International Journal on Advanced Science Engineering Information Technology

Vol.9 (2019) No. 3
ISSN: 2088-5334

Effects of *Leucaena leucocephala* Supplementation to Total Mixed Ration Based on Ammoniated Rice Straw Onfiber Digestibility and Rumen Fermentation Characteristics *in vitro*

Rusmana Wijaya Setia Ningrat[#], Mardiati Zain[#*], Erpomen[#], Ezi Masdia Putri[+], Malik Makmur[+]

[#]*Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Indonesia.*
E-mail: [*]mardiati@ansci.unand.ac.id

[+]*Postgraduate Student, Faculty of Animal Science Andalas University, Padang, 25163, Indonesia.*

***Abstract*— Legume is a feed ingredient that contains high protein for ruminants. The tannin content in legumes can provide a bypass protein for ruminants. This study was done to find out the effect of legume supplementation (*Leucaena leucocephala*) on fiber digestibility, characteristic rumen, protozoa population and methane production from total mixed ration (TMR) based on ammoniated rice straw. The study was conducted *in vitro* using a randomized block design with 3 treatments and 5 replications. The treatment was A. 40% ammoniated rice straw + 60% concentrate, B. 40% ammoniated rice straw + 50% concentrate + 10% *L. leucocephala*, C. 40% ammoniated rice straw + 40% concentrate +10, + 20% *L.leucocephala*.The results showed that the addition of *L. leucocephala* increased digestibility of NDF, ADF, and Cellulose, RUP (rumen undegradable protein) and reduced protozoa population and methane production (p <0.05). Increased doses of *L.leucocephala* up to 20% reduce fiber digestibility of feed substances compared to a dose of 10% but are still higher than controls. The results of this study concluded that *L.leucocephala* supplementation in TMR based on ammoniated rice straw *in vitro* improved digestibility, fermentability, and reduced methane gas production. Supplementation of 10% and 20% *L. leucocephala* needs further research, to see the effect on livestock *in vivo*.**

***Keywords*— digestibility; *Leucaena leucocephala*; completed feed; ammoniated rice straw.**

I. INTRODUCTION

Use of rice straw as animal feed in general has several disadvantages, because of the low crude protein content and digestibility. Furthermore Ref. [1] said that rice straw is known to contain nutrient values that are not sufficient for the growth of cattle as a business. Field grass generally contains arround20%dry matter. The crude protein content is around 8.4% and 52% TDN content. Grass if given singly to livestock on a dry matter base around 2.5% of body weight is only expected sufficient energy needs for just the basic life and a little for growth.

Lack of nutrients in animal feed can be overcomed by adding feed supplements. In general, feed supplements are beneficial for livestock to meet the nutrients required by the animal body so that there is a balanced composition for production optimally. The optimal composition of feed supplement will increase livestock productivity.

Feed supplement used in this study was *Leucaena leucocephala* leaf. *L. leucocephala* are one type of legume that can be used as an animal feed. *L. leucocephala* has a balanced protein, mineral, amino acid content, has low crude fibre and low tannin content which provides added value because it can function to protect the excess protein degradation in the rumen (by pass protein) so the amount of protein absorbed in the small intestine can be higher [2]. This research was conducted to know the effect of supplementation of *L. leucocephala* on fiber digestibility, characteristic rumen, microbial protein synthesis, and methane production from TMR based on ammoniated rice straw.

II. MATERIAL AND METHODS

This study was done in the Laboratory of Ruminant Nutrition, Faculty of Animal Science, University of Andalas. This experiment assigned in Randomized Block Design with three treatments and five groups as replicates. The treatments were A. 40% rice straw ammoniated + 60% concentrate, B. 40% rice straw ammoniated + 50% concentrate + 10% *L. leucocephala*, C. 40% rice straw ammoniated + 40% concentrate + 20% *L.leucocephala* respectively. Concentrate consist of rice brain, palm kernel cake, cassava and mineral. Chemical composition of each treatment can be seen in Table 1.

Ruminal fluid from a cannulated steer was collected and strained with four layers of cheese cloth. The *in vitro* digestibility was measured according to [3]. Each of fermentation tubes contained of 50 ml of rumen fluid and 200 ml McDougall buffer solution. Three fermentation tubes containing no substrate as control were also incubated. Tubes were incubated in a shaker water bath for 48 h at a temperature of 39ºC. After 48 h incubation, the fermentation activity was stopped immersed in iced water to stop the activities of microbes. Rumen fluid pH was measured with pH meter. Fermentation tubes were then centrifuged at 1500 rpm for 30 min and the supernatant was retained. Residual samples were dried in oven with the temperature set up at 60^{o}C for 24h and retained for Neutral Detergent Fiber (NDF), and Acid Detergent Fiber (ADF), cellulosa digestibility and RUP (rumen undegradable protein) analysis according to [4;5], The total VFA (volatile fatty acids) concentration, partial VFA and rumen NH_3 were analyzed by distillation according to [6,7], respectively. Population of rumen protozoa was counted by [8] method. Methane gas production was measured based on VFAs production [9]. Data obtained were analyzed by Analysis of Variance using a Completely Randomized Design with sub-samplings. Any differences among means were tested using LSD [10].

TABLE I
THE INGREDIENT AND THE CHEMICAL COMPOSITION (% DRY MATTER)

Ingredient composition (%)	Diet		
	A	B	C
Ammoniated rice straw	40	40	40
Concentrate	60	50	40
Leucaena leaf meal	-	10	20
Total	100	100	100
Chemical composition (%)			
Protein	10.93	12.21	13.48
TDN	67,79	68,20	68.60
NFE	5,07	4,91	4.74
NDF	46.73	44.49	42.26
ADF	49,31	51,53	52,01
Lignin	36,77	40.04	43,26

III. RESULT AND DISCUSSION

A. Fiber Digestibility and Rumen Undegradable Protein (RUP)

The results showed that the treatments had a significant effect on the fibre fraction digestibility (NDF, ADF and cellulose) and RUP (P <0.05) (Table 2).

TABLE II
FIBER FRACTION DIGESTIBILITY AND RUP

No	Treatments	Digestibility			RUP (g/100g sample DM)
		NDF	ADF	Cellulose	
1	A	50.66^{b}	47.33^{b}	48,66^{b}	286^{b}
2	B	60.54^{a}	53.66^{a}	58.54^{a}	542^{a}
3	C	58.50^{a}	47.83^{b}	56.50^{a}	562^{a}
	SE	2.4	2.2	2.1	33.77

Description: Means within row bearing different superscripts differ significantly (p <0.05).

Supplementation of 10% and 20% *L. leucochepala* in ammoniated rice straw ration significantly increased digestibility (NDF, ADF, cellulose) and RUP compared to treatment without supplementation of *L. leucochepala*. This could occur because the nutrient content of treatments B and C also increased compared to treatment A, hence more nutrients available for rumen microbes. Besides, *L. leucochepala*also contains sulfur and phosphorous required by rumen microbes to develop mainly cellulolytic bacteria and fungi [11]. Supplementation of 10% *L. leucochepala* provides better digestion than supplementation of 20%. The decrease in nutrient digestion in treatment C was due to the high content of lignin in the ration. Lignin binds to cellulose and hemicellulose and cannot be digested by enzymes produced by microbes. The higher the lignin contents in the ration, the lower the digestibility of the ration as explained by [12].

Beside lignin, the tannin content which was slightly higher in this treatment can reduce digestibility because the tannin would bind protein and carbohydrate so that it cannot be degraded in the rumen. Complex tannins with protein can be released in acidic conditions in accordance to [13] that tannins in the rumen can bind proteins and carbohydrates so that the digestion in the rumen reduced.

As can be seen in Table 2, the addition of *L. luecochepala* significantly affected the amount of by-pass protein (RUP). This increase was caused by an increase in the protein content of TMR because *L. leucochepala* is a feed ingredient with a good quality protein content [14] and as stated by [2] that *L. leucochepala* has its tannin content which can provide added value as it can protect the degradation of the protein inside rumen (bypass protein), hence more protein can be absorbed in the small intestine. This is also shown by the increase in the post-rumen protein digestibility and the amount of bypass protein.

B. Concentration of Total VFA and Partial VFA

Volatile Fatty Acids (VFA) is the result of carbohydrate degradation in the rumen. VFA is the main energy source for ruminants. VFA production will increase if the digestibility of food substances also increases. The production of individual VFA and total VFA in this study was presented in Table 3. From Table 3, it can be seen that the treatment had significant effects (P <0.05) on total VFA, propionate production and acetate to propionate ratio but not significantly on acetate and butyrate production.

TABLE III
EFFECT OF TREATMENTS ON VFA PRODUCTION IN THE RUMEN

Treatments	**Acetate**	**Propionate**	**Butirate**	**Valerate + iso-valerate + iso-butirate**	**Total VFA**	**Ratio Acetate to propionate**
A	41.19	19.00^{c}	9.43	2.89^{cd}	72,00^{b}	2.11^{b}
B	40.70	25,50^{a}	9.75	4.35^{a}	79,25^{a}	1.60^{a}
C	37.00	23.75^{b}	9.50	3.76^{ab}	71.45^{b}	1.53^{a}
SE	1.25	0.34	0.4	0.45	1.3	0.3

Description: Means within row bearing different superscripts differ significantly (p <0.05).

The LSD test results showed that the addition of *L. leucochepala* to the TMR-based on ammoniated rice straw was able to increase VFA production to a level of 10% but a decrease in VFA production at the level of 20%. VFA is the main energy source for ruminants and also the final product of rumen microbial fermentation [15]. The composition of feed ration may affect the partial concentration of VFA. Production of acetic acid (C2), propionic (C3) and butyrate

(C4) relied on carbohydrate fermentation and a small portion of protein fermentation. Supplementation of *L. leucochepala* containing tannin increased the production of propionate (C3). The proportion of propionic acid tends to increase in feed supplement that contain tannin compared to control. This was showed by the decrease in ratio of acetic acid to propionic. The effect of tannin supplementation on rumen fermentation was mainly on a short chain fatty acid conversion pattern which increased the proportion of propionate and decreased of acetate to propionate ratio [16].

The propionate production in Table 3 showed that the highest average was found in the treatment of the addition of 10% *L. leucochepala* compared to other treatments. The production of propionate was influenced by tannin supplementation. Contrary to other substances e.g. essential oils,monensin and saponins, the effect of tannin on methanogenesis is not always associated with increased propionate production [17,18]. Inhibition of the cellulolytic bacterial growth in the presence of tannins turns out to be a causative factor of VFA such as reducing acetate production as the main VFA resulting from cellulolytic bacterial fermentation [18] Although propionate generally decreases due to purified tannins, the amount of reduction is relatively small compared to decrease in acetate. Decreasing acetate to propionate ratio due to the addition of purified tannins is advantageous to mitigating methanogenesis since glucose fermentation into acetate produces H_2, the main substrate required for methane formation, and vice versa, glucose fermentation into propionate consumes H_2[19.20]. The decrease in acetate production in treatment C shows depression *in vitro* fermentation, which is in line with the decreasing digestibility of dry matter and organic matter [21,22].

Table 3 also showed that the production butyrate did not differ between treatments, but for the production of valerate, iso-valerate and iso-butirate had a significantly different between treatments. This suggested that tannin supplementation of up to 20% of dry matter did not interfere with the rumen microbial work to digest feed carbohydrates. The ratio of acetic acid to propionic acid (C2/C3) can be used as an indication of the efficiency of energy used by the ruminants. C2 is a non-glucogenic compound, and almost all body tissues can oxidize it because after being absorbed it is not accumulated but is oxidized directly. As a result of the oxidation process creates a high heat increment so that the efficiency value is low. Conversely, C3 is a compound of sugar precursor or major glucogenic feed [23]. The results of this study indicated that the ratio of C2/C3 feed treatment ranged from 1.53 – 2.11. Treatment D had the lowest C2/C3 ratio (1.52) and the highest at treatment B (2.11).

C. Rumen pH, Protozoa population, NH3 and Methane Production

The results of research on rumen pH can be seen in Fig 1. The addition of *L. leucochepala* does not affect the pH of rumen fluid. Relatively stable rumen pH with *L. leucochepala* supplementation occurs because the concentration of lactic acid in the rumen is low so that *L. leucochepala* can stimulate the growth of users of lactic acid bacteria. The value of pH produced in this study ranged from 6.82 to 6.97. The value obtained sufficient to support optimal microbial rumen growth, where the rumen of the normal pH for microbial activity is from 6.0-7.0 [24].

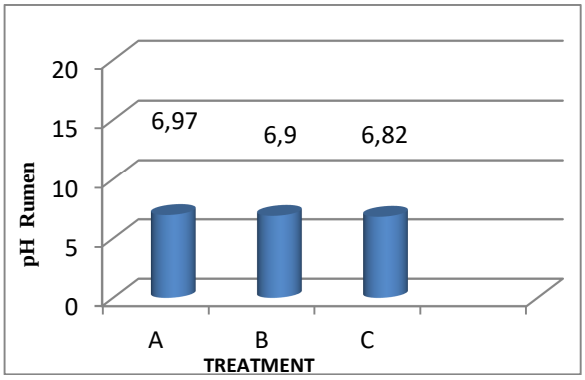

Fig. 1 Effect of treatments on rumen pH

The concentration of rumen NH_3 varied depending on the amount of feed protein, the rate of protein degradation and the time after feeding [25]. Feed proteins that enter the rumen were fermented by proteolytic microorganisms (bacteria and protozoa). Bacteria and protozoa produced proteolytic enzymes such as proteases, peptidases and deaminases to degrade proteins into amino acids, peptides and eventually become ammonia [26]. Ammonia is the main product of the process of deamination of amino acids and their adequacy in the rumen to supply most N for microbial growth and protein synthesis [27]. The average rumen NH3 concentration due to different feed treatments can be seen in Fig 2.The results of the analysis of variance showed that the treated feed and rumen fluid collection time had significant effect ($P< 0.05$) on rumen NH_3 concentration.

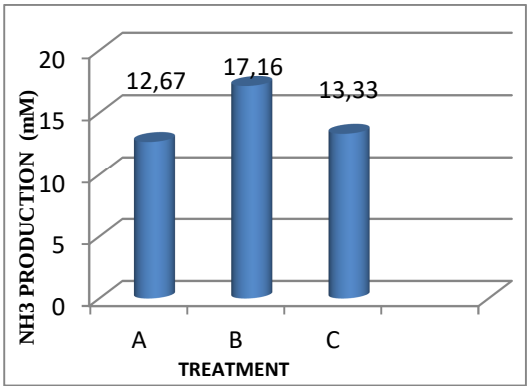

Fig. 2 Effect of treatments on NH3 Production

The results of the study in Fig 2 showed that the concentration of NH_3-N from the ammoniatedrice straw without *L. leucochepala* supplement (A) had significant effect ($P< 0.05$) compared to the *L. leucochepala*supplement. Supplementation of *L. Leucochepala*tends to increase NH_3-N concentration. The concentration NH_3indicated the degradation process of feed proteins in the rumen. It is also known that supplementation of *L. leucochepala* increased the protein content of diet but hingher supplementation of L. leucochepala in treatment C reduced NH3 concentration. It due to the hight of tannin conten in that treatment. It is also known that tannins bind proteins, thereby reducing their

degradation in the rumen. CP degradation decreased in all tannin treatments. This is in agreement with the low production of NH_3-N in supplementation if more than 10% of *L. leucochepala*due to higher tannins in the ration. In this study, the NH_3-N produced ranged from 8.50-10.93 mM and considered as normal. The optimum range of NH_3-N in the rumen for maximum microbial growth was 3.75 mM - 15.00 mM[28].

The supplementation of *L. leucochepala* decreased the population of protozoa in incubation media significantly (P <0.05) Fig. 3. This result can be attributed to secondary metabolites present in forages that affect rumen microorganisms. The defaunation effect of *L. leucochepala* has been demonstrated *in vitro* and *in vivo*, and this has been associated with the presence of Tannin Condensation (CT) and saponins [29]. This coincides with the results where the effectiveness of *L. leucochepala* in removing protozoa can be attributed to the saponin content of forages that appears to be positive in the froth test analysis. Saponins high in molecular glycosides consisting of sugar units (s) associated with triterpene or steroid aglycones and widely distributed in higher plants. The sensitivity of protozoa ciliate to saponin can be attributed to sterols present in protozoa; it's different in bacterial membranes. Therefore, the ability of sterol-binding saponins is likely to result in damage to the protozoa cell membrane [30].

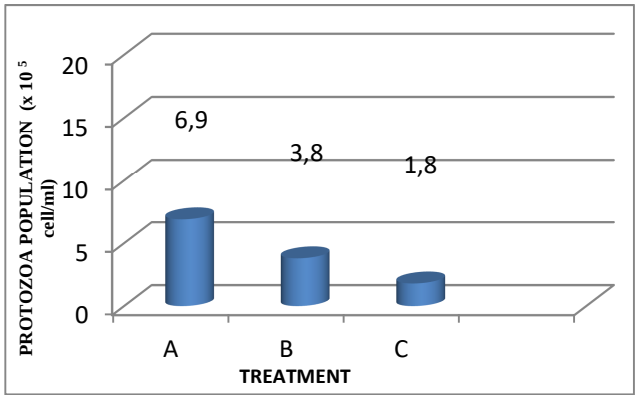

Fig. 3 Effect of treatments on protozoa population

Some literature that suspects tannin is responsible for removing the protozoa population. Condensed tannin reduces the total population of protozoa in the rumen. *L. leucocephala* has tannin compounds and has anti-protozoa effects by damaging the protozoa cell membrane. The ability of tannins to bind to sterols on protozoan cell membranes or their ability to change the permeability of cell membranes can destroy protozoa cells. Therefore, the tannin function is similar to saponin which is a lipid compound that changes the structure of the protozoan cell membrane which has the potential to destroy protozoan growth and change the pattern of fermentation in the rumen system. The use of tannins in rations can reduce the population of ruminal protozoa. Protozoa population is proportional to production of methane, due to methanogenic bacteria symbiosis with protozoa in the rumen. The effect of 10% supplementation of *L. leucochepala*on protozoal population was significantly different (P> 0.05) compared to control (A). This shows that the presence of tannins in*L. Leucochepala* reduced the protozoal population. This is in line with the decrease in methane gas production (12.67mM) compared to other treatments. The decrease in the protozoan population has an effect on the increase in the bacterial population because protozoa are predators for bacteria to meet protein needs. Furthermore, partial defaunation in the rumen was able to increase the population of rumen bacteria [31].

Apart from the partial VFA production ratio (C2 / C3 ratio), feed efficiency in ruminants can be seen from CH_4 gas production. CH_4 is one of the final products of feed fermentation in the rumen where CH_4 gas is formed from H2 and CO_2 by methanogenic bacteria. The amount of H2 used in methanogenesis is 4 mol / mol CH_4 and the change in free energy from this reaction is -134 kj / mol CH_4. The reaction from the conversion of pyruvate to acetate when combined with ΔF = -55 kj / mol of the substrate is 1 mole of ATP. Therefore, the estimation of methanogenesis with ΔF = -134 kj / mol CH_4 will produce equivalent energy of at least 3 moles of ATP / mol CH_4[32].The higher the production of CH_4, the more energy is released (wasted energy). [33] stated that the higher the CH_4gas produced the more inefficient the feed was. The molar average of $CH_4$and CO_2 can be seen in Table 3. The results obtained between treatments were the molar production of CO_2 and CH_4gas, the lowest is produced by treatment B and C which means it is more efficient to use as feed. This is in accordance with the opinion of [33] that feed efficiency in ruminants can be seen from the production of CH_4gas in the rumen, namely the higher the CH_4 gas produced, the more inefficient the feed is. Feed ingredients that contain high carbohydrates or high cell walls produce a lot of CO_2 gas which ultimately results in a large total gas when compared to feed containing high protein [34].

L. leucochepala supplementation was able to decrease methane gas production compared to controls (Fig. 4). Tannin is known to affect the protozoa population [35] since parts of methanogens attached to protozoa [19] and contribute to reduce methane emissions. The greater decrease in methane in this study due to higher tannins content compared to other studies [36]. Some literature reports the relationship of symbiosis of rumen protozoa and methanogens. It is evident that defaunation results in a decrease in CH4 gas production.

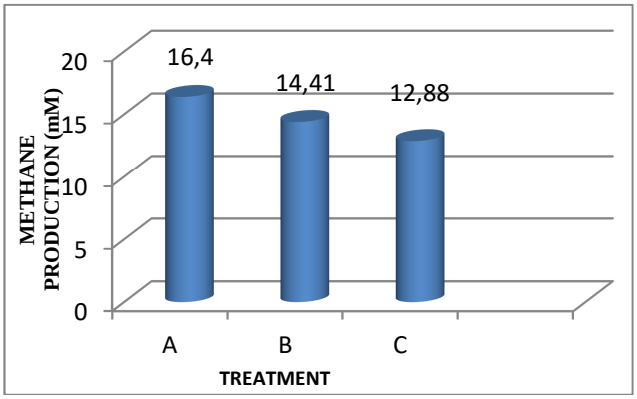

Fig. 4 Effect of treatments on methane production

Retnani *et al* [37] presented a study of the effectiveness of *L. leucocephala* in reducing methane gas production, which

gave 27% *L. leucocephala* in P. purpureum-based basal ration to reduce methane gas production by 15.6% in L/kg DM consumed without affecting digestibility nutrition in sheep. Therefore, this study provides the initial idea that reducing protozoan populations will reduce methanogenic bacteria, thereby reducing methane gas production. This can lead to developing further research to explore various sources and dosages of tannins to be used.

The inhibition of metanogenesis by the presence of tannins may also be the result of a reduction in fiber degradation. The mechanism of tannin ability to reduce methane gas emissions in the rumen, through reducing digestive fibers which in turn indirectly reduces H_2 production as a methane precursor [38], and or directly through inhibition of growth or methanogen activity, thus also with the formation of complex compounds by tannins with proteins and carbohydrates in the rumen [39, 40 and 41].

IV. Conclusion

Supplementation of *L. leucochepala* on TMR based on ammoniated rice straw can increase fiber degradability, rumen fermentability, and reduce methane gas production *in vitro*. Supplementation of 10% and 20% *L. leucochepala* were suitable to be used for further studies, therefore *in vivo* experiment is required to study the effects on animal production.

Acknowledgment

The authors gratefully acknowledge the support by University of Andalas to provide the Professor Research Grant Cluster Award, with contract No. 33/ UN.16.17/ PP.KP.RGB/ LPPM/2018. The authors would like also to thank our post-graduated students and technical assistance of the staffs in the Laboratory of Ruminant Nutrition, Faculty of Animal Science, University of Andalas.

References

[1] Sutardi, T., 1991. Aspek Nutrisi Sapi Bali. In Prosiding Seminar Pengembangan Sapi Bali. Universitas Hasanudin. Ujung Pandang.

[2] Panjaitan, T.S., 2002. Mengenal Potensi Lamtoro Hibrida F2 sebagai Sumber Pakan Hijauan Ternak.BPTP Nusa Tenggara Barat.

[3] Tilley, J.M. and Terry, R.A. 1963. A two stage technique for in vitro digestion of forage crops. Journal of British Grassland Society, 18: 104-111

[4] AOAC, 2007.Official Method of Analysis.Association of Official Analytical Chemist, Washington D.C.

[5] Van Soest, P. J., Robertson, J. B. & Lewis, B. A. (1991). Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74: 3579 – 3583.

[6] General Laboratory Prosedure, 1996. Departement of Dairy Science, University of Wisconsin.

[7] Conway, E.J. and E. O'Malley, 1942. Microdiffusion: Ammonia and urea using bufferedabsorbents (revised methods for ranges greaterthan 10 μg N). Biochem. J., 36: 655-566.

[8] Ogimoto, K. and S. Imai. 1981. Atlas of RumenMicrobiology. Japan Scientific Societies Press, Tokyo.

[9] Makkar, H.P.S., O. P. Sharma, R.K.Dawra and S.S.Negi. Simple determinination of microbial protein in rumen liquor. 1981. J. Dairy Sci 65: 2170-2173.

[10] Steel, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics, A Biometrical Approach. McGraw Hill, New York.

[11] Aregheore, E.M. 1999. Nutritive and antinutritive value of some tree legumes used in ruminant livestock nutrition in Pacific island countries. Journal of South Pacific Agriculture. 6: 50-61

[12] Hariadi BT, Santoso B. 2010. Evaluation of tropical plants containing tannin on in-vitro methanogenesis and fermentation parameters using rumen fluid. J Sci Food Agri. 90:456–461.

[13] Patra, A.K., 2010. Meta-analyses of effects of phytochemicalson digestibility and rumen fermentation characteristicsassociated with methanogenesis.J. Sci. Food. Agric., 90. p. 2700-2708.

[14] Galindo, J., Marrero, Y., Ruiz, T., González, N., Díaz, A., Aldama, A., Moreira, O., Hernández, J., Torres V., Sarduy, L. 2009. Efecto de una mezcla múltiple de leguminosas herbáceas y Leucaenaleucocephala en la población microbiana y productos fermentativos del rumen de añojos mestizos de Cebú. Revista Cubana de Ciencia Agrícola. 43: 256-264

[15] Jayanegara, A., N. Togtokhbayar, H. P. S.Makkar& K. Becker.2008. Tannins determined by various methods as predictors of methane production reduction potential of plants by an in vitro rumen fermentation system. Anim. Feed Sci. Technol. 150: 230- 237

[16] Wina, E., Muetzel,S. and Becker, K. 2005. The Impact of Saponin-Containing PlantMaterials on RuminantProduction-A review: J Agric Food Chem. 19:53(21):8093-105.

[17] Ningrat, RWS., Zain, M., Erpomen, and Suryani, H. 2016. Effects of Doses and Different Sources of Tannins on in vitro Ruminal Methane, Volatile Fatty Acids Production and on Bacteria and Protozoa Populations.Asian J. AnimSci 11: 47-53

[18] Jayanegara, A., F. Leiber, and M. Kreuzer. 2012. Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from in vivo and in vitro experiments. Journal of Animal Physiology and Animal Nutrition 96, 365-375.http://dx.doi.org/10.1111/j.1439-0396.2011.01172.x

[19] Morgavi, D. P., E. Forano, C. Martin, and C. J. Newbold. 2010. Microbial ecosystem and methanogenesis in ruminantsAnimal. 4:1024-1036.http://dx.doi.org/10.1017/ S1751731110000546

[20] Hristov, A. N., J. Oh, J. L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H. P. S. Makkar, A. T. Adesogan, W. Yang, C. Lee, & P. J. Gerber. 2013. Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91:5045-5069. http://dx.doi.org/10.2527/jas.2013-6583

[21] McSweeney, CS, Palmer B, McNeill DM and Krause DO.2001. Microbial interactions with tannins: nutritional consequences for ruminants. Animal Feed Science and Technology 91, 83–93.

[22] Heni Suryani, M Zain, RWS Ningrat, N Jamarun. 2016. Supplementation of Direct Fed Microbial (DFM) on in vitro Fermentability and degradability of ammoniated palm frond. Pakistan Journal of Nutrition 15 (1): 89-94

[23] Susanti, S., Chuzaemi, S dan Soebarinoto. 2001. Pengaruh pemberian konsentrat yang mengandung bungkil kedelai, biji kapuk terhadap kecernaan ransum, produk fermentasi dan jumlah protozoa rumen sapi perah Peranakan Friesian Holstein jantan. Thesis. Fakultas Peternakan Universitas Brawijaya, Malang.

[24] Calsamiglia, S., Ferret, A., Reynolds, C., Kristensen,N., van Vuuren, A. 2010. Strategies for optimizing nitrogen use by ruminants. Animal. 4: 1184-1196.

[25] Hungate, R. E. 1966. The Rumen Microbial Ecosystem. Elsvier Applied science. London and New York.

[26] Chuzaemi, S. Dan J.V. Bruchem. 1990. Fisiologi Nutrisi Ruminansia. Universitas Brawijaya. Malang.

[27] Leng, R.A., 1991. Application of Biotechnology toNutrition of Animal in Developing countries. FAOProduction and Health Paper.

[28] Roffler RE and Satter LD 1975.Relationship between ruminal ammonia and non protein nitrogen utilization by ruminants. I. Development of a model for predicting non protein nitrogen utilization by cattle. Journal of Dairy Science 58, 1880-1888

[29] Jayanegara, A., Goel, G., Makkar, H.P.S. & Becker, K.2010. Reduction in Methane Emissions from Ruminants by Plant Secondary Metabolites: Effects of Polyphenols and Saponins. IN N.E. Odongo, M. Garcia & G.J. Viljoen (eds), Sustainable Improvement of Animal Production and Health. Food and Agriculture Organization of the United Nations, Rome, 2010: 151–157

[30] Patra, A., and Saxena, J. 2009. The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. Nutrition Research Reviews 22(2):204-19

[31] Gutierrez, J. 2007. Observations on bacterial feeding by the rumen ciliate Isotricha prostoma," Journal Eukaryotic Microbiology, vol. 5, pp. 122-126.

[32] Czerkawski, J.W. 1986. An Introduction to Rumen Studies. Robert Maxwell, M. C., Oxford, UK

[33] Mc.Donald, P., R. A. Edwards, J. F. D. Greenhalgh, and C. A. Morgan. 2002. Animal Nutrition. 6th ed. Ashford Colour Press Ltd, Gosport. pp. 515-535.

[34] Wolin, M.J., T.L Miller and C.S Stewart. 1997.Microbe-microbe Interactions. In: The RumenMicrobial Ecosystem. HOBSON, P.N. and C.SSTEWART (Ed.).

[35] Bhatta, R., Y. Uyeno, K. Tajima, A. Takenaka, Y. Yabumoto, I. Nonaka, O. Enishi, and M. Kurihara. 2009. Difference in the nature of tannins on in vitro ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. Journal of Dairy Science 92:5512-5522.http://dx.doi.org/10.3168/jds.2008-1441

[36] Jayanegara, A., F. Leiber, and M. Kreuzer. 2012. Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from in vivo and in vitro experiments. Journal of Animal Physiology and Animal Nutrition 96, 365-375.http://dx.doi.org/10.1111/j.1439-0396.2011.01172.x

[37] Retnani Y, Kamesworo S, Khotidjah L, Saenab A. 2010. Pemanfaatan Wafer Limbah Sayuran Pasar Untuk Ternak Domba.Seminar Nasional Teknologi Peternakan dan Veteriner, 2010 Agustus 2-3; Bogor, Indonesia. Bogor (ID): Pusat Penelitian dan Pengembangan Peternakan. 503-510.

[38] Tavendale, M.H., Meagher, L.P., Pacheco, D., Walker, N., Attwood, G.T. Sivakumaran, S. 2005. Methane production from in vitro rumen incubations with Lotus pedunculatus and Medicago sativa, and effects of extractable condensed tannin fractions on methanogenesis, Animal Feed Science and Technology, 123/124: 403-419.

[39] Makkar, H.P.S. 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin rich feeds.Small Ruminant Research. 49: 241-256

[40] Mueller-Harvey, I. 2006.Unravelling the conundrum of tannins in animal nutrition and health. Journal of the Science of Food and Agriculture 86:2010-2037.http://dx.doi.org/10.1002/jsfa.2577.

[41] Monforte-Briceño, G., Sandoval-Castro, C., Ramírez-Avilíes, L., Capetillo-Leal, M. 2005. Defaunating capacity of tropical fodder trees: Effects of polyethylene glycol and its relationship to in vitro gas production. Animal Feed Science and Technology. 123/124: 313-327.