Mangrove (Avicennia marina) leaves as an alternative feed resources for ruminants

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Abstract. The aim of this research was to get the best treatment for preserving of mangrove (Avicennia marina) leaves as an alternative feed resources for ruminants. This research used experimental method using a completely randomized design (CRD) with 2 treatments and 5 replications for each treatment. The treatments are: P1 (Mangrove leaves silage) and P2 (Mangrove leaves hay). The variables observed in the in-vitro experiment were in-vitro rumen fluid characteristics (pH, NH₃, VFA), total gas production and methane gas production. The results of the in-vitro research showed that the P2 treatment (mangrove hay) produced: pH 6,67, VFA 83 Mm, NH₃ 5,44 mg/100 ml, total production gas for 48 hours 99,7 ml/hour, and methane gas production for 48 hours 65,05 ml/gr DM. From this research can be concluded that the best treatment for preservation of mangrove leaves (Avicennia marina) was the hay treatment based on the total gas and methane gas production. It can be concluded that the hay mangrove leaves (Avicennia marina) can be used as an alternative resource feed for ruminant animals.

Keywords: Mangrove (Avicennia marina), Silage, Hay, Rumen fluid characteristics, Total gas and Methane gas production.

1. Introduction
Mangrove trees are a type of tropical mangrove plant from genus Avicennia. Mangrove are very potential as animal feed due to the best nutritional content includes vitamins, fats, calories, amino acids, protein, fiber, carbohydrates, and minerals (Fe, Mg, Ca, K, Na) are quite high amounts in leaves and fruit [1]. Nutrition content of mangroves based on proximate analysis were: 69,2% water, 14,91% ash, 11,04% protein, and 2,21% fat based on dry weight [2]. Mangroves (Avicennia marina) could be used for goat feed by people around the coast [3]. Because of these, mangrove leaves are potential as feed for ruminant.

The basic principle of hay was preservation it with drying method used sunlight (naturally) or using a dryer. The goals for make it sure the forage should containing 12-20% water content to prevent fungus growth. Another preservation technique was silage method. Silage is method for preserving fresh forage...
through anaerobic fermentation process with the final product wishes containing 60-70% water content to maximize the preservation of the nutritional content [4].

This research aimed to determining the effect of preserving of mangrove (*Avicennia marina*) leaves using silage and hay methods on rumen fluid characteristics, total gas and methane gas production.

2. Material and Methods

2.1. Materials
The materials used in this study were mangrove leaves (*Avicennia marina*), filter paper, oven, kiln, dessicator, whatman filter paper no.41, beaker, buchner funnel, vacuum pump, Tecator Scrubber, digestion tube, soxhlet extractor tube, and chemicals for nutrients analysis.

2.2. Research design
The research design in this research was an experimental method, for making silage and hay using a completely randomized design (CRD) with 2 treatments and 5 replications, which P1: Silage Mangrove leaves, and P2: Hay Mangrove leaves, the data analysis using T test.

Mangrove leaves are taken at coastal areas of Tiram Beach area of Padang Pariaman Regency, West Sumatra. Dry matter was measured by drying method Thermogravimetri.

2.2.1 Measurement of rumen fluid characteristics
- **pH measurement**
  pH measurements were made after each incubation period was stopped. pH is measured using a pH meter. Before using the tool, it has been standardized with a standard buffer solution of pH 7. The value on the pH meter scale indicates the degree of acidity or alkalinity of the rumen fluid. After that the sample is centrifuged at a speed of 1200 rpm for 20 minutes. The supernatant was taken to be analyzed for VFA and NH₃ levels.

- **NH₃ production measurement**
  Rumen fluid NH₃ levels were measured according to the Conway and O'Malley procedure using a Conway dish (Jamarun and Zain, 2013). On one side, 1 ml supernatant was added, while on the other side was added with a boric acid solution after which it was kept for 24 hours at room temperature. Ammonia bound to boric acid is irritated with 0.005 N H₂SO₄ until the color changes from blue to reddish.
  The concentration of NH₃ can be calculated by the formula:
  \[\text{NH₃} = \text{ml of titration} \times \text{N H₂SO₄} \times 17 \times 100 \text{ (mg/100ml)}\]

- **Volatile Fatty Acid (VFA) production measurement**
  VFA determination was carried out using steam distillation techniques (General Laboratory, 1996). 5 ml of the supernatant was piped and put into the distillation tube, then 1 ml of 15% H₂SO₄ was added and the distillation tube was immediately closed. The distillation tool is heated, then the distillation results are accommodated in an Erlenmeyer containing 5 ml of 0.5 N NaOH. Then 3 drops of pp indicator (Phenolptaline) were added and titrated with 0.5 N HCl until the purple color changed to a clear color.

2.3. Variables
Variables Observed on this research was rumen fluid characteristics (pH, NH₃, VFA) and total gas and methane gas production.

3. Discussion

3.1. Rumen fluid characteristics (pH, NH₃, VFA)
3.1.1. pH. The effect of preserving mangrove leaves (Avicennia marina) in the form of silage and hay on the value of the acidity (pH) of the rumen fluid can be seen in Table 1.

**Table 1.** Average value of pH measurement.

| Treatment | pH Rumen Fluid |
|-----------|----------------|
| P1        | 6.70±0.1028<sup>a</sup> |
| P2        | 6.67±0.0371<sup>a</sup> |

Note: The same superscript in the same column shows that the treatment indicated insignificant difference (P>0.05).

The results of the analysis using the T test showed that the treatments had no different effect (P>0.05) on the degree of acidity (pH) of the rumen fluid. This means that the rumen conditions of the goats in both treatments were in an ideal atmosphere for rumen microbes. The pH values of the liquid obtained in the study were 6.7 for P1 and 6.67 for P2. It can be seen from the results obtained that the pH value is still normal conditions for growth and development of rumen microbes in accordance [5] that the normal degree of acidity in the rumen is between 6.5 - 7.0.

In addition, the average pH value of the rumen fluid was different for each treatment. This difference can be due to the different content of VFA and NH3 in each treatment. The highest pH at P1 (mangrove leaf silage) was 6.70 compared to P2 6.67, presumably because of the higher VFA content. [6] The pH value of the rumen fluid is determined by the amount of bicarbonate (HCO3) and phosphate (HPO4)2 which comes from the salivary flow that enters the rumen. [7] Besides that, the balance between the different VFA and NH3 values in each treatment can also affect on the pH value of the rumen fluid because VFA is acidic and NH3 is alkaline.

The degree of acidity (pH) of the rumen obtained in each treatment is quite optimal for the growth and development of rumen microorganisms in digesting food substances, especially carbohydrates originating from cellulose and hemicellulose and for synthesizing microbial protein in the rumen. This is accordance with the opinion of [8] that the optimum rumen pH value for digestive activity ranges from 6.0 to 7.0.

3.1.2. NH3. NH3 is product of protein degradation by rumen microbes. NH3 as a source of nitrogen for body protein synthesis. The increase in NH3 can increase the growth and development of microbes, thereby increasing the digestibility of the feed in the rumen. The effect of preserving mangrove leaves (Avicennia marina) in the form of silage and hay on the NH3 concentration of rumen fluid can be seen in Table 2.

**Table 2.** In-vitro rumen NH3 values.

| Treatment | NH3 Rumen Liquid Concentration (mg/100ml) |
|-----------|-----------------------------------------|
| P1        | 6.97±1.0235<sup>a</sup> |
| P2        | 5.44±0.5541<sup>b</sup> |

Description: Different superscripts in the same column showed significantly different effects (P<0.05).

The results of the analysis using the T test showed that the treatments had a significantly different effect (P<0.05) on the NH3 concentration of rumen fluid. Table 2 shows the average concentration of NH3 in the rumen fluid in the range from 6.97-5.44 mg/100 ml. The concentration of this research is mostly still in the normal range. According to [9] that the NH3 concentration of 4.58 mM of rumen fluid is the minimum limit of NH3 which can support rumen growth.

The highest NH3 concentration was in the P1 treatment that is 6.97 mg/100 ml. This shows that different preservation methods on mangrove leaves have an effect on the NH3 concentration. This is because the fermentation process in making mangrove leaf silage is able to produce higher protein degradation compared to P2 (mangrove leaf hay). [10] fermentation is able to break down the bonds
between tannins and proteins so that the soluble protein will increase. The higher soluble protein made the higher of the NH$_3$ concentration produced. [11] High ammonia concentrations can be caused by the process of degradation of feed protein which is faster than the process of forming microbial protein. Thus, the ammonia concentration of the rumen fluid is influenced by the protein consumed and the protein degradation process in the rumen.

The low concentration of NH$_3$ in P2 (mangrove hay) is thought to be due to the low degradation of crude protein in the rumen. This is in accordance with the opinion of [12] and [13] that the higher degradation of crude protein in the rumen will increase the concentration of NH$_3$, and vice versa. [14] Besides that, the low NH$_3$ in the rumen is also influenced by the availability of N in feed. Low NH$_3$ will result in the slow growth of microorganisms because microbes need NH$_3$ for protein synthesis.

3.1.3. VFA. Volatile Fatty Acid (VFA) is a fermented product of carbohydrates and fats which can be used as an energy source and as a carbon framework for rumen microbes. The effect of preserving mangrove leaves (Avicennia marina) in the form of silage and hay on the value of Volatile Fatty Acid (VFA) can be seen in Table 3.

| Table 3. Average value of rumen fluid VFA production by in-vitro. |
|------------------|---------------|
| Treatment        | VFA (mM)      |
| P1               | 104±13.4164$^a$ |
| P2               | 83±8.3666$^b$  |

Note: Different superscripts in the same column showed significantly different effects (P <0.05).

The results of the analysis using the T test showed that the treatment had a significantly different effect (P <0.05) on VFA production. In table 3 it can be seen that the average VFA production in this study was 104 and 83 mM. Based on this, VFA production in this study can support microbial growth and activity. [15] The optimal VFA production is 80-160 mM to support microbial growth. High VFA production provides a sufficient source of energy for rumen microbes to reproduce so that more microbial cells are formed to produce enzymes and caused the higher the level of feed degradation in the rumen and the digestibility increases. [16] The high VFA production is sufficient energy for livestock. The less VFA production is produced, the less protein and carbohydrates that are easily dissolved and the lower the digestibility.

In table 3, it can be seen that the VFA production is different for each treatment. The VFA production in treatment P1 was higher than that in treatment P2. This difference is thought to have occurred due to the difference in the value of P1 protein degradation which was higher so that VFA production was also higher. This is in accordance with the opinion [17] that protein degradation in the rumen will result in amino acids which will then undergo deamination to produce ammonia, VFA and CO2. Ammonia is used to meet the needs for rumen microbial protein synthesis.

The lowest VFA production at P2 (mangrove leaf hay) can happened cause the lower degradation of organic matter. This is supported by [18] that the results of fermentation of organic matter include VFA, so that the more organic matter is fermented, the total VFA of rumen fluid produced will increase.

3.2. Total gas production and methane gas production

3.2.1. Total gas production. The effect of preserving mangrove leaves (Avicennia marina) in the form of silage and hay on the average value of total gas production can be seen in Table 4.
The results of the analysis using the T test showed that the method of preserving silage and hay in mangrove leaves had a significant effect (P <0.05) on the total gas production. From Table 4 it is known that the total average gas production for 48 hours at P1 (mangrove leaf silage) was 111.65 ml/hour and P2 (mangrove leaf hay) 48 hours was 107.25 ml/hour. Gas production is the result of the fermentation process that occurs in the rumen which can show microbial activity in the rumen and illustrates the amount of digestible organic matter. In addition, gas production from fermented feed can reflect the quality of the feed [19].

The highest gas production for rumen microbial growth was the 48 hours incubation of treatment P1 (111.75 ml/hour), presumably because the composition of the existing food substances was utilized properly by rumen microbes. This is in accordance with what was explained by [19] that the 48 hours incubation is the longest time for the adaptation phase between the composition of food substances and rumen microbes so that microbes can grow and develop by utilizing protein, carbohydrates, structural and starch. The higher the gas production, the better the quality of the feed ingredients, in the sense that it has high digestibility. In addition, gas production is also affected by the higher degradation of P1 organic matter. This is according to the opinion of [20] that the higher the gas production, can conclude the higher microbial activity in the rumen and can describe the digested organic matter so that it reflects the quality of the feed ingredients. The rate of gas production has a positive correlation with the digestibility of organic matter and consumption of digestible organic matter and vice versa. [21] That gas production is a description of organic matter that ferments in the rumen. [22] Feed components in the form of fiber and protein can affect gas production during the fermentation process.

Meanwhile, the lowest total gas production was found in P2 (mangrove leaf Hay), which is the 48 hours incubation hour (99.70 ml/hour), this is thought to have occurred because the content of anti-nutritional substances in the form of tannins in P2 treatment was higher than that in P1. Which affects gas production, where tannins can bind to organic compounds. This is in accordance with the opinion of [23] that the factor that causes low gas production is the role of anti-nutritional substances contained in feed ingredients. [24] and [25] One of the anti-nutritional substances is tannins which can bind various kinds of organic compounds (carbohydrates, proteins and fats) so that rumen microbes cannot digest them.

3.2.2. Methane gas production. The effect of preserving mangrove leaves (Avicennia marina) in the form of silage and hay on the average value of total gas production can be seen in Table 5.

Table 5. Average Total Methane Gas Production ml/gr DM.

| Treatment | 12 hours | 24 hours | 36 hours | 48 hours |
|-----------|---------|---------|---------|---------|
| P1        | 27.1±4.2375<sup>a</sup> | 51.3±6.2859<sup>a</sup> | 68.2±10.8909<sup>a</sup> | 74.95±11.3228<sup>a</sup> |
| P2        | 21.85±3.8103<sup>b</sup> | 42.95±6.6248<sup>b</sup> | 57.5±8.7981<sup>a</sup> | 65.05±10.3568<sup>a</sup> |

Note: Different superscripts in the same column show significantly different effects (P <0.05).

The results of the analysis using the T test showed that the method of preserving silage and hay in mangrove leaves had a significant effect (P <0.05) on methane gas production in the 12 and 24 hours. The highest methane gas production was in P1 (mangrove leaf silage) of 74.95 ml / gr BK, which was higher than P2 (mangrove leaf hay), might happened because the tannin content in P1 was lower than P2. It is known that a decrease in methane gas production can occur in the presence of tannins. This is
in accordance with the statement of [23] that total phenols and tannins affect the decrease in gas production.

The lowest methane gas production at P2 (mangrove leaf Hay) resulted in methane gas production of 65.05 ml/gr DM at the 48 hours incubation time, this is thought to be related to tannins because the higher the tannin content will result in lower methane gas production. The presence of tannins in mangrove leaf hay plays a direct role in inhibiting growth and methanogenic activity. This is in accordance with the statement of [26] that in the formation of energy sources such as VFA, propionate requires hydrogen gas (H2), while in the formation of acetate and butyrate it produces hydrogen gas (H2), meaning that the formation of acetate and butyrate triggers the formation of gas. hydrogen (H2) so that it will be used by methanogenic bacteria to be converted into methane gas (CH4), meanwhile high propionate production requires hydrogen gas (H2) so that methane gas formation decreases. [27] Decreased the population of protozoa in the rumen will be followed by a decrease in methane gas, because protozoa are hosts for methanogenic bacteria in the process of transferring hydrogen gas (H2), which then utilizes hydrogen gas (H2) produced by protozoa and converted to CH4 (methane).

Tannins can reduce methane emissions from the rumen fermentation system during in-vitro, has been described by [28, 29, 26] using different types of forages and treatments. The mechanism of methane production inhibition in ruminants was initiated by [30] indirectly through inhibition of fiber digestion which reduces the production of hydrogen gas (H2) and directly inhibits the growth and activity of methanogens. Tannins besides having the ability to bind protein, carbohydrates and fats. Tannins can also reduce methane gas production either by reducing the production of hydrogen gas (H2) or inhibiting the growth of methanogens and protozoa. With the presence of tannins in the ration, it can inhibit the digestion of fiber by rumen bacteria due to the lack of availability of protein (N) sources for the growth of these bacteria due to the presence of tannins with protein bonds. The decrease of fiber digestion, the production of hydrogen gas (H2) will decrease, as a result of which methanogenic bacteria will decrease due to the lack of supply of hydrogen gas (H2) for their growth. This is in line with [30] that condensed tannins reduce methane through a mechanism indirectly by inhibiting fiber digestion which reduces the production of hydrogen gas (H2), while tannins that are easily hydrolyzed play a more direct role in the mechanism of directly inhibiting growth, and methanogenic activity.

4. Conclusion
The results of the in-vitro research showed that the mangrove hay results: pH 6,67, VFA 83 Mm, NH3 5,44 mg/100 ml, total production gas for 48 hours 99,7 ml/hour, and methane gas production for 48 hours 65,05 ml/gr DM. From this research can be concluded that the best treatment for preservation of mangrove leaves (Avicennia marina) was the hay treatment based on the total gas production, and methane gas production. It can be concluded that mangrove leaves (Avicennia marina) can be used as an alternative resource feed for ruminant animals.

References
[1] Wibowo, C Kusmana, Suryani, C A Hartati, Y dan Oktadiyani P 2009 Pemanfaatan Pohon Mangrove Api-Api (Avicennia sp.) sebagai Bahan Pangan dan Obat IPB Bogor 160-165
[2] Handayani, S 2013 Kandungan Flavonoid Kulit Batang daun Pohon Api-api (Avicennia marina (Forks.) Vierh) sebagai Senyawa Aktif Antioksidan Skripsi Institut Pertanian Bogor Bogor
[3] Zandi, K., Taherzadeh, M., Yaghoubi, R., Tajbakhs, s., Rastian, Z, Fouladvand, M., and Sartavi, K. 2009. Antiviral Activity of Avicennia marina Against Herpes Simplex Virus Type I and Vaccine Strain of Poliovirus (an In Vitro Study). Journal Of Medicinal Plants Research. 3 (10): 771-775.
[4] Kartasudjana, R 2001 Modul Program Keahlian Budidaya Ternak, Mengawetkan Hijauan Pakan Ternak Jakarta Departemen Pendidikan Nasional, Proyek Pengembangan Sistem dan Standar Pengelolaan SMK Direktorat Pendidikan Menengah Kejuruan
[5] Indrayanto, D 2013 Degradasi Bahan Kering, Nilai pH dan Produksi Gas Sistem Rumen In Vitro
Terhadap Kulit Buah Kakao (Theobroma cacao L.) dengan Lama Fermentasi yang Berbeda

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[6] Ismail, R 2011 Kecernaan In vitro, http://rismanismai2.wordpress.com/2011/05/22/nilai-
kecernaan-part-4/#more310. (diakses pada tanggal 2 Januari 2021)

[7] Jamarrun, N dan Zain, M 2013 Dasar Nutrisi Ruminansia Diklat Edisi I CV Jaya Surya Padang

[8] Paengkoum, P, Liang, J B Jelan Z A dan Basery, M 2006 Utilization of steamtreaded oil palm
frond in Growing Saunan Goats: II Supplementation with Energy and Urea. Asian-Aust. J Aim
Sci 19 (11): 1623-1631

[9] Bunglavan, S J and Dutta N 2013 Use of tannins as organic protectan of protein in digestion of
ruminant J Livestock Sci 4 : 67-77

[10] Putri, L D N A Rianto E dan Arifin M 2013 Pengaruh Imbangan Protein dan Energi Pakan
Terhadap Produk Fermentasi di Dalam Rumen Pada Sapi Madura Jantan Animal Agriculture
Journal 2(3): 94-103

[11] Cahyani, R D Nuswatara, L K dan Subrata A 2012 Pengaruh Proteksi Protein Tepung Kedelai
Dengan Tannin Daun Bakau Terhadap Konsentrasi Amonia, Undregraded Protein dan Protein
Total secara In Vitro Animal Agriculture Journal I:(1) 2012, p 159-166

[12] Gumilar, D A K W Rianto, E dan Arifin, M 2017 The Concentration of Rumen Fluid Volatile
Fatty Acids And Ammonia, And Rumen Microbial Protein Production In Sheep Given Feed
During The Day And Night Time IOP Conf Series: Eart and Environmental Science 119 12045
doi: 10.1088/1755-1315/119/1/012045

[13] Rahmadi, D Sunarso, J Achmadi, E Pangestu, A Muktiani, M Christiyanto, Surono dan
Suarahmanto 2010 Ruminologi Dasar Universitas Diponegoro Press, Semarang

[14] Cammack, K M Austin, K J Lamberson, W R Conant, G and Cungnigham H C 2018 Ruminant
Nutrition Symposium: Tiny but mighty: The role of the ruminant microbes in livestock
production J Anim Sci 96 : 752-770

[15] Sakinah, D 2005 Kaflian Suplementasi Probiotik Bermineral Terhadap VFA, NH3, dan Kecernaan
Zat Makanan Pada Domba Skripsi Fakultas Peternakan Institut Pertanian Bogor: Bogor

[16] Kozloski, G V Ribeiro, H M N and Rocha, J B T 2000 Effect of the substitution of urea for soybean
meal on digestion in steer Can J Anim Sci 80 : 713-719

[17] Yulia, O 2007 Pengujian Kapasitas Antioksidan Ekstrak Polar, Nonpolar, Fraksi Protein Dan
Nonprotein Kacang Konak (Lablab purpureus (L.) sweet) Departemen Ilmu Dan Teknologi
Pangan Institut Pertanian. Bogor

[18] Ella, A Hardjosoewignya, S Wiradaryadyan, T R dan Winugroho, M 1997 Pengukuran Produksi
Gas dari Hasil Proses Fermentasi Beberapa Jenis Leguminosa Pakan Dalam : Prosiding Sem
Nas II-INMT Ciawi, Bogor

[19] Gusasi, A 2014 Nilai pH, Produksi Gas, Konsentrasi Amonia dan VFA Sistem Rumen In Vitro
Ransum Lengkap Bahan Jerami Padi, Daun Gamal dan Urea Mineral Molasses Liquid
Makasar: Skripsi Fakultas Peternakan Universitas Hasanuddin

[20] Pellikaan, W F Hendriksa, W H Uwimanaa, G Bongersa, L J G M Beckerc, P M dan Conea, J
W 2011 A novel method to determine simultaneously methane production during in vitro gas
production using fully automated equipment Animal Feed Science and Technology 168 196-
205

[21] Makkar, H P Francis, G and Becker, K 2007 Bioactivity of phytochemicals in some lesser known
plants and their effects and potential applications in livestock and aquaculture production
systems Animal 1 :1371-1391

[22] Jayanegara, A dan Sofyan, A 2008 Penentuan Aktivitas Biologis Tanin Beberapa Hijauan Secara
In-vitro Menggunakan “Hohenheim Gas Test” dengan Polietilen Glikol sebagai Determinan
Media Peternakan 32 (3) : 44-52

[23] Hariadi, B T and Santoso, B 2010 Evaluation Of Tropical Plants Containing Tannin on In Vitro
Methanogenesis and Fermentation Parameters Using Rumen Fluid. J Sci Food Agric 90 : 456-
461
[24] Firsoni dan Yunita, R 2014 *Uji Degradabilitas Pakan Komplit yang Mengandung Daun Chromolaena odorata secara in-vitro* Jurnal Peternakan Indonesia 16(2):89-93

[25] Martin, C Doreau, M dan Morgavi, D P 2008 *Methane Mitigation in Ruminants: From Rumen Microbes To The Animal* Inra, Ur 1213 Herbivores Research Unit, Research Centre of Clermont-Ferrand-Theix, F-63122 France (FR) St Genès Champanelle

[26] Susanti, S dan Marhaeniyanto, E 2014 *Kadar Saponin Daun Tanaman yang Berpotensi Menekan Gas Metana Secara In-Vitro* Jurnal Peternakan Indonesia 16(2):89-93

[27] Martin, C Doreau, M dan Morgavi, D P 2008 *Methane Mitigation in Ruminants: From Rumen Microbes To The Animal* Inra, Ur 1213 Herbivores Research Unit, Research Centre of Clermont-Ferrand-Theix, F-63122 France (FR) St Genès Champanelle

[28] Puchala, R Min, B R Goetsch, A L and Sahlu T 2005 *The effect of a condensed tannin-containing forage on methane emission in goats* J Anim Sci 83: 182-186

[29] Tavendale, M H Meagher, L P Pacheco, D Walker, N Attwood, G T and Sivakumar, S 2005 *Methane Production From In Vitro Rumen Incubation with Lotus pedunculatus and Medicago sativa, and Effects of Extractable Condensed Tannin Fractions on Methanogenesis* Anim Feed Sci Technol 123/124: 403-419

[30] Jayanegara, A Sofyan, A Makkar, H P S dan Becker, K 2009 *Kinetika Produksi Gas, Kecernaan Bahan Organik dan Produksi Gas Metana In Vitro pada Hay dan Jerami yang Disuplementasi Hijauan Mengandung Tanin* Media Peternakan 32: 120-129

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