Sina and Preston, 2017).

Some researchers have intensively encouraged to explore various types of plant and use the extract as an alternative of antioxidant, antimicobial, antifungal and antibiotic growth promoters (AGPs), which have the potential to reduce negative impact of the environment (Makkar *et al*., 2007; Kavitha *et al*., 2023). Many plants that are classified as a group of tree legume species (*fodder trees species =FTS*), such as Sengon (*Albizia falcataria*), Gamal (*Gliricidia sepium*), Turi (*Sesbania grandiflora*), Kaliandra (*Calliandra calothyrsus*), Moringa (*Moringa oleifera*) and Lamtoro (*Leucaena leucocephala*), are used to fed to livestock in Indonesia. The FTS group of plants generally contains secondary metabolites such as tannins and saponins, which has the potential to reduce methane gas emissions in ruminants (Patra *et al*., 2006; Puchala *et al*., 2012).

This study aims to evaluate the chemical composition, dry matter digestibility (DMD), organic matter digestibility (OMD), ME, gas production and methane gas using *in vitro gas techniques*. The potential candidates from the FTS can be obtained in addition to having a high nutritional content, also potentially as a methane gas *inhibitor* that

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can developed as a supplement as well as as part of ruminant fodder.

Materials and Methods

The laboratory experiment was conducted at the Faculty of Animal Science at University of Jambi during 2019-2020. Leaves of FTS were collected from the the teaching and research farm of Faculty of Animal Science, University of Jambi. The sample was sun-dried, then dried in force oven at temperature of 60°C for 24 h, ground with milling machine to pass through a 1 mm sieve and used for chemical analysis and *in vitro* gas production test.

In vitro gas production procedures

A total of 200 mg of FTS flour was put into 100 ml a serum bottle and to be kept into the incubator at a temperature of 39°C.

Rumen liquor was taken from the rumen-cannulated Bali cow before morning fed grass and concentrate mix approximately 3 kg DM basis. Rumen liquor was collected and inserted into pre-warmed thermos, homogenised in laboratory, strained using four layers nylon cloth. Added with buffer McDougalls solution with a ratio of 1:4, inserted into 2500 ml serum bottle, flowed with CO_2 and be assembled with an 100 ml dispenser pipette.The sample of FTS in serum bottle was added with 40 ml mix solution of rumen liquor and buffer McDougalls then incubated into incubator at temperature of 39°C.

Gas production were measured at each time interval of 3, 6, 9, 12, 24, 48 and 72 hours by injecting *a hyphodermic glass syringe* in the rubber cover of the serum bottle. Gas production was observed through the movement of the piston into glass syringe and read through the scale on the glass syringe. Total gas production is calculated after correction by incubation of blank. The gas production rate is measured by the following exponential model of Orskov and McDonald 's (1979):

$$P = a + b\,(1 - e^{-ct})$$

P = Gas produced at t.

a = GP from soluble fraction.

b = GP from unsoluble fraction but potential to degraded.

c = Rate of GP from b.

All parameters were calculated using the NEWAY Excel program (Chen, 1996).

Energy metabolism (MJ/kg DM) and OM digestibility from FTS were measured by model the of Menke and Steingass (1988) as followed:

$$ME\left(\frac{MJ}{kgDM}\right) = 1.68 + 0.1418GP + 0.073CP + 0.21CF - 0.028A$$

$$OMD\,(\%) = 14.88 + 0.8893GP + 0.448CP + 0.651A$$

Where;

GP = Gas production for 24 h (ml/200 mg).

CP = Crude protein (%), CF is crude fat and A is ash (%).

The methane produced was measured using the procedure described by Fieves *et al*. (2005). A syringe filled with gas is inserted into the in-let of the washing gas system (Duran™). The gas flows through a 4 M NaOH solution and then an out-let connected to another syringe is used to determine the amount of methane and total GP after subtracting with blank.

Chemical analysis consisting of dry matter, ash, crude protein, crude fat, crude fiber and nitrogen free extract based on AOAC (1995) procedure. The content of NDF and ADF of FTS flour is carried out on the basis of the procedure of Van Soest *et al*. (1991).

Statistical analysis

Observation data were carried out a variety analysis (ANOVA) to see the species effects of the FTS. The Duncan test was used to identify the mean differences of FTS species using a confidence level of $P<0.05$. Statistic analysis using the SPSS Version 17 program.

Results and Discussion

Chemical composition of *FTS*, consisting of *P. falcataria*, *G. sepium*, *S. grandiflora*, *C. calothyrsus*, *M. oleifera* and L. *leucocephala* are presented in Table 1. The chemical composition is significantly affected by the species of FTS ($P<0.05$). The crude protein content varies from 14.07% to 26.81%, with the highest levels found in *S. grandiflora* and the lowest in *P. falcataria*. Crude fat content ranges from 3.61% to 11.20%, with the highest levels in *M. oleifera* and the lowest in *P. falcataria*. Crude fiber content varies from 11.51% to 14.64%, with the highest levels observed in *P. falcataria* and the lowest in *L. leucocephala*. The nitrogen-free extract ranges from 44.71% to 63.21%, with the highest levels in *P. falcataria* and the lowest in *S. grandiflora*. The chemical composition varies among different FTS species, particularly in their ability to extract and accumulate nutrients from the soil and to fix nitrogen from the atmosphere (Yusuf and Muritala, 2013).

Important components regulate forage quality is fiber (acid detergent fiber and neutral detergent fiber) and crude protein (Horrocks and Vallentine (1999). Tree foliage was generally higher in terms of lignin and fiber content then the other finctional groups. The ranged of value of protein (10-25%), fibre (as defined by NDF: (33-68%), minerals (4-22%) and lignin content (3-21%) (Lee, 2018). Crude protein contained in forage is one of the important substances which will affects the performance of livestock. The protein content must be >8% of the DM in the feed to be able to meet the basic living requirements of livestock (Norton, 1994). Livestock consumption will be less if the protein content <10% (Raanjhman, 2001). Table 1 showed that all FTS has suitable crude protein content as a feed supplement for low-quality forage in improving the productivity of ruminants.

The FTS contains several types of cell walls, such as NDF and ADF. This means it serves not only as a source of protein but also provides fiber for ruminants, where it is essential for stimulating rumen function, promoting chewing activity, encouraging rumination and increasing saliva production (Parish and Rhinehart, 2008). The concentration of ADF is typically higher in the stem material of forages compared to the leaves (Belesky, 2006). According to the NRC (1989), dairy feed should contain 25% NDF in dry matter, with 75% of that NDF coming from forage. Meanwhile Arelovich *et al*. (2008) and Zamora-Natera *et al*. (2020) when NDF content increases ranging from 22.5% to 45.8% both feed consumption and digestibility tend to decrease. Therefore, lower NDF contents are preferred, as they are associated with increased animal intake (Wang *et al*., 2021).

Parish and Rhinehart (2008) classify forage quality standards based on the content of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF). The classification is as follows: Excellent Quality (Prime) NDF < 40% and ADF < 31%,Quality Standard (QS) 1: NDF 40-46% and ADF 31-35%,QS 2: NDF 47-53% and ADF 36-40%,QS 3: NDF 54-60% and ADF 41-42%, QS 4: NDF 61-65% and ADF 43-45% and QS 5: NDF > 65% and ADF > 45%. According to these QS, L. *leucocephala* and *M. oleifera* fall under the excellent quality (prime) category. *G. sepium* and *S. grandiflora* are classified as QS 1, while *P. falcataria* and *C. calothyrsus* are placed in QS 2.

Gas production profile

The results of measuring the gas production profile of FTS fermentation at incubation time of 3, 6, 9, 12, 24, 48 and 72 hours are depicted in Fig 1. Fig 1 showed that cumulative gas production has increased as the incubation time increases. Cumulative gas production (GP) at 3-24 h incubation of *C. calothyrsus, P. falcataria*, *G. sepium* is relatively the same, but lower compared to *S. grandiflora, M. oleifera* and L. *leucocephala*. Cumulative GP at the incubation time 72 h was consecutively *S. grandiflora* (57.02 ml), *M. oleifera* (53.94 ml), *L. leucocephala* (48.29 ml), *C. calothyrsus* (37.21 ml), *G. sepium* (27.44 ml) and the lowest was *P. falcataria* (24.08 ml). This difference is closely related to the difference in chemical composition, especially the fractions of NDF and ADF among FTS (Table 1) as well as the content of OM that has the potential to be fermented. GP is the result of the fermentation process in the rumen. It can reflect the amount of OM which can be ingested by the rumen microbes and the amount of energy produced from the fermentation process (Menke and Steingass, 1988; Zhang *et al*., 2022). Low GP is also related to the presence of secondary metabolite found in the FTS, especially in C. *calothyrsus, G. sepium* and *P. falcataria*. The rate of GP is relatively slow in forages containing high

Table 1: Chemical composition of fodder tree species (FTS).

Species	Dry matter	Crude protein	Crude Fat	Crude Fiber	NNFE	NDF	ADF	Ash
P. falcataria	92.02[b]	14.07[f]	3.61[e]	14.64[c]	63.21[a]	51.22[e]	25.86[d]	4.47[d]
G. sepium	88.43[e]	19.34[e]	5.44[d]	12.56[b]	56.27[b]	45.06[c]	25.60[d]	6.38[c]
S. grandiflora	93.17[a]	26.81[a]	8.39[b]	11.59[a]	44.71[d]	45.40[d]	29.66[e]	8.50[b]
C. calothyrsus	88.17[e]	19.98[d]	7.38[c]	12.00[ab]	56.47[b]	51.21[e]	24.88[c]	4.17[d]
M. oleifera	91.19[c]	21.54[c]	11.20[a]	11.55[a]	45.17[d]	11.30[a]	6.86[a]	10.54[a]
L. leucocephala	90.45[d]	25.05[b]	5.47[d]	11.51[a]	51.89[c]	36.64[b]	11.42[b]	6.07[c]
SEM	0.172	0.187	0.132	0.281	0.521	0.30	0.157	0.297
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Description: [a,b,c,d,e] The number followed by different superscripts in the same column is different at (P<0.05), NNFE: Non nitrogen free extract (%), NDF: Neutral detergent fiber (%), ADF: Acid detergent fiber (%).

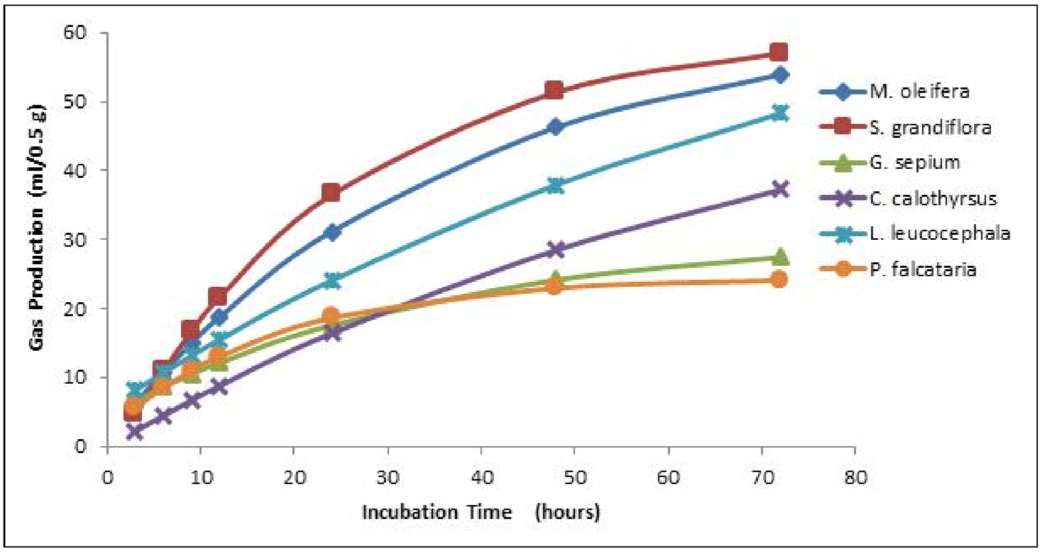

Fig 1: The gas production profile of FTS fermention in *in vitro*.

secondary metabolite, especially tannins. The presence of secondary metabolite such as tannins and phenolics has a negative correlation to the GP (Gemeda and Hassen, 2015; Afzalani *et al*. 2022), because tannins are toxic to rumen bacteria (Bhata *et al*., 2009).

Methane gas production profile

The profile of methane GP from FTS incubated at times of 3, 6, 9, 12, 24, 48 and 72 hours is shown in Fig 2. The results of FTS measurements on methane GP showed a greater cumulative production of methane gas with a large amount of incubation time (Fig 2). At an incubation time of 3-12 h, gas production is relatively the same among FTS. However, at an incubation time of 12-24 h, the production of methane gas from *M. oleifera, S. grandiflora* and *L. leucocephala* is higher than *L. leucocephala, G. sepium* and *C. calothyrsus.* Meanwhile, at the incubation time of >24 hours, *C. calothyrsus* produces higher methane gas than *P. falcataria* and *G. sepium*, but lower than *M. oleifera, S. grandiflora* and *L. leucocephala*. This difference is possible due to differences in chemical composition, differences in interistic properties and the presence of differences in the secondary metabolic content of the FTS. Enteric methane production is strongly influenced by feed quality (Beauchemin *et al*., 2008). Feedstuff with high content of cell wall fractions tend to cause increased in methane GP. However, in tannin-rich forage, it has bacteriocidal and bacteriostatic properties that affect the rumen microbes and inactivation of the enzymes produced, suppressing fermentation process and resulting in a decrease in methane production (Beauchemin *et al*., 2008).

Rumen fermentation products

The results of FTS measurements on the *in vitro* (IVDMD), *in vitro* OM digestibility (IVOMD), total GP, methane gas (CH_4) and ammonia NH_3 are presented in Table 2.

The results showed that the FTS had not significant effect (P>0.05) on IVDMD and IVOMD, but signifcantly (P<0.05) affect total gas, methane gas and ammonia. The total gas produced varies in a range of 25.58-53.80 ml, with the highest GP in *M. oleifera* and the lowest in *P. falcataria*. The difference in gas produced among FTS is indicated by the difference in chemical composition,

Table 2: Effect of FTS on rumen fermentation parameters.

Species	IVDMD	IVOMD	TG	CH_4	NH_3	ME
P. falcataria	69.35	74.06	25.58^{e}	9.26^{a}	3.48^{a}	4.24^{d}
G. sepium	63.13	71.27	29.36^{d}	9.72^{a}	5.85^{a}	4.20^{d}
S. grandiflora	64.37	70.13	56.80^{a}	16.06^{c}	11.23^{b}	7.16^{a}
C. calothyrsus	64.16	69.94	45.50^{c}	13.58^{b}	10.70^{b}	4.70^{c}
M. oleifera	66.22	68.13	53.80^{b}	17.08^{c}	12.58^{b}	5.98^{b}
L. leucocephala	66.39	71.39	48.28^{c}	15.99^{c}	12.23^{b}	4.99^{c}
SEM	1.79	1.526	1.016	0.281	0.887	0.099
P	0.215	0.179	<0.001	<0.001	<0.001	<0.001

Description: ^{a,b,c,d,e} The numbers followed by different superscripts in the same column are different P<0.05), IVDMD = *In vitro* dry matter digestibility (%), IVOMD = *In vitro* organic matter digestibility (%), TG = Total gas (ml), CH_4 = Methane (ml), NH_3 = Ammonia (mM), ME = Metaboliszable energy (MJ/kg DM).

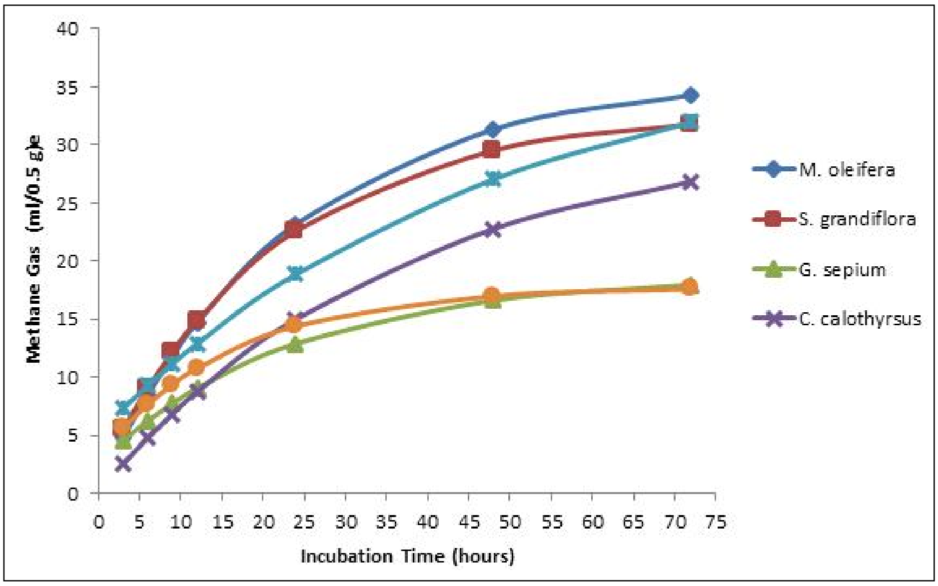

Fig 2: Methane gas production profile of FTS fermention in *in vitro.*

especially the content of NDF and ADF (Table 1). Uslu *et al.* (2018) reported that NDF and ADF content have a negative correlation to GP.

The gas produced in feed fermentation process in the rumen, is an indicator of the availability of carbohydrates for microbes. The high production of the resulted gas depending on the availability of the soluble carbohydrates (Blûmmel and Orskov, 1993). Apart from that, it also influenced by presence of of the metabolite secondary such as tannins (Gemeda and Hassen, 2015; Afzalani *et al*., 2022).

The methane GP measured in this study aims to find out the potential of the FTS as a low methane gas-producing forage and the potential as an anti-methanogenic. There was a significant effect of FTS (P<0.05) on methane GP at 72 h of incubation time. The highest methane production in *M. oleifera* (17.08 ml), *S. grandiflora* (16.06 ml), *L. leucocephala* (15.99 ml), *C. calothyrsus* (13.58 ml) and the lowest in *P. falcataria* (9.26 ml), *G. sepium* (9.72 ml) of the same of total amount of organic matter ingested. The FTS has different characteristics in producing rumen microbial fermentation products from digested organic matter. The difference in the ratio of acetic acid: propionate produced. The formation of acetic acid leads to H_2 and CO_2 gases produced, which will be converted by metanobacteria to methane gas.

The results on the production of rumen ammonia (Table 2) showed that FTS significantly (P<0.05) affects the ammonia concentration of rumen fluid. Ammonia is the result of proteins feed digested by rumen microbes for the purposes of microbial protein synthesis. The highest N-ammonia is produced by *M. oleifera,* L. *leucocephala, S. grandiflora, C. calothyrsus and* the lowest *P. falcataria* and *G. sepium*. The amount of ammonia produced ranges from 3.48-12.58 mM. The optimal N-NH_3 concentration for microbial activity in the rumen ranges from 3.57-7.14 mM, or equivalent to 5 mg% (Satter and Slyter, 1974). The excess ammonia produced will further encourage the amount of ammonia excreted through urine and have the potential to increase the production of N_2O release to the atmosphere. *P. falcataria* and *G. sepium* exhibit their protein properties that are resistant to digestion in the rumen and have the potential to be digested and absorbed in the intestines.

The FTS species has a significant effect (P<0.05) on the results of calculating the content of the ME value. The highest ME content in *S. grandiflora* (7.16 MJ/kg BK) and the lowest in *G. sepium* (4.20 MJ/kg BK). This difference occurs mainly due to differences in chemical composition such as fats, proteins and cell walls (NDF and ADF). Uslu *et al.* (2018) reported that proteins and fats had a positive correlation to ME values, in contrast NDF and ADF had a negative correlation to ME.

CONCLUSION

Fodder tree species have a varied chemical composition and potential nutritional value and provide not only protein but also a source of carbohydrates for ruminant. *P. falcataria and G. sepium* have the potential as low methane-producing feeds and also have protein characteristic which are resistant to rumen digested. Further research is needed on testing the use of various species of tree legumes (FTS) as feed supplements in ruminants.

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Disclaimers

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Informed consent

The use of animals in this study was under the approval and supervision of the animal care committee of the Faculty of Animal Science.

Conflict of interest

The researchers state that they have no conflicts of interest related to the publication of this study. They declare that the research, including its design, data collection, analysis, publication decision and manuscript preparation, was not influenced by any financial support or sponsorship.

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