Nutritive Value and Fermentation Characteristics of *Tithonia diversifolia* and *Moringa oleifera* Evaluated by Gas Production Technique *in vitro*

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ABSTRACT
Paitan (*Tithonia diversifolia*) and kelor (*Moringa oleifera*) leaves contain high crude protein, which can increase the protein content of the feed. This study aims to determine the role of pain leaf (*Tithonia diversifolia*) and kelor (*Moringa oleifera*) as a concentrate in vitro. Another feed ingredient used is rice bran. A completely randomized design with five treatments and five replications was applied in this study. The treatments were A (*Tithonia diversifolia* leaves without rice bran), B (*Tithonia diversifolia* leaves 75% + rice bran 25%), C (*Tithonia diversifolia* leaves 37.5% + *Moringa oleifera* leaves 37.5% + 25% rice bran), D (*Moringa oleifera* leaves 75% + rice bran 25%) and E (*Moringa oleifera* leaves without rice bran). Parameters observed were nutrient content, in vitro gas production and rumen fermentation products. The results showed that the crude protein content of treatments B, C and D was lower than A and E due to the use of rice bran which had a low crude protein content, but the addition of rice bran in the feed resulted in higher gas production ($P<0.05$). The combination of the use of rice bran and *Moringa oleifera* leaves (treatment D), resulted in the highest gas production up to 48 hours of incubation ($P<0.05$). The highest gas production from the fermentation of soluble (GPSF) and insoluble (GPNSF) fractions resulting from treatment D were 11.01 ml and 137.48 ml respectively. Anti-nutrients found in Tithonia diversifolia leaves make gas production lower than anti-nutrients found in *Moringa oleifera* leaves. 25% rice bran on feed could increase metabolizable energy (ME), organic matter digestibility (OMD), microbial protein (MP) and single-chain fatty acid (SCFA) production. It can be concluded that the use of 25% rice bran in feed containing *Tithonia diversifolia* and *Moringa oleifera* can reduce the anti-nutritional effect.

Keywords: *Tithonia diversifolia, Moringa oleifera, Rice bran, in-vitro.*

1. INTRODUCTION

The tropical climate reduces the quality of grasses and forages that grow in Indonesia, because they contain high crude fiber. This condition makes it more difficult to be digested by microbes in the rumen, due to its high crude fiber [1], so it is necessary to use alternative feeds with high nutrition to increase livestock productivity. *Tithonia diversifola* and *Moringa oleifera* are shrubs that are widely grown in Indonesia. The leaves of these plants contain high crude protein and are very useful for food, feed and medicine [2]. These plants are widely available and grow well in Indonesia, but their use as ruminant feed is still limited. The use of dry *Moringa oleifera* and *Tithonia diversifolia* leaves as ruminant feed can increase the crude protein content of ruminants concentrates.

Jamarun *et al* [3] reported that the nutritional content of the whole plant of *Tithonia diversifolia* (leaves + stem) is 25.5% dry matter, 84.01% organic matter, 22.98% crude protein, and 18.17% crude fiber. *Tithonia diversifolia* leaves could be used as a feed supplement for ruminants, especially during the dry season because it contains high crude protein [4]. *Tithonia diversifolia* leaves contain protein about 24.46% of the total dry matter and also contain various types of macro-mineral elements such as minerals Na, K, P, Ca, Mg and some very useful micro-mineral elements [5].

The use of *Moringa oleifera* leaves flour as a source of protein in fortifying feeds (concentrates) has been applied at the farmer level. The results of previous studies showed that *Moringa oleifera* leaves flour can be used as a feed supplement (as much as 20% of the total ration) in growing sheep [6]. *Moringa oleifera* leaves contain complete and balanced essential amino

acids, vitamins A, B, C and E and minerals Ca, Mg, P, K, Cu, Fe, and S, and contains crude protein of about 26.4% [7], 28.05% [5]. The availability of *Moringa oleifera* is limited due to the low amount of biomass, but the availability of *Tithonia diversifolia* is widely available in the countryside, as it does not suit human needs.

Rice bran is an agricultural by-product with enough availability in Indonesia. Rice bran is often used as a ruminant concentrate by small farmers and an agricultural by-product that is more easily absorbed. Rice bran is a waste of rice processing from rice milling factories with various qualities according to rice varieties. Rice bran is a by-product of rice processing from rice mills with various qualities according to rice varieties. Rice bran is a favorite animal feed because it has a high nutritional content, is cheap and easy to obtain and does not compete with human needs. Rice bran can be used as concentrate feed that contains energy and is favored by livestock. Rice bran produced around 5-6.5% of the weight of dry rice grains and it contains crude protein 11.9- 13.4%, crude fiber 10-16%, Total Digestible Nutrient (TDN) 70.5-81.5%, metabolic energy 2730 kcal/kg, and minerals Ca 0, 1% and P 1.51% [8]. Rice bran has the nutritional content that is crude protein 10.01-12.41%, crude fiber 14.62-19.53%, extract ether 6.10-8.57%, nitrogenless extract material (BETN) 50.93-54.95% [9].

Tithonia diversifolia and *Moringa oleifera* leaves are very suitable as protein sources in concentrate, because of their high protein content, but *Moringa oleifera* and *Tithonia diversifolia* leave also contain antinutrients that must be considered; tannins, flavonoids, alkaloids, phytat acids, saponins and polyphenols [5]. The main problem is the antinutrient content which has an effect on the utilization of feed by ruminants [7].

To determine the effect of anti-nutritional substances contained in *Tithonia diversifolia* and *Moringa oleifera* leaves, this study was conducted to examine the role of the amount of bran used in feed on the effect of anti-nutrients contained in *Tithonia diversifolia* and *Moringa oleifera* leaves *in vitro*.

2. MATERIALS AND METHODS

2.1 Preparation of samples

Rice bran was collected from a local agricultural market. *Moringa oleifera* dan *Tithonia diversifolia* were cut and hung until they wither, then chopped to 2-3 cm and dried in the hot sun for 1 day. *Tithonia diversifolia* leaves were obtained from roadside bushes around Cisarua (Puncak) Bogor Regency. *Moringa oleifera* leaves were harvested from the experimental garden of Agricultural division, Center for Isotope and Radiation Application (CIRA), National Nuclear Energy Agency of Indonesia (BATAN). All leaves were water-dried by hanging in an open room with a roof, cut 2-3 cm after wilting and dried in an oven at 55-60ºC for 3 to 4 days. Then, the leaves are finely ground with a blender.

2.2 Nutrient content analysis

Analyze dry matter (DM), ash, organic matter (OM), crude protein (CP), extract ether (EE) using proximate analysis procedure [10], but to analyze neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined by a detergent fiber analysis methods [11].

2.3 In vitro incubation and analysis

The practical method used for in vitro evaluation is based on the gas production technique described by Menke and Steingass [12]. Rumen fluid was obtained from fistulous buffalo fed twice a day (08.00-15.30) with 100% field grass. It was taken from the fistula hole manually, filled into pre-warmed thermos flasks and sealed properly. The slurry was filtered through 4 layers of nylon cloth and flushed with carbon dioxide (CO2). Rumen liquor was kept at approximately 39ºC before use. The glass syringe containing a total of 200 mg DM samples was added with 30 ml rumen liquor- buffer (2:1). The incubation was carried out at 39ºC for 48 h. Total gas production was performed at 0, 3, 6, 9, 12, 24 and 48 hours incubation times. Cumulative gas production data were fitted by NEWAY computer software described by Ørskov and McDonald [13].

$$y = A + B\,(1 - \exp^{(-ct)}) \quad (1)$$

Where, a, the gas production from the immediately soluble fraction (mL); b, the gas production from the insoluble fraction (mL); c, the gas production rate constant for the insoluble fraction (mL/h); A+B, potential gas production (mL); t, incubation time (h); y, gas produced at the time “t”.

Gas production caused by fermentation of soluble fraction (GPSF) and an insoluble fraction (GPNSF) was calculated by total gas produced at an incubation time of 3 and 24 hours, calculated as described by Van Gelder *et al*. [14]. Organic matter digestibility (OMD) and Metabolizable energy (ME) were calculated from gas production at 24-hour incubation, crude protein (CP), ether extract (EE) and ash values, according to Menke and Steingass [12]. Microbial protein (MP) was calculated as described by Czerkawski [15]. The concentration of short chain fatty acids was calculated as follows Menke and Steingass [12].

$$GPSF = (\text{gas at } 3\text{ h} \times 0.99 \times 5) - 3 \quad (2)$$

$$GPNSF = (1.02 \times ((\text{gas } 24\text{ h} \times 5) - (\text{gas } 3\text{ h} \times 5)))$$

2 (3)

OMD = 14.88+(0.889 x gas 24 h) + (0.45 x CP) + (0.0651 x ash) (4)

MP = OMD x 19.3 x 6.25 (5)

SCFA = (0.0222 x gas at 24 h) – 0.00425 (6)

ME = 2.2 + (0.136 x gas at 24 h) + (0.057 x CP) + (0.0029 x EE) (7)

NE = (2.2+(0.0272 x gas at 24 h) + (0.057 x CP) +(0.149 x EE)) /14.64 (8)

Where
1 Mcal = 4.184 MJ; 1 kg = 2,2050717 lbs

2.4 Design of experiment and statistic

A completely randomized design with five treatments and five replications was used in this study. The treatments were A (*Tithonia diversifolia* leaves without rice bran), B (*Tithonia diversifolia* leaves 75%+ rice bran 25%), C (*Tithonia diversifolia* leaves 37.5% + *Moringa oleifera* leaves 37.% + 25% rice bran), D (*Moringa oleifera* leaves 75% + rice bran 25%) and E (*Moringa oleifera* leaves without rice bran). The data obtained were processed manually with the excel program from Microsoft, if there was a significant difference at the 5% level in the analysis of variance (ANOVA), then the Least Significant Different (LSD) further test was carried out to see the effect between the treatments

3. RESULTS AND DISCUSSION

3.1 Nutrient content

The chemical composition of feed treatments is presented in Table 1. The lowest crude protein was treatment B (17.30%) and the highest was treatment E (23.21%). The lower crude protein content in treatments B, C and E was caused by the use of rice bran in the feed. The crude protein content of level 3 rice bran was not more than 8% [16]. Rice bran contains crude protein around 9.7% [17], 10.01 – 12.41% [9]. The higher crude protein concentration was due to the use of *Tithonia diversifolia* and *Moringa oleifera* leaf flour had a higher crude protein content. The crude protein content of *Tithonia diversifolia* was 22.4% [3], 24.46±0.06% [5], while the crude protein content of *Moringa oleifera* was 26.4% [7], 28.05±0.06% [5]. The protein content is preferred by ruminant breeders because the price of protein source feed is more expensive than energy source feed. The use of *Tithonia diversifolia* and *Moringa oleifera* leaf flour is one part of finding good quality alternative feeds, which are abundantly and continuously available and do not compete with human needs [18].

The results of the measurement of extract ether (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were lowest in treatment E, (4.52%, 39.03% and 17.38%, respectively) (Table 1). ADF and NDF are constituents of plant cell walls that low digestibility of feed [1]. Aderinola and Binuomote [19] reported that the NDF for *Moringa oleifera* was 46.97% lower than the NDF for treatment E, but the NDF for *Tithonia diversifolia* was 45.43% [20], almost the same as the NDF for treatment A (Table 1), which was 45.43. %. The higher NDF value could be caused by the use of young green stems when sampling *Tithonia diversifolia* and *Moringa oleifera* leaves. The use of fat in the diet of ruminants is recommended to be lower than 5%, and the use of fat is directed to be a source of energy that is not degraded in the rumen [21].

3.2 In vitro gas production

Cumulative gas production, gas characteristics and gas production kinetics of fermented rice straw are presented in Table 1.

Table 1. Nutrient Content of Feed Treatments

Treatments	DM	ASH	CP	EE	NDF	ADF
	%					
A	90.07	13.97	20.15	4.92	44.60	22.24
B	89.60	15.43	17.30	5.51	46.71	26.01
C	89.85	15.63	18.45	5.35	44.62	24.19
D	90.11	15.83	19.60	5.20	42.53	22.36
E	90.76	14.49	23.21	4.52	39.03	17.38

A: *Tithonia diversifolia* leaves only, B: *Tithonia diversifolia* leaves 75% + rice bran 25%, C: *Tithonia diversifolia* leaves 37.5% + *Moringa oleifera* leaves 37.5% + 25% rice bran, D: *Moringa oleifera* leaves 75% + rice bran 25% and E: *Moringa oleifera* leaves only. DM: dry matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber.

Table 2. *In vitro* Gas Production of Feed Treatments

Treatments	Total gas production (ml/200 mg DM)								Gas kinetics	
	3	6	9	12	24	48	GPSF	GPNSF	A+B	C
A	0,75^{a}	2,19^{a}	3,59^{a}	4,97^{a}	10,17^{a}	19,28^{a}	0,71^{a}	50,02^{a}	76,01^{c}	0,007^{a}
B	2,28^{b}	6,14^{b}	9,35^{b}	12,17^{b}	19,53^{b}	27,94^{b}	8,30^{b}	89,98^{b}	34,61^{a}	0,038^{b}
C	2,77^{c}	7,49^{c}	11,50^{c}	15,01^{d}	23,88^{c}	35,36^{d}	10,73^{c}	109,62^{c}	42,20^{a}	0,043^{c}
D	2,83^{c}	8,64^{d}	13,42^{d}	17,60^{e}	29,40^{d}	41,00^{e}	11,01^{c}	137,48^{d}	48,40^{b}	0,045^{c}
E	2,97^{c}	7,15^{c}	10,87^{c}	14,17^{c}	24,10^{c}	34,17^{c}	11,72^{c}	109,74^{c}	41,61^{a}	0,039^{b}
SEM	0,20	0,46	3,59	0,89	1,34	1,52	0,98	5,96	3,71	0,003

A (*Tithonia diversifolia* leaves without rice bran), B (*Tithonia diversifolia* leaves 75% + rice bran 25%), C (diversifolia leaves 37.5% + *Moringa oleifera* leaves 37.% + 25% rice bran), D (*Moringa oleifera* leaves 75% + rice bran 25%) and E (*Moringa oleifera* leaves without rice bran), GPSF: the gas production caused by fermentation of soluble fraction and GPNSF: the gas production caused by fermentation of insoluble fraction. ^{a,b,c,d,e}: Different superscripts in the same column indicate significant differences (P<0.05), SEM: standard error of the means, A+B: potential gas production (ml/200 mg DM). c: Gas production rate (ml/h).

Table 3. Organic Matter Digestibility, Microbial Protein (MP) and Energy of Feed Treatments

Treatments	OMD (%)	MP (g/kg OMD)	SCFA (mmol/L)	ME (MJ/kg DM)	NE (MJ/kg DM)
A	33,89^{a}	40,89^{a}	18,25^{a}	4,80^{a}	1,54^{a}
B	41,04^{b}	49,50^{b}	35,06^{b}	5,89^{b}	1,90^{b}
C	45,43^{c}	54,79^{c}	42,86^{c}	6,53^{c}	2,09^{c}
D	50,86^{e}	61,35^{e}	52,76^{d}	7,33^{e}	2,31^{e}
E	47,69^{d}	57,53^{d}	43,26^{c}	6,81^{d}	2,14^{d}
SEM	1,23	1,48	2,41	0,18	0,05

A (*Tithonia diversifolia* leaves without rice bran), B (*Tithonia diversifolia* leaves 75% + rice bran 25%), C (diversifolia leaves 37.5% + *Moringa oleifera* leaves 37.% + 25% rice bran), D (*Moringa oleifera* leaves 75% + rice bran 25%) and E (*Moringa oleifera* leaves without rice bran). OMD: organic matter digestibility (%), MP: microbial protein (g/kg OMD), SCFA: single-chain fatty acid (mmol/L), ME: metabolizable energy (MJ/kg DM), NE: net energy (MJ/kg DM). ^{a,b,c,d,e}: Different superscripts in the same column indicate significant differences (P<0.05), SEM: standard error of the means.

The total gas production up to 48 hours of incubation time showed a significant difference (P<0.05) and the highest gas production was obtained by treatment D (Table 1). Treatment D resulted in total gas production after 3, 6, 9, 12, 24 and 48 hours of incubation were 2.83, 8.64, 13.42, 17.60, 29.40 and 41.00 ml/200 mg DM respectively. The highest gas production due to soluble fraction fermentation (GPSF) (P<0.05) was produced by treatment E, but it was not significantly different from treatments C and D, it was in accordance with the value of the highest gas production rate at 3 hours of incubation produced by treatment E is 2.97 ml/200 mg DM, but not significantly different from treatments C and D. The highest potential gas production (A+B) was obtained from treatment A, which was 76.01 ml/200 mg DM significantly (P<0.05), this is reinforced by the lowest value of gas production rate, which is 0.007 mL/h (P<0.05). High potential gas production indicates that rumen microbial activity inhibits gas production in the rumen; this is indicated by a low gas production rate (c). The use of rice bran in *Tithonia diversifolia* leaf feed can increase the rate of gas production, GPSF and GPNSF, meaning that rice bran can suppress the activity of anti-nutritional substances contained in *Tithonia diversifolia* leaves. Treatment A produced the lowest gas production during incubation up to 48 hours (P<0.05), but the use of rice bran could significantly increase gas production (P<0.05) in treatment B (Table 2). The use of rice bran in feed containing *Tithonia diversifolia* and *Moringa oleifera* can significantly increase gas production (P<0.05). Rice bran is a by-product of carbohydrate-rich

feed in rice mills in producing rice, an excellent source of energy for animal feed [22]. The low gas production in treatment A was caused by the effect of anti-nutrients on the *Tithonia diversifolia* leaves which were heavier than the *Moringa oleifera* leaves. The gas production of *Tithonia diversifolia* leaves after 24 hours was 9.67 ml/200 mg DM [20], meanwhile the gas production of *Moringa oleifera* leaves after 24 hours of incubation was 36.4 ml/200 mg DM after conversion [6]. Utilization of *Moringa oleifera* leaves as much as 20% in the ration of growing sheep resulted in a 20% increase in body weight gain [5]. The use of *Moringa oleifera* to partially replace *Tithonia diversifolia* could significantly increase gas production, total VFA, and dry matter and organic degradation ($P < 0.05$) [23]. The effect of sesquiterpene lactones such as tagitinin in *Tithonia diversifolia* leaves which have broad pharmacological activities, among others, can be anti-inflammatory and anticancer so that it inhibits some microbial activities [24]. The leaves and roots of the *Tithonia diversifolia* plant are the most important antimicrobial agents against *E. coli* and *Candida albicans* [25].

3.3 Organic matter digestibility, energy and microbial protein synthesis

The use of rice bran as a mixture of *Tithonia diversifolia* and *Moringa oleifera* can increase the value of OMD, MP, SCFA, ME and NE ($P<0.05$), and the use of *Moringa oleifera* leaves can increase OMD, MP, SCFA, ME and NE ($P<0.05$) was also compared with feed containing *Tithonia diversifolia* leaves (Table 3). In Table 2 it is explained that the anti-nutritional effect of *Tithonia diversifolia* leaves on inhibiting the activity of some rumen microbes is higher than that of *Moringa oleifera* leaves. The less use of *Tithonia diversifolia* leaves, the higher the gas production, OMD, MP, SCFA, and NEL values (Table 3). The efficiency of microbial growth is influenced by the availability and balance of protein and carbohydrates fermented in the rumen [1]. The microbial activity could be described from the rumen microbial biomass production in vitro [1, 13]. The results of statistical analysis showed that the addition of rice bran and the use of *Moringa oleifera* significantly ($P > 0.05$) suppressed the effect of anti-nutritional substances contained in the leaves of *Tithonia diversifolia* and supported the production of microbial biomass in the rumen. Microbial biomass production is an illustration of the level of fermentation of feed ingredients in the rumen, the higher the fermentation activity, the higher the microbial production [15]. The main factors that affect the synthesis of microbial protein in the rumen are the availability of precursors for microbial cell formation such as glucose, amino acids, ammonia, peptides, and minerals in the rumen fluid, energy [13].

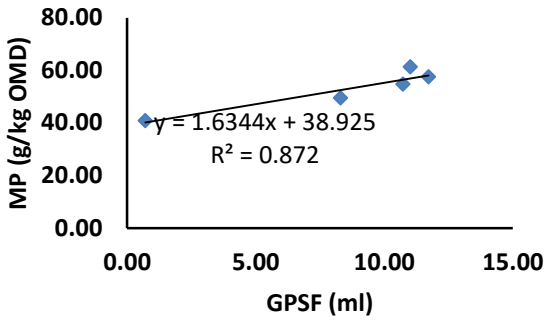

Figure 1. The relationship between "GPSF" and "MP"

Figure 1 shows that there is a positive correlation between GPSF and MP with a value of r2= 87.2%, each increase in GPSF will increase microbial protein synthesis (MP). Chemical content and feed digestibility and microbial biomass production can be used to determine the quality of the feed early [20]. Microbial biomass is one of the results of the fermentation process that can be used as a source of protein for livestock [13]. The efficiency of microbial growth is influenced by the availability and balance of protein and carbohydrates fermented in the rumen [1]. Microbial biomass production is an illustration of the level of fermentation of feed ingredients in the rumen, the higher the fermentation activity, the higher the microbial production [26].

4. CONCLUSION

Utilization of rice bran can increase gas production, OMD, MP, SCFA, ME and NE of treated feed. The use of *Moringa oleifera* leaves increased gas production, OMD, MP, SCFA, ME and NE higher than *Tithonia diversifolia* leaves. The use of *Tithonia diversifolia* as animal feed needs to be re-examined, to determine its anti-nutritional effect which is resistant to several types of microbes. Further research is needed on the optimal use of *Tithonia diversifolia* leaves as a source of high protein feed.

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