Said Mirza Pratama et al./Animal Production. 24 (3): 150-160, November 2022
Accredited by Kemenristek Dikti No 32a/E/KPT/2017. ISSN 1411-2027

Evaluation of Some Forage as Feed for Ruminant Animal: Chemical Composition, In Vitro Rumen Fermentation, and Methane Emissions

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Abstract. This experiment aimed to evaluate chemical composition, in vitro rumen fermentation, digestibility, and methane emissions of forages including bede grass, gamal (*Gliricidia sepium*), Indigofera, lamtoro (*Leucaena leucocephala*), elephant grass, mini elephant grass and Thai elephant grass. Forage samples were dried at 60°C for 24 hours, then ground to 1 mm sieve. The ground samples were used subsequently to determine the chemical composition and in vitro rumen fermentation test. Incubation was carried out in a water bath at 39°C for 48 h without replication. The results showed that all forages contained various crude protein at a range of 9-20% DM. The proportion of neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) in forage was generally low, but the highest was found in bede grass. The highest NH_3 concentration was found in Indigofera incubation, and lowest was in bede grass. All forage samples, except for mini elephant grass and Indigofera, had IVDMD and IVOMD below 70%. We identified low methane emissions from forage at 48 hours of incubation. It was concluded that legumes had a higher crude protein than in grass species and had low methane emissions. However, the relatively low digestibility of legume may limit its utilization.

Keywords: elephant grass, forage, indigofera, *Leucaena leucocephala*, rumen fermentation

Abstrak. Penelitian ini bertujuan untuk mengevaluasi komposisi kimia, fermentasi rumen secara in vitro, kecernaan dan emisi metana dari beberapa hijauan, yaitu rumput Bede, gamal, Indigofera, lamtoro, Rumput Gajah, Rumput Gajah Mini dan Rumput Gajah Thailand. Sampel hijauan dikeringkan pada suhu 60°C selama 24 jam, kemudian digiling hingga melewati ayakan 1 mm. Sampel tersebut selanjutnya digunakan untuk penentuan komposisi kimia dan uji fermentasi rumen secara in vitro. Inkubasi dilakukan dalam *waterbath* pada suhu 39°C selama 48 jam tanpa replikasi. Hasil penelitian menunjukkan bahwa semua hijauan yang diuji mengandung berbagai protein kasar yaitu 9-20% BK. Proporsi CP tidak larut deterjen netral (NDICP) dan CP tidak larut deterjen asam (ADICP) pada hijauan rendah, tertinggi di rumput bede. Konsentrasi NH_3 tertinggi terdapat pada Indigofera dibandingkan dengan yang lain dan terendah pada rumput bede. Semua sampel hijauan memiliki KCBK dan KCBO di bawah 70%, kecuali Rumput Gajah Mini dan Indigofera. Emisi metana dari hijauan pada 48 jam tergolong rendah. Disimpulkan bahwa protein kasar pada leguminosa lebih tinggi daripada jenis rumput dan memiliki emisi metana yang rendah. Namun, daya cernanya agak rendah, dapat membatasi pemanfaatannya.

Kata kunci: fermentasi rumen, hijauan, indigofera, lamtoro, rumput gajah

Introduction

Increasing livestock production, especially ruminants, will be successful if the supply of forage as feed is available in sufficient and sustainable quality and quantity. Forage for livestock is sourced either from natural pastures or planted greens. The type and quality of forage are influenced by the ecological and climatic conditions in particular area. Providing adequate amount of feed for livestock is a serious challenge in livestock development. An indication of a shortage of feed and nutrition is the low production level of livestock. In addition, land use for other purposes and extreme climate change may cause feed production to decline (Smith and Gregory, 2013). This problem is also followed by a condition where most ruminant livestock businesses have not followed by efforts to develop quality of forage (Agus and Widi, 2018). Forage is the source of feed for ruminants because it contains the nutrients needed by livestock and the mains source of energy (Afrizal et al., 2014). Livestock productivity must be balanced with the feed quality. According to

Nurlaha et al. (2015), forage takes up 40-80% of the total dry matter in the ruminant ration, or about 1.5-3.0% of the live weight of livestock, and the rest is concentrates and additional feed (feed supplement). Therefore, to achieve optimal productivity, the supply of forage must be increased in order that achieve the level of sufficient quantity, quality, and sustainability (Muhakka et al., 2013).

Aceh Province has many forage resources to supply feed ingredients for ruminants, such as bede grass, gamal (*Gliricidia sepium*), Indigofera, lamtoro (*Leucaena leucocephala*), elephant grass, mini elephant grass and Thai elephant grass. Information about the potential of local forage and local feed ingredients for animal feed has been published (Afrizal et al., 2014; Nurhayu and Saenab, 2019; Ramadhani and Suprayogi, 2020; Pratama et al., 2019; Samadi et al., 2020) with limited data for ration formulation; consequently, feed formulation in Indonesia still follow the reference from the National Research Council (NRC). Meanwhile, the nutritional content of feed ingredients varies greatly depending on soil conditions, climate and other environmental factors (Slegers et al., 2021) and is not suitable for generalized use to all animals because they need different nutritional values. Therefore, determining the nutritional value for local feed ingredients is crucial to support nutritionists in formulating feed using forages in Aceh Province.

Determining the nutritional quality of feed ingredient requires test of both nutritional content and digestibility. Both of this information are very important in the development of ruminant nutrition (Syamsi et al., 2020). The purpose of this study was to evaluate several forages, namely bede grass, gamal, Indigofera, lamtoro, elephant grass, mini elephant grass and Thai elephant grass related to their potential as ruminant feed ingredients based on their chemical composition, in vitro digestibility, and methane gas production.

Materials and Methods

Sample Collection and Preparation

The forages used in this study were bede grass, gamal, Indigofera, lamtoro, elephant grass, mini elephant grass and Thai elephant grass obtained from Banda Aceh and Aceh Besar. After being collected, the forage was first chopped to a size of ±5 cm and then dried in an oven at 60°C for 48 hours. The dry sample was ground using a hammer mill to pass through 1 mm sieve. The mashed sample was then analyzed to determine the chemical composition and in vitro digestibility test.

Chemical composition determination

Chemical composition of samples were determined by proximate analysis consisting of determination of dry matter (DM), ash, organic matter (OM), crude protein (CP), ether extract (EE), and crude fiber (CF) based on AOAC procedures (AOAC, 2005). For a short information, DM content was determined by drying samples in an oven set at 105°C for 24 h. The content of ash was determined by burning samples in a furnace at 500°C for 3 h. OM was determined by calculating the difference between DM and ash. CP was determined based on Kjeldahl method with the following consecutive procedures; destruction, distillation and titration. Determination of CF was conducted by sequential boiling of samples in acid and alkali solutions each for 30 min. EE was determined by applying soxhlet extractor and petroleum ether to extract the fat content of samples.

In Vitro Rumen Fermentation

Samples were incubated in vitro with buffered rumen fluid mixture by following the procedure from Theodorou et al. (1994). Rumen fluid was collected before morning feeding from a rumen fistulated Friesian Holstein cow in Indonesian Research Center for Animal Production, Bogor. Before use, rumen fluid was filtered through four layers of gauze. An amount of 0.75 g sample was inserted into a 125 ml serum bottle and added with 75 ml buffered rumen fluid (ratio rumen fluid:buffer was 1:4 v/v). The serum bottles were sealed with butyl rubber stoppers and aluminum crimp seals to start the incubation which was performed in a water bath maintained at 39ºC for 48 h. Gas production was vented and recorded at 2, 4, 6, 8, 12, 24, 36 and 48 h after incubation using a syiringe. Manual shaking was conducted at each time of gas production recording. Methane measurement was also performed at each time of gas production recording by following the method described in Fievez et al. (2005). After 48 h incubation, serum bottles were centrifuged to separate supernatant and residue. Supernatant obtained after 48h incubation was analyzed for pH by using a pH meter, and total volatile fatty acid (VFA) and ammonia concentrations were determined as described in Jayanegara *et al.* (2016a). Residue was further incubated with 75 ml pepsin-HCl 0.2 N solution for another 48 h (Tilley and Terry, 2006). In vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) were obtained by subtracting DM and OM residues from their initial DM and OM amounts prior to incubation, respectively. In vitro incubation was carried out once and represented by two bottles of serum.

Data analysis

All data used in this study were derived from one sample data, presented in descriptive analysis, and expressed in percentage and average values. Data on gas production kinetics were fitted against a modified equation of Orskov and Mcdonald (1979) as follow:

GP = b (1 – e–ct)

where GP is gas production (ml/g DM), b is gas production potential (ml/g DM), c is gas production rate (/h) and t is incubation period (h).

Results and Discussion

Chemical Composition and Fiber Fraction

Based on the results of the proximate analysison several types of forage (bede grass, gamal, Indigofera, lamtoro, elephant grass, mini elephant grass and Thai elephant grass) in Table 1, all forages contained 90.33% to 91.51% dry matter (DM) high. The highest organic matter content (OM) was 84.65% found in lamtoro (LT), while the lowest was 77.78% in mini elephant grass (MEG). The highest ash content in this study was 12.55% in mini elephant grass (MEG), which was lower than 14.45% as reported by Dumadi *et al.* (2021). The lowest ash content was 6.86% found in lamtoro (LT).

Table 1. Chemical composition (% dry matter) of bede grass (BG), gamal (GM), Indigofera (IF), lamtoro (LT), elephant grass (EG), mini elephant grass (MEG) and Thai elephant grass (TEG)

Component	EG	MEG	TEG	BG	GM	IF	LT
DM (%)	91.08	90.33	90.31	91.83	90.81	90.63	91.51
OM (%)	79.65	77.78	77.95	83.32	83.3	80.84	84.65
Ash (%)	11.43	12.55	12.36	8.51	7.51	9.79	6.86
CP (%)	10.65	12.45	10.51	5.90	14.42	9.28	20.04
CL (%)	2.18	2.06	1.96	1.31	3.34	2.70	3.84
CF (%)	27.03	27.09	26.45	26.40	12.52	18.77	11.97
NFE (%)	48.71	45.85	48.72	57.88	62.21	59.46	57.29
NDF (%)	82.03	82.27	50.3	76.56	49.32	35.24	47.24
ADF (%)	45.86	42.7	43.98	39.1	33.32	30.03	31.9
NDICP (%CP)	0.24	1.89	0.14	2.38	0.26	0.22	0.33
ADICP(%CP)	1.21	1.56	1.24	2.09	1.04	0.86	1.24

ADF = acid detergent fiber; ADIC = acid detergent insoluble crude protein; NFE = nitrogen free extract; OM = organic matter; DM = dry matter; CL = crude lipid; CP = crude protein; CF = crude fiber; NDF = neutral detergent fiber; NDICP = neutral detergent insoluble crude protein.

The highest crude protein (CP) content was in lamtoro, namely 20.04%. These data were lower than those reported by Kasiga and Lochmann (2014), Argadyasto et al. (2015), Eniolorunda (2010), namely 25.2%, 25%, and 21.8%, respectively. Lamtoro (LT) is a tropical forage that is superior source of protein with 23.20% CP and suitable for use as ruminant feed (Ahmed et al., 2018). The lowest crude protein content was 5.90% found in bede grass (BG) this is in line with its high fiber content. In general, legumes contain high crude protein (CP), but in this study, the highest crude fiber (CF) content (27.09) was found in mini elephant grass (MEG), which was lower than that reported by Andis *et al.* (2020), namely 31.12%. Agustono *et al.* (2018) stated that the difference in the content of feed ingredient depends on the climate, soil conditions, and other environmental factors, so it is necessary to analyze the feed first before formulating the ration.

The highest crude lipid content (CL) was 3.84% found in lamtoro (LT). These data were higher than that reported by Eniolorunda (2010) and Putri *et al.* (2014), namely 3.1% and 2.55%, resepectively. The level of lipid in plants is influenced by species, age, and differences in the parts of plants used as the sample. In this study, the lowest lipid content was found in bede grass (BG). The highest content of Nitrogen Free Extract (NFE) was observed in gamal (GM). Gamal is a type of plant which farmers often combine with grass for animal feed. The lowest content of Nitrogen Free Extract (NFE) in this study was found in mini elephant grass (MEG)

Fiber Fraction and Protein Fraction in Fiber

NDF fraction is the cell wall fraction consisting of hemicellulose, cellulose, and lignin, while ADF only contains cellulose and lignin (Christiyanto and Utama, 2021). The highest NDF content was 82.27% mini elephant grass (MEG) and 82.03% in elephant grass (EG), while the highest ADF content was found in elephant grass (EG) and Thai elephant grass (TEG), namely 45.86% and 43.98 %, respectively.

NDF in this study is below the maximum standard value according to the National Research Council (2001), namely 35-36% of the dry weight. The maximum ADF value is 17-22%. High content of NDF and ADF is influenced by cellulose and hemicellulose which bind to lignin to form a matrix and are difficult to separate and decompose. However, hemicellulose has the ability of delignification by binding to polysaccharides for easier comparison with cellulose (Nisa *et al.,* 2018).

The lowest NDF and ADF values were found in Indigofera (IF) because the cell walls did not contain much lignin. Lignin is a basic chemical element that is often known as the biggest inhibitor in the digestion of fiber and feed as a whole. Yu *et al.* (2017) stated that lignin is a polymer that binds to phenylpropane, so it is difficult to decompose. Lignin works by attaching to hemicellulose and cellulose in cell walls. Low lignin content can increase the activity of fibrolytic bacteria in degrading fiber (Pizzol et al., 2017).

There has been limited analysis of NDICP and ADICP content related to forage feed ingredients in Indonesia. Compared to findings by Jayanegara et al. (2016b), the ADICP for indigofera leaves (IF) and lamtoro in this experiment were lower. ADICP is a protein fraction that is insoluble in acid detergents and usually represents lignin-associated proteins and heat-damaged proteins (Pelletier *et al.* 2010). Therefore, this fraction is considered indigestible in the digestive tract of ruminants. The higher proportion of ADICP than CP is indicative of low protein quality in feed ingredients. NDICP is a protein fraction that is insoluble in neutral detergents and is considered slow or non-degradable in the rumen. Since part of the NDICP is ADICP, the reduction of the latter to the former (NDICP–ADICP) can provide important information about the amount of protein that passes through the rumen. The NDICP–ADICP value is referred to as the B3 fraction in the Cornell Net Carbohydrate and Protein System (CNCPS; Higgs *et al.,* 2012). NDICP and ADICP appear to be valuable parameters for determining the protein quality of ruminant feed ingredients so we recommend this analysis as a routine procedure, in addition to proximate and Van Soest analysis, in ruminant nutrition-related laboratories in Indonesia.

In Vitro Rumen Fermentation

Based on the data presented in Table 2, regarding to the characteristics of rumen fermentation and in vitro digestibility of several types of forage, the VFA concentrations of all the tested feed ingredients ranged from 82.7 - 138.41 mmol/l, with the highest concentrations in indigofera (IF) and lowest in bede grass (BG). In the NH_3 measurement, values ranged from 7.7 to 14.37 mmol/l, with the highest number in indigofera (IF) and the lowest in bede grass (BG) (Table 2). The digestibility of Indigofera (IF), both IVDMD and IVOMD, was the highest of all forage species tested. Among all forage ingredients tested, the highest IVDMD and IVOMD were found in indigofera and the lowest in Thai elephant grass (TEG).

The rumen pH of the treated feed ingredients after 48 hours incubation ranged from 6.8 to 7.0. The pH value for each treatment was within in the normal range, which was in accordance with the ideal pH standard according to Mariani and Suryani, (2016), namely 6-7. The highest pH values were found in elephant grass (EG) and mini elephant grass (MEG) (Table 2). High rumen pH value was probably due to low digestibility which indicates less-than-optimal fermentation, and hence low organic acids. According to Kara *et al.*, (2018) organic acids affect the degradation of proteins from complex molecules into simple and dissolved molecules, thereby accelerating the decrease in pH.

Volatyle Fatty Acid (VFA) concentration is an indicator to control and optimize the anaerobic digestion process (Jin *et al.,* 2016). The production of Volatyle Fatty Acid (VFA) is strongly influenced by pH (Table 1). Based on Table 2, the value of VFA concentration in the rumen fluid of the research feed ingredients has met the needs of rumen microbial growth as proposed by Dhia *et al.* (2019) that the total VFA range for rumen microbial growth was between 70-150 mM. Furthermore, Samadi *et al.* (2020) reported that the optimal total Volatyle Fatty Acid (VFA) that supports rumen microbial growth is 60-120 mmol/l. Feed that enters the rumen is fermented to produce the main products in form of Volatyle Fatty Acid (VFA), microbial cells, methane gas, and CO2. VFA is used as a carbon skeleton for rumen microbial growth and as a source of energy for livestock. The results of the Volatyle Fatty Acid (VFA) measurement in this study were higher than that by Hambakodu *et al.* (2021) which used natural grass for grazing.

Table 2. Characteristics of Rumen Fermentation and In vitro Digestibility of some Forages during an incubation period of 48 hours

Feedstuff	pH	VFA (mmol/l)	NH_3 (mmol/l)	IVDMD (%)	IVOMD (%)
EG	7	101,03	9,61	66,21	63,98
MEG	6,9	108,46	9	72,17	70,38
TEG	6,9	94,24	11,33	63,48	61,82
BG	7	82,7	7,7	66,58	63,54
GM	6,9	94,92	14,37	71,02	69,33
IF	6,8	138,41	18,97	81,95	81,56
LT	6,9	115,81	11,31	66,16	64,36

BG, bede grass; GM, gamal; IF, Indigofera; IVDMD, in vitro dry matter digestibility; IVOMD, in vitro organic matter digestibility; lamtoro (LT), NH_3, amonia; EG, elephant grass; MEG, mini elephant grass; TEG, thai elephant grass; dan VFA, volatile fatty acid.

Ruminants are capable of utilizing non-protein nitrogen compounds to meet their protein needs. While rumen bacteria require ammonia as the main nitrogen source, protozoa need N-amino as the main nitrogen source. The value of NH_3 in this study was similar to that expressed by Dhia *et al.* (2019), namely 6-21 mM. High concentration of NH_3 in indigofera (IF) (Table 2) is directly proportional to the CP content (Table 1). Dhia *et al.* (2019) explained that high NH_3 concentrations indicate a faster process of feed protein degradation than microbial protein formation process, and therefore the ammonia yield accumulates in the rumen. The NH_3 concentration in lamtoro (LT) (Table 2) was not the highest despite having the highest CP content (Table 1), which may be due to under-optimum protein digestibility. In general, high NH_3 content was found in legumes. The results of the Volatyle Fatty Acid (VFA) measurement in this study were higher than those of rice straw silage made with the addition of rumen fluid reported by Saputra *et al.* (2019).

IVDMD value can be used to determine the feed quality and livestock's capacity to use a particular feed. The presence of antinutrients that prevent rumen bacteria from destroying the feed is indicated by a low IVDMD and high CF content in the feed. Amount of lignin that binds cellulose and hemicellulose often increases after a high CF concentration, lowering the digestibility value. Table 2 shows that the TEG IVDMD is the lowest, which is consistent with the high CF content (Table 1). The results of the IVDMD measurement in this study were higher than those reported by Harahap *et al.* (2020). High IVDMD values in ruminants indicate high digested nutrients, especially those digested by rumen microbes (Costa and Costa, 2017).

Since organic matter is a component of dry matter, factors affecting the level of IVDMD will also affect the high and low of IVOMD in a feed. The TEG (Table 2) shows that IVOMD is the lowest compared to other feed ingredients because of high CF (Table 1). The ash content of TEG (Table 1) is also high. Fathul and Wajizah (2010) stated that ash content can slow down or inhibit the digestion of DM feed ingredients, meanwhile, OM will increase with DM. The IVOMD data of this study was higher than Pamungkas *et al.* (2014).

Gas Production Total and Kinetics

The in vitro gas production technique is a method to evaluate the quality of ruminant feed ingredients (Firsoni and Lisanti, 2017). Nutrient fermentation in rumen will produce gas which is mainly sourced from organic matter degraded by rumen microbes (Zakariah *et al.,* 2016). Table 3 shows that the longer the incubation process of feed ingredients, the more gas produced (Figure 1). It is indicative of the increasing ability of rumen microbes to digest feed ingredients.

Table 3. In vitro gas production of some forage

Feedstuff	Gas 12 (ml/g DM)	Gas 24 (ml/g DM)	Gas 48 (ml/g DM)	b (ml/g DM)	c (/h)
RG	46	57	70.5	67.48	0.100
RGM	93	125	138	136.57	0.103
RGT	102	136	162	156.78	0.098
BD	70.5	140.5	200.5	296.96	0.024
GM	62.5	73.5	87	81.99	0.127
IF	121.25	136.25	148.25	141.23	0.177
LT	97.5	123	138	137.26	0.102

Gas12, gas production after 24 hours of incubation; Gas24, gas production after 24 hours of incubation; Gas48, gas production after 48 hours of incubation; b, potential gas production; c, the value of gas production.

The highest gas production is found in bede grass (Table 3) which showed a high content of CF in this study (Table 1). Another contributing factor could be low ADF content compared to other feed ingredients (Table 1) because the more amount of substrates that are degradable and digestible by rumen microbes, the more gas will be produced. Khoiriyah *et al.* (2016) stated that high gas production is positively correlated with the level of digestibility of feed ingredients.

The lowest gas production is found in EG (Table 3) because of lower OM content than the other forage ingredients. According to Mukmin *et al.* (2014) low gas production indicates small amount of organic matter degraded by rumen microbes. The content of anti-nutritional substances, such as lignin, is also thought to inhibit substrate degradation by microbes, causing low gas production. Samadi *et al.*, (2020) stated that the higher the microbial population in the rumen, the higher the level of degradation of organic matter in feed ingredients, and therefore, higher production of gas.

Mukmin *et al.* (2014) stated that the value of b was gas production from the insoluble but potentially fermentable portion of the feed (ml). The high value of parameter b indicates high feed particles that are not dissolved but have fermentation potential in the rumen to produce gas. The value of c is the constant rate of gas production during the incubation period (ml/h). A high c value indicates that feed is rapidly degraded in a certain time unit. The value of c is the constant rate of gas production during the incubation period (ml/h).

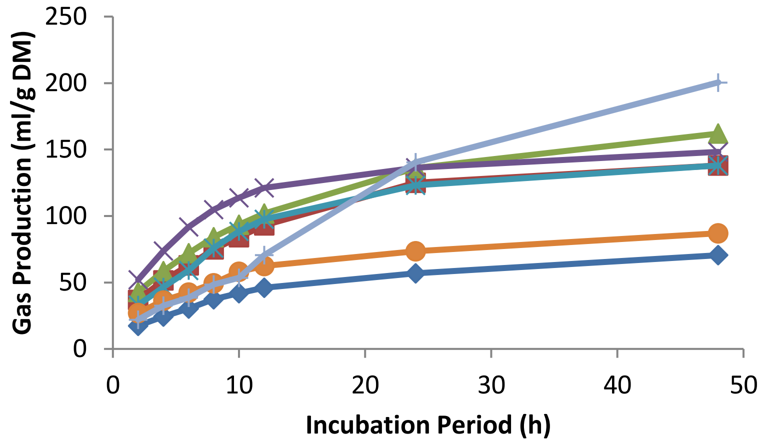

Figure 1. Gas production from elephant grass (-◊-), mini elephant grass (-▪-), thai elephant grass (-Δ-), indigofera (-x-), lamtoro (-Ж-), gamal (- -), and Bede grass (-I-).

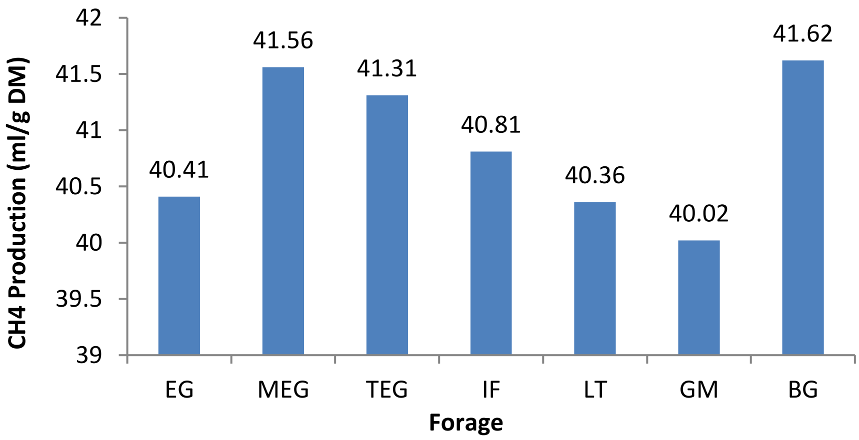

Figure 2. Emissions of methane gas from some forages for ruminants after rumen fermentation

In Table 3, the highest b value was 296.96 ml/g DM in bede grass and the lowest was in elephant grass 67.48 ml/g DM. Meanwhile, the highest c value was found in indigofera 0.177 ml/h and the lowest was in Bede grass 0.024 ml/h. The high b value of Bede grass was due to the high content of CF (table1) so that its digestibility was high. In elephant grass, low OM levels (Table 1) compared to legumes indirectly affect the b value, the less OM content contained, the less can be utilized by rumen microbes. Ramdani *et al.* (2017) stated that the value of c is the value of the gas production rate which describes the dynamics of the increase in gas production that occurs at an incubation time of 0-48 hours. The resulting gas production indicates the level of microbial activity in the rumen fluid to degrade feed.

Methane Emissions

Methane gas production expressed as a percentage of the total gas production incubation for 48 hours is shown in Figure 2. The highest methane production was found in bede grass 41, 62 ml/g DM. Fat content has an influence on methane gas emissions (Table 1). Fats reduce methane emissions through several mechanisms. The number of protozoa decreases along with the increasing number of double bonds contained in long chain unsaturated fatty acids (Alfiansyah and Hartutik, 2021). The percentage of methane gas production varies depending on different factors, including the type of livestock, the content of organic matter and fiber components in the feed, degradability of these fiber components by rumen microbes, and rumen environmental conditions (Alfiansyah and Hartutik, 2021).The relatively similar average value of methane gas from the research feed ingredients is relatively the same because the method of measuring methane gas was different from that of gas production. The delay in measuring methane gas due to technical problems may have caused the release of methane gas before it was measured, thereby reducing the actual value of methane gas from the research feed ingredients.

Conclusion

Legume have better nutritional contents than grass. There was no significant difference in digestibility between grass and legumes, except for Indigofera, which had a higher digestibility. Special treatment is needed to degrade the fiber fraction from forage so that the feed ingredients can be digested properly. Methane gas emissions from all forages are low in vitro. Further research is needed for in vivo testing.

Acknowledgement

This study was supported by Syiah Kuala University Grant under Profesorship Scheme in 2018. Authors express their gratitude to the group research team, Ilham and Hanafiah for their contribution in the field and laboratory analysis. Sincere gratitude goes to Ms. Dian Anggraeni, Ms. Endang Rosmalia, Ms. Eneh Maisaroh and Ms. Ratih Windyaningrum at IPB for their assistance in chemical analysis.

References

Afrizal, A, R Sutrisna, and M Muhtarudin. 2014. Potensi Hijauan sebagai Pakan Ruminansia di Kecamatan Bumi Agung Kabupaten Lampung Timur. Jurnal Ilmiah Peternakan Terpadu. 2(2): 233366.

Agus, A and TSM Widi. 2018. Current situation and future prospects for beef cattle production in Indonesia - A review. Asian-Australasian Journal of Animal Sciences. 31(7): 976–983. https://doi.org/10.5713/ajas.18.0233

Agustono, B, M Lamid, A Ma'ruf, and MTE Purnama. 2018. Identifikasi Limbah Pertanian dan Perkebunan Sebagai Bahan Pakan Inkonvensional Di Banyuwangi. Jurnal Medik Veteriner. 1(1): 12. https://doi.org/10.20473/jmv.vol1.iss1.2017.12-22

Ahmed, MA, S Jusoh, AR Alimon, M Ebrahimi, and AA Samsudin. 2018. Nutritive and anti-nutritive evaluation of Kleinhovia hospita, Leucaena leucocephala and Gliricidia sepium with respect to their effects on in vitro rumen fermentation and gas production. Tropical Animal Science Journal. 41(2): 128–136. https://doi.org/10.5398/tasj.2018.41.2.128

Alfiansyah, AH, and H Hartutik. 2021. Tren Produksi Gas, Produksi Gas Total dan Degradasi Secara In Vitro Dengan Penambahan Aditif Dengan Level Berbeda Pada Silase Tebon Jagung (*Zea Mays L.*). Jurnal Nutrisi Ternak Tropis. *4*(2): 77–87. https://doi.org/10.21776/ub.jnt.2021.004.02.2

Andis, MF, N Sandiah, and S Syamsuddin. 2020. Produksi Rumput Odot (*Pennisetum purpureum Cv . Mott*) sebagai Pakan Ternak pada Berbagai Dosis Pupuk Kandang Sapi. JIPHO (Jurnal Ilmiah Peternakan Hulu Oleo), 2(2), 156–159.

AOAC. 2005. Official Methods of Analysis. 15th Edition, Association of Official Analytical Chemist, Washington DC.

Argadyasto, D, Y Retnani, and D Diapari. 2015. Pengolahan daun lamtoro secara fisik dengan bentuk mash, pellet dan wafer terhadap performa domba. Buletin Ilmu Makanan Ternak, 102(1): 19–26. https://jurnal.ipb.ac.id/index.php/bulmater/article/view/12484%0Ahttps://jurnal.ipb.ac.id/index.php/bulmater/article/download/12484/9547

Costa, ND and CD Costa. 2017. The Level Of Legumes Supplementation In The Basal Diet Of Rice Straw On Intake And Digestibility Of Ongole Crossbred Cows. 7(10): 13–19.

Dal Pizzol, JG, HMN Ribeiro-Filho, A Quereuil, A Le Morvan, and V Niderkorn. 2017. Complementarities between grasses and forage legumes from temperate and subtropical areas on in vitro rumen fermentation characteristics. Animal Feed Science and Technology, 228(April), 178–185. https://doi.org/10.1016/j.anifeedsci.2017.04.020

Dhia, KS, KA Kamil, and H Tanuwira. 2019. Kecernaan Dan Fermentabilitas Substrat Kombinasi Mineral–Fungi Dalam Rumen. Jurnal Ilmiah Peternakan Terpadu, *7*(2): 217-222. https://doi.org/10.23960/jipt.v7i2.p217-222

Dumadi, EH, L Abdullah,and HA Sukria. 2021. Kualitas Hijauan Rumput Gajah (Pennisetum purpureum) Berbeda Tipe Pertumbuhan: Review kuantitatif. JINTP, 19(1): 6–13.

Eniolorunda, OO. 2010. Evaluation of biscuit waste meal and Leucaena leucocephala leaf hay as sources of protein and energy for fattening “yankassa” rams. African Journal of Food Science, *5*(2): 57–62. http://www.academicjournals.org/ajfs

Fathul, F and S Wajizah. 2010. Penambahan Mikromineral Mn dan Cu dalam Ransum terhadap Aktivitas Biofermentasi Rumen Domba Secara In Vitro. JITV, *215*(1): 9–15. https://doi.org/10.1136/bmj.2.2853.388

Fievez, V, OJ Babayemi, and D Demeyer. 2005. Estimation of direct and indirect gas production in syringes: A tool to estimate short chain fatty acid production that requires minimal laboratory facilities. Animal Feed Science and Technology, 123-124 Part 1(March 2018): 197–210. https://doi.org/10.1016/j.anifeedsci.2005.05.001

Firsoni and E Lisanti. 2017. Potensi Pakan Ruminansia dengan Penampilan Produksi Gas Secara In Vitro Bahan Penelitian Bahan pakan yang diujikan di dalam penelitian ini yaitu kulit kopi , kulit kacang tanah , daun turi kering yang diperoleh dari propinsi Nusa Tenggara Barat digiling h. *Jurnal Peternakan Indonesia*, *19*(3), 136–144.

Hambakodu, M, EP Ranja, and MA Sudarma. 2021. Nilai VFA dan NH3 Rumput Alam Padang Penggembalaan Kecamatan Haharu Kabupaten Sumba Timur. Jurnal Ilmu Peternakan Terapan. *5*(1): 8–12. https://doi.org/10.25047/jipt.v5i1.2588

Harahap, N, E Mirwandhono, and HD Hanafi. 2020. Uji Kecernaan Bahan Kering, Bahan Organik, Kadar Nh3 Dan Vfa Pada Pelepah Daun Sawit Terolah Pada Sapi Secara In Vitro. JURNAL PETERNAKAN. *1*(1): 13–21.

Higgs, RJ, LE Chase, and ME Van Amburgh. 2012. Development and evaluation of equations in the Cornell Net Carbohydrate and Protein System to predict nitrogen excretion in lactating dairy cows. Journal of Dairy Science. 95(4): 2004–2014. https://doi.org/10.3168/jds.2011-4810

Jayanegara, A, SP Dewi, N Laylli, EB Laconi, E. B., Nahrowi, and M Ridla. 2016. Determination of cell wall protein from selected feedstuffs and its relationship with ruminal protein digestibility in vitro. Media Peternakan, *39*(2), 134–140. https://doi.org/10.5398/medpet.2016.39.2.134

Jayanegara, A, SP Dewi, and M Ridla. 2016. Nutrient content, protein fractionation, and utilization of some beans as potential alternatives to soybean for ruminant feeding. Media Peternakan, 39 (3): 195–202. https://doi.org/10.5398/medpet.2016.39.3.195

Jin, X, I Angelidaki, and Y Zhang. 2016. Microbial Electrochemical Monitoring of Volatile Fatty Acids during Anaerobic Digestion. Environmental Science and Technology. 50 (8): 4422–4429. https://doi.org/10.1021/acs.est.5b05267

Kara, K, S Özkaya, S Erbaş, and E Baytok. 2018. Effect of dietary formic acid on the in vitro ruminal fermentation parameters of barley-based concentrated mix feed of beef cattle. Journal of Applied Animal Research. 46*(*1): 178–183. https://doi.org/10.1080/09712119.2017.1284073

Kasiga, T and R Lochmann. 2014. Nutrient Digestibility of Reduced-Soybean-Meal Diets Containing Moringa or Leucaena Leaf Meals for Nile tilapia, Oreochromis niloticus. Journal of the World Aquaculture Society. 45(2): 183–191.

https://doi.org/https://doi.org/10.1111/jwas.12102

Khoiriyah, M, S Chuzaemi, and H Sudarwati. 2016. Effect Of Flour And Papaya Leaf Extract (Carica Papaya L.) Addition To Feed On Gas Production, Digestibility And Energy Values In Vitro. J. Ternak Tropika. 17(2): 74–85.

Mariani, NP and NN Suryani. 2016. Kecernaan Dan Produk Fermentasi Rumen (in Vitro) Ransum Sapi Bali Induk Dengan Level Energi Berbeda the Digestibility and Product of Rumen Fermentation (in Vitro) Ration of Female Bali Cattle At Different Level of Energy. Majalah Ilmiah Peternakan. 19(3): 93–96.

Muhakka, A Napoleon, and H Isti'adah. 2013. Pengaruh Pemberian Asap Cair Terhadap Pertumbuhan Rumput Raja. Pastura. 3(1): 30–34.

Mukmin, A, H Soetanto, Kusmartono, and Mashudi. 2014. Produksi gas in vitro asam amino metionin terproteksi dengan serbuk mimosa sebagai sumber condensed tannin (CT). J. Ternak Tropika. 15(2): 36–43. http://www.elsevier.com/locate/scp

National Research Council. 2001. Nutrient requirement of dairy cattle. The National Academy Press, Washington DC.

Nisa, F, A Subrata, and E Pangestu. 2018. Kehilangan Bahan Kering, Acid Detergent Fiber dan N-Acid Detergent Fiber Daun Moringa oleifera Secara In Vitro. Jurnal Sain Peternakan Indonesia. 13(3): 282–286. https://doi.org/10.31186/jspi.id.13.3.282-286

Nurhayu, A and A Saenab. 2019. Pertumbuhan, Produksi dan Kandungan Nutrisi Hijauan Unggul pada Tingkat Naungan yang Berbeda. Jurnal Agripet. 19(1): 40–50. https://doi.org/10.17969/agripet.v19i1.13250

Nurlaha, L Abdullah, and D Diapari. 2015. Kecukupan Asupan Nutrien Asal Hijauan Pakan Kambing PE di Desa Totallang-Kolaka Utara (Forage based Nutrient Intake Sufficiency for Etawah Crossbred Goat in Totallang Village-North Kolaka). Jurnal Ilmu Pertanian Indonesia (JIPI). 20(1): 18–25. journal.ipb.ac.id/index.php/JIPI

Orskov, ER and I Mcdonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. The Journal of Agricultural Science. 92(2): 499–503. https://doi.org/10.1017/S0021859600063048

Pamungkas, Y, M Christiyanto, and A Subrata. 2014. Kecernaan Bahan Kering Dan Bahan Organik Secara In Vitro Ampas Aren Yang Difermentasi Dengan Penambaha Nitrogen,Phospor Dan Potassium. Animal Agriculture Journal. 3(2): 353–361.

Pelletier, S, GF Tremblay, A Bertrand, G Bélanger, Y Castonguay, and R Michaud. 2010. Drying procedures affect non-structural carbohydrates and other nutritive value attributes in forage samples. Animal Feed Science and Technology. 157(3–4): 139–150. https://doi.org/10.1016/j.anifeedsci.2010.02.010

Pratama, SM, S Wajizah, A Jayanegara, and S Samadi. 2019. Evaluation of Agro-Industrial by Products as Potential Local Feed for Ruminant Animals: Chemical Composition, Fiber Fractions and In Vitro Rumen Fermentation. Animal Production. 20(3): 155. https://doi.org/10.20884/1.jap.2018.20.3.715

Putri, A, Purnomoadi, and E Purbowati. 2014. Bobot badan, tinggi pinggul, lebar pinggul dan panjang pinggul kambing kacang betina di kabupaten karanganyar. Animal Agriculture Journal. 3(2): 221–229.

Ramadhani, E and A Suprayogi. 2020. Analisis potensi hijauan bahan pakan ternak ruminansia di Desa Sukawening Kecamatan Dramaga Kabupaten Bogor Jawa Barat. Jurnal Pusat Inovasi Masyarakat. 2(3): 451–454.

Ramdani, D, M Marjuki, and S Chuzaemi. 2017. Pengaruh perbedaan jenis pelarut dalam proses ekstraksi buah mengkudu (Morinda citrifolia L.) pada pakan terhadap viabilitas protozoa dan produksi gas in-vitro. Jurnal Ilmu-Ilmu Peternakan. 27(2): 54–62. https://doi.org/10.21776/ub.jiip.2017.027.02.07

Samadi, SM Pratama, S Wajizah, and A Jayanegara. 2020. Evaluation of agro-industrial by products as potential local feed for ruminant animals: Volatile fatty acid and NH3 concentration, gas production and methane emission. IOP Conference Series: Earth and Environmental Science, 425(1), 0–6. https://doi.org/10.1088/1755-1315/425/1/012010

Saputra, IKTA, AAAS Trisnadewi, and IGLO Cakra. 2019. Kecernaan In Vitro dan Produk Fermentasi dari Silase Jerami Padi yang Dibuat dengan Penambahan Cairan Rumen. Journal of Tropical Animal Science *7*(2): 647–660. https://simdos.unud.ac.id/uploads/file_penelitian_1_dir/80a62e1b18443e312ea393947017b283.pdf

Slegers, PM, RJK Helmes, M Draisma, R Broekema, M Vlottes, and SWK van den Burg. 2021. Environmental impact and nutritional value of food products using the seaweed Saccharina latissima. Journal of Cleaner Production. *319*(August), 128689. https://doi.org/10.1016/j.jclepro.2021.128689

Smith, P and PJ Gregory. 2013. Climate change and sustainable food production. Proceedings of the

Nutrition Society. *72*(1): 21–28. https://doi.org/10.1017/S0029665112002832

Syamsi, AN and HS Widodo. 2020. Synchronization protein-energy index of various forages for dairy livestock: an in vitro study. Animal Production. 22(2): 92-97.

Theodorou, MK, BA Williams, MS Dhanoa, AB McAllan, and J France. (1994). A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology*, *48*(3), 185–197. https://doi.org/https://doi.org/10.1016/0377-8401(94)90171-6

Tilley, J and RA Terry. 2006. A Two-Stage Technique for the in vitro Digestion of Forage Crops. *Grass and Forage Science*, *18*, 104–111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

Yu, J, N Paterson, J Blamey, and M Millan. 2017. Cellulose, xylan and lignin interactions during pyrolysis of lignocellulosic biomass. *Fuel*, *191*, 140–149. https://doi.org/https://doi.org/10.1016/j.fuel.2016.11.057

Zakariah, MA, R Utomo, Z Bachruddin. 2016. Pengaruh Inokulasi Lactobacillus plantarum dan Saccharomyces cerevisiae terhadap Fermentasi dan Kecernaan In Vitro Silase Kulit Buah Kakao. Buletin Peternakan. *40*(2): 124. https://doi.org/10.21059/buletinpeternak.v40i2.9294