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Effects of Supplementing *Gliricidia Sepium* on Ration based Ammoniated Rice Straw in Ruminant Feed to Decrease Methane Gas Production and to Improve Nutrient Digestibility (In-Vitro)

Mardiati Zain[a1], Ezi Masdia Putri[a2], Rusmana W.S.N[a3], Erpomen[a4], Malik Makmur[a5]

[a] *Department of Animal Nutrition, Faculty of Animal Science, Andalas University, Padang, West Sumatera, Indonesia*
e-mail: [1]mardiati@ansci.unand.ac.id; [2]ezimasdia@gmail.com; [3]r-ningrat@faterna.unand.ac.id; [4]erpomen897@yahoo.co.id; [5]malikmakmur27@gmail.com

Abstract*—Rice straw as ruminant feed has low digestibility makes ruminants difficult to digest. Since ammoniated rice straw can increase its digestibility, it is necessary to supplement it with Gliricidia sepium. This research aimed to determine the effect of Gliricidia sepium supplementation on decreasing methane gas production and improving nutrient digestibility in complete ration with ammoniated rice straw. The treatments of this research were: A = 40% ammoniated rice straw + 60% concentrate, B = 40% ammoniated rice straw + 50% concentrate + 10% Gliricidia sepium, C = 40% ammoniated rice straw + 40% concentrate + 20% Gliricidia sepium, D = 40% ammoniated rice straw + 30% concentrate + 30% Gliricidia sepium. This research showed supplementation of G. sepium significantly decreased methane gas production from 27.22 mM to 13.13 mM and the number of protozoa from 6.3 x 105 cell/ml rumen fluid to 4.7 x 105 cell/ml rumen fluid. Gliricidia sepium supplementation increased digestibility significantly (P < 0.05). Digestibility of dry matter increased from 58.83% to 68.54% and digestibility of organic matter also increased from 59.50% to 69.50%. Total VFA (Volatile Fatty Acid) concentration did not differ significantly (P > 0.05) among treatments. Higher levels of G. sepium supplementation increased N-NH3 concentration from 7.33 mM to 10.50 mM and microbial protein synthesis from 74.33 mg/100ml rumen fluid to 108.25 mg/100ml rumen fluid. The treatment had a significant effect (P <0.05) on propionate production and the ratio of acetate: propionate. It can be concluded that 30% of Gliricidia sepium supplementation decreased methane gas production and the number of protozoa. Besides, 30% of Gliricidia sepium supplementation increased digestibility and rumen fermentation.

***Keywords*— gliricidia sepium; rice straw ammoniated; complete feed, digestibility; fermentation characteristic.**

I. INTRODUCTION

Rice straw is a post-harvest waste that is currently widely used as a ruminant feed. However, rice straw has several disadvantages, including a high content of lignin and silica, and its low content of energy, protein, minerals, and vitamins [1]. Additionally, rice straw is delicate for ruminants to digest [1,2]. Ammoniating rice straw increased its digestibility [3]. Hence, it is necessary to supplement it with a soluble protein from legume, e.g., *Gliricidia sepium*. *Gliricidia sepium* have 15-30% protein content [4], making them suitable to use as a feed supplement in waste-based feeds [5,6]. It also contains sufficient amounts of minerals (except phosphorus and copper) to meet the needs of livestock in the tropics [7]. Also, the addition of *Gliricidia sepium* provides a nitrogen source for rumen microorganisms. This is important because ruminants depend on rumen microorganisms to supply enzymes that digest the crude fiber in rice straw [8].

Protein is the major factor determining the productivity of ruminant. The protein flowing to the abomasum consists of protein dietary and microbial protein. Microbial protein synthesis depends on nitrogen degraded from soluble protein by rumen microorganism. Not only degraded proten, ruminant also need by pass protein. Tannin can protect protein from degrading and flow to abomasum. Tannin have been shown to inhibit endogenous enzyme activity from rumen mirobe. Tannin interact with proteins by forming hydrogen bonds between the phenolic groups of tannins and carboxyl groups of aliphatic and aromatic side chainsog proteins and through hydrophobic interaction. The binding strength of tannin-protein interactions determine the responses of tannin on protein digestibility in the digestive tract [9]. Tannin from *Gliricidia sepium* can slow down the rate of protein degrading because of tannin-protein bond [10].

Gliricidia sepium in ration have a role in reducing methan gas because it contains condensed tannin and saponin that

modify the numbers of rumen microbes such as archaea, protozoa, and fibrolytic bacteria affecting fermentative processes and methane gas production [10]. Tannin has shown to lower protozoal numbers; it means decreasing protozoal-associated methanogenesis. Oligomeric condensed tannin fractions can be inactive rumen methanogens *Methanobrevibacter ruminantium* strains YLM-1 and DSM1093, as determined by methane production measurements. Hydrolyzable tannin extracts such as gallotanic acid inhibited 50% methane production [9].

Tannin with adequate mount in the ration can increase the digestibility of nutrients and fiber fractions, which means increasing a number of fibrolytic bacterial populations in the rumen. Higher concentrations of tannin in diets, which remain free after binding with proteins, may decrease fiber digestion by complexing with lignocellulose, thus preventing microbial digestion or by inhibiting cellulolytic microorganism. Tannin and saponins interaction were found to be additive. They reduced the populations of fiber-degrading bacteria, but the efficiency of microbial protein synthesis was not affected [9]. This research aims to determine the effect of supplementing ammoniated rice straw with *Gliricidia sepium* on methane gas production, nutrient digestibility and characteristics of rumen fermentation in vitro.

II. Materials and Methods

A. Sampling

We used ammoniated rice straw, concentrate, and *Gliricidia sepium*. Concentrate consisted of palm kernel cake, corn, rice bran, and mineral. This research was conducted in the Ruminant Nutrition Laboratory of the Faculty of Animal Science, Andalas University, Padang. We used a randomized block design with four treatments, replicated five times each, as in Table 1.

TABLE I
INGREDIENT COMPOSITION AND NUTRITION OF EXPERIMENTAL DIET (% DM)

Item	Diet			
	A	B	C	D
Rice straw ammoniated	40	40	40	40
Concentrate	60	50	40	30
Gliricidia	-	10	20	30
Total	100	100	100	100
Nutrition (%)				
Protein	11.51	12.54	13.56	14.59
TDN	68.85	69.19	69.15	69.05
Lemak	2.55	2.47	2.38	2.29
BETN	46.63	46.70	46.77	46.83
NDF	52.20	51.53	51.23	51.01
ADF	43.21	40.50	38.17	36.78
Lignin	11.86	11.65	11.15	10.46

The details of each diet aspects are as follows:

- A = 40% ammoniated rice straw + 60% concentrate
- B = 40% ammoniated rice straw + 50% concentrate + 10% *Gliricidia sepium*
- C = 40% ammoniated rice straw + 40% concentrate + 20% *Gliricidia sepium*
- D = 40% ammoniated rice straw + 30% concentrate + 30% *Gliricidia sepium*

The treatments, utilizing dry matter, were: A = 40% ammoniated rice straw + 60% concentrate, B = 40% ammoniated rice straw + 50% concentrate + 10% *Gliricidia sepium*, C = 40% ammoniated rice straw + 40% concentrate + 20% *Gliricidia sepium*, D = 40% ammoniated rice straw + 30% concentrate + 30% *Gliricidia sepium*. We measured 1) digestibility of dry matter and rganic matter, 2) digestibility of NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber), 3) microbial protein synthesis, 4) concentration of VFA (total and partial), 5) N-NH_3 rumen fluid, 6) ruminal pH, 7) Total population of protozoa and 8) methane gas production.

B. In vitro method

Measurement of digestibility in vitro was carried out as per the protocol established in Tilley and Terry [11], with slight modifications. Fermentation was carried out in a 250 mL Erlenmeyer flask. Sample as much as 2.5 g (based on dry matter) is used as a substrate. CO_2 gas was added into the mixture of 250 mL rumen fluid with buffer by comparison 1:4 into the Erlenmeyer flask. We also added a blank treatment which only contained rumen fluid and buffers without substrate. All treatments had five replications. Incubation proceeded for 48 hours at 39 °C. After incubation, the fermentation activity was stopped by immersion in the Erlenmeyer flask with ice water to stop the microbial activity. We measured pH with a pH meter, then separated the supernatant from the residue. The supernatant mixture and residual particles in the tube were centrifuged for 30 min at a speed of 1200 rpm to obtain residue and supernatant.

The residue was filtered with Whatman paper No. 41 and dried in an oven at 60 °C in 48 h, then analyzed the digestibility of DM and OM using the method established by Blummel [12]. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed using the methods established by Kjeldahl [13]. The supernatant obtained was collected in a bottle and stored in the freezer to be analyzed for total VFA, partial VFA, N-NH_3, and a total number of protozoa. The VFA analysis was performed using gas chromatography [14]. The determination of N-NH_3 production was carried out according to the procedure of Conway and O'Malley [15]. The calculation of a number of protozoa was carried out based on the methods established by [16]. We measured methane gas production according to the methods established by [17].

C. Statistical analysis

Data obtained from this research were statistically analyzed using SPSS software version 21.0 [18]

III. Results and Discussion

All levels of treatment decreased (*Gliricidia sepium* supplementation =10, 20 and 30%) total protozoa and methane production ($P < 0.05$); Table II).

TABLE II
EFFECT OF TREATMENT ON FERMENTATION (TOTAL PROTOZOA AND METHANE GAS PRODUCTION)

Treatments	Total Protozoa (cell/ml)	Methane Gas Production (mM)
A	6.3 X 10^{5a}	27.22^{a}
B	5.8 X 10^{5b}	23.64^{b}
C	4.9 X 10^{5c}	12.67^{c}
D	4.7 X 10^{5c}	13.14^{c}
SE	0.21	2.98

^{a,b,c,d} significantly different in a column (P<0.05).

All levels of treatment increased (*Gliricidia sepium* supplementation =10, 20 and 30%) N-NH_3 and microbial protein synthesis (P < 0.05); Table III).

TABLE III
EFFECT OF TREATMENT ON FERMENTATION (PH, NH3 AND PROTEIN MICROBIAL SYNTHESIS)

Treatments	pH	NH3-N (mM)	Protein microbial synthesis (mg/100 ml)
A	6.97	7.33^{b}	74.33^{a}
B	6.94	8.53^{b}	94.17^{b}
C	6.86	9.20^{a}	107.50^{c}
D	6.80	$10,50^{a}$	108.25^{c}
SE	0.03	0.44	2.48

^{a,b,c,d} significantly different in a column (P<0.05).

In vitro fermentation of treatment ration aims to evaluate the ability of feed to provide substrate for rumen microbes both for growth and activity. Nutrient digestibility was affected by treatment (Table IV and V).

TABLE IV
EFFECT TREATMENTS ON DIGESTIBILITY OF DRY MATTER (DM) AND ORGANIC MATTER (OM)

No	Treatments	Digestibility (%)	
		DM	OM
1	A	$58,83^{c}$	59.50^{c}
2	B	62.50^{b}	63.72^{b}
3	C	66.33^{a}	68.66^{a}
4	D	68.54^{a}	69.50^{a}
5	SE	0.27	1.20

^{a,b,c,d} significantly different in a column (P<0.05). DM = dry matter, OM = organic matter

TABLE V
EFFECT OF TREATMENTS ON DIGESTIBILITY OF CRUDE PROTEIN (CP), NEUTRAL DETERGEN FIBER (NDF), ACID DETERGEN FIBER (ADF)

No	Treatments	Digestibility (%)		
		CP	NDF	ADF
1	A	60.10^{c}	$49,66^{c}$	48.33^{c}
2	B	64.75^{b}	54.54^{b}	52.76^{b}
3	C	70.25^{a}	58.50^{a}	57.13^{a}
4	D	71.05^{a}	59.50^{a}	58.23^{a}
5	SE	1.44	2.6	2.3

^{a,b,c,d} significantly different in a column (P<0.05) CP = crude protein, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber.

Treatment had no significant effect on the total amount of VFA, acetate, butyrate and valerate + isovalerate + isobutyrate ((P > 0.05; Table VI). Supplementation of *Gliricidia sepium* (B, C, and D) in complete rations based on ammoniated rice straw significantly increased propionate and decreased the ratio of acetate propionate (P < 0.05; Table VII).

TABLE VI
EFFECT OF TREATMENTS ON VFA PRODUCTION IN RUMEN

Treatments	Valerat+ isovalerat+ isobutirat	VFA Total	Ratio Acetate propionate
A	1.06	72,00	2.14^{b}
B	1.69	74,25	1.50^{a}
C	1.96	75.45	1.70^{a}
D	2.03	76,80	1.33^{a}
SE	0.35	1.21	0.3

^{a,b,c,d} significantly different in a column (P<0.05).

TABLE VII
EFFECT OF TREATMENTS ON VFA PRODUCTION IN RUMEN

Treatments	Acetat	Propionat	Butirat
A	37.29	19.20^{b}	10.23
B	39.10	$25,70^{a}$	10.27
C	40.70	26.75^{a}	10.65
D	42.45	30.42^{a}	10.80
SE	1.63	0.57	0.4

^{a,b,c,d} significantly different in a column (P<0.05).

A. Effects of Supplementing Ammoniated Rice Straw with Gliricidia sepium on Methan Gas Production and Number of Protozoans

Gliricidia sepium supplementation of up to 30% showed a marked pattern of decline in protozoa populations and methane gas production (Table II). Tropical plants such as *Gliricidia sepium* can be used to reduce methane and protozoa populations [19]. The population density of protozoa is very closely related to the production of methane gas in the rumen [20]. The results obtained by [21, 22] indicated that the addition of Gliricidia sepium reduced rumen protozoa. *Gliricidia sepium* has the potential to reduce methane because it contains saponins and tannins. Saponin is able to reduce methane because it interacts with cholesterol in the protozoa cell membrane and causes cell lysis [23]. This result is also supported by the results obtained by [24, 25], which stated that saponins can reduce methane gas emissions, increase the efficiency of feed use, and increase livestock productivity. Furthermore, [26] stated that saponin supplementation is reported to improve rumen fermentation efficiency through a mechanism of population decline of protozoa. The reduced protozoa population results in reduced H_2 availability for methanogens [27], resulting in decreased methanogen numbers. Reduction of the protozoa population supports an increase in cellulolytic microorganism populations and stabilization of the rumen pH. However, it decreased levels of free ammonia, reduced methanogenesis, but increased digestibility efficiency in high fiber rations [28].

B. Effects of Supplementing Ammoniated Rice Straw with Gliricidia sepium on pH, N-NHx, and Microbial Protein Synthesis

An increasing percentage of *Gliricidia sepium* (10, 20 and 30%) supplementation significantly increased N-NH_3 and microbial protein synthesis, reducing total protozoa and methane production significantly when compared to the control. Supernatant pH values in this research were not significantly different between treatments. The range of pH of all treatments was 6.80-6.97. *Gliricidia sepium* supplementation tended to reduce pH even though it was not

significantly different from the control. The decrease in pH was caused by an increase in the soluble carbohydrates in the ration with the content of tannins in *Gliricidia sepium*. However, the pH value from this research was still within the normal range of normal pH for rumen microbial activity [29].

Ammonia concentration (N-NH3) can be used as an indicator of the quality of feed protein in ruminants. The addition of *Gliricidia sepium* had a significant effect, increasing N-NH_3. Treatment D (addition of 30% *Gliricidia sepium*) with a concentration of 10.50 mM had the highest value but was not statistically significantly different from treatment C. Ammonia (N-NH_3) is the result of decomposition of feed proteins by rumen microbes and assimilation of NH_3 for microbial growth. The resulting ammonia is used for the synthesis of rumen microbial proteins [30]. Increasing of *Gliricidia sepium* content as a source of RDP in rations (B, C and D) tends to increase N-NH_3. This happened because the content of soluble protein from *Gliricidia* at treatment D was higher. Also, the high N-NH_3 in treatment D was caused by the high digestibility of the dry matter produced. Increasing N-NH_3, the utilization of N-NH_3 as a source of N for bacterial growth was also higher. This could be seen from the increase in protein digestibility (Table V) so that rumen fermentation products (N-NH_3) increased. [31] states that the optimal range of NH_3 concentrations for the synthesis of rumen microbial proteins is 6-21 mM.

Microbial protein synthesis increased with increasing *Gliricidia sepium* supplementation (Table III). This is supported by the statement by [32], which states that 66% of the total protein contained in *Gliricidia sepium* can stimulate the synthesis of microbial protein. Increasing rumen microbial population can lead to increase of ammonia utilization, fiber digestibility, and microbial protein synthesis. Thus, the feed can be degraded more optimally so that overall feed digestibility can be increased. The results showed that microbial protein synthesis increased with decreasing protozoan population numbers. The decrease in protozoa population increases the bacterial population. The resulting decrease in protein turnover in the rumen causes less protein to enter the duodenum [33], finally triggering an increase in microbial protein synthesis. *Gliricidia sepium* in the ration can be used as a source of easily degraded protein (RDP), which contain 66.04% of RDP [34]. It results in a high availability of nitrogen in the rumen and supports the synthesis of rumen microbial proteins. This is supported by the results obtained by [35], which state that that the addition of RDP at certain levels in feed containing low quality forages can increase microbial protein synthesis. According to [36], the presence of tannins has a positive effect if added to feeds that are high in protein both in quantity and quality. The high-quality proteins because this can be protected by tannins from the degradation of rumen microorganisms so that its availability increases in the post-rumen digestive tract. *Gliricidia* leaves have a crude protein content and TDN (True Digestible Nutrient) is high with a high rate of aminogenesis, so that it can increase the growth of rumen microbes and their activity in producing high rumen metabolites [37].

C. Effects of Supplementing Ammoniated Rice Straw with Gliricidia sepium on Nutrient Digestibility

Gliricidia *sepium* supplementation increased nutrient digestibility in all treatments. Nutrient digestibility in the C and D treatment was significantly higher than A and B treatments. The D treatment (30% *Gliricidia sepium* supplementation) had the highest mean DMD (Dry Matter Digestibility) and OMD (Organic Matter Digestibility) values (68.54% and 69.50%, respectively), followed by increased protein digestibility, NDF and ADF. The D treatment did not differ from the C treatment (addition of 20% *Gliricidia sepium*). This showed that the higher the supplementation of *Gliricidia sepium*, the better the digestibility. *Gliricidia sepium* contains degraded protein sources (proteins needed by rumen microbes). These protein sources provide an optimal situation for rumen microbial growth, resulting in increased digestion by microbial activity. This agrees with previous results [35].

Treatment D contained 30% *Gliricidia sepium* as a source of RDP making it suitable to meet the needs of rumen microbes, especially in the provision of N-NH_3 for microbes. The addition of RDP at a certain level in feed containing low quality forages can improve the quality of DM, OM, OMD [35]. Table I shows that the D treatment (with the highest *Gliricidia* supplementation of 30%) had a high protein content of (14.59%) and high digestibility value. Protein degradation of *Gliricidia sepium* produces N-NH_3, which is needed by rumen microorganisms to synthesize microbial proteins. Thus, it was expected that the population and activity of rumen microorganisms would increase, in turn increasing the digestibility of feed containing rice straw. This agrees with [6] results, who found that *Gliricidia sepium* increases digestibility of agricultural waste-based rations. Furthermore, [20] stated that increasing levels of *Gliricidia sepium* supplementation in low-quality feeds increases the rate of feed degradation, thus *G. sepium* supplementation may increase consumption and providing organic material that is easily degraded. In this research, 30% *Gliricidia* supplementation gave optimal results for the digestibility of DM, OM, CP, NDF and ADF. To obtain maximum growth in livestock, the proportion of *Gliricidia* in the ration is not more than 50% [38]. Increasing *Gliricidia* supplementation will reduce consumption caused by saponin and tannin contained in *Gliricidia* which began to affect supplementation above 50%.

The proportion of 30% *Gliricidia sepium* in the complete ration in this research was still in amounts that do not exceed the optimum. It was demonstrated that the inhibitors contained in *Gliricidia sepium* had no effect on the digestibility value produced. This is also supported by [39] stating that tannin is an anti-nutrient for cattle feed, but in amounts that do not exceed the optimum level, it has a positive effect that helps the intestines digest and absorb proteins directly by forming tannin-protein bonds that can prevent degradation protein in the rumen. High-quality proteins can be protected by tannins from the degradation of rumen microorganisms, making them more available in the post-rumen digestive tract.

The addition of *Gliricidia sepium* increased the digestibility of NDF and ADF from 49.66% to 57.51% and 48.33% to 56.04%, respectively. Treatment D yielded the

highest NDF digestibility, and ADF, (59.50% and 58.23%, respectively), and was not significantly different from treatment C (addition of 20% *Gliricidia sepium*). These results are supported by the results of the study [40], where the digestibility of NDF and ADF increased with the addition of *Gliricidia sepium* which had better effect in digestibility compared to *Leucaena* [41].

D. Effects of Supplementing Ammoniated Rice Straw with Gliricidia sepium on Rumen Fermentation

The VFA content in rumen fluid can be used as a measure of the efficiency of the fermentation process in the rumen [42]. The results of statistical analysis showed that the treatment had no significant effect on the total amount of VFA, acetate, butyrate and valerate + isovalerate + isobutyrate. Table VI shows that the highest mean VFA was in treatment D with supplementation of 30% *Gliricidia sepium* and not significantly different between treatments. This result is supported by the results obtained by [43], who determined that the addition of *Gliricidia* was able to reduce the CH_4 concentration with a slight change in the VFA profile. Tannin in *Gliricidia sepium* is useful in reducing the number of protozoa in the rumen, improving rumen fermentation, and maximizing rumen microbial protein synthesis, slow down the rate of feed degradation, and reduce nutrient availability. However, nutrients are still available in large portions for microbial protein synthesis compared to VFA formation [44].

Supplementation of *Gliricidia sepium* (B, C, and D) increased propionate and decreased the ratio of acetate propionate. However, there was no significant differences among levels of *Gliricidia sepium* supplementation (B, C, and D). The higher the supplementation of *Gliricidia sepium*, the higher the propionate content and the lower the propionate-acetate ratio. The high concentration of propionate in the ration with supplementation of *Gliricidia sepium* may be due to the high fraction of the feed being easily soluble, leading to quick fermentation. This result was also supported by lower rumen pH values of B, C, and D treatment compared to A treatment (without *Gliricidia* supplementation). This condition stimulates the growth of propionic acid-producing bacteria and instead inhibit the growth of acetic acid-producing bacteria [45]. The concentration of acetic acid showed that the addition of *Gliricidia sepium* (B, C, D) to feed had a different fermentability. Acetic acid is one of the free fatty acids absorbed through the rumen wall [46] during the absorption process, causing a high heat increment so that the efficiency value is low.

The propionate acetate ratio can be used as an indication of the efficiency of energy use and the quality of the product produced. This study showed that the ratio of acetate:propionate ranges from 1.33-2.14. Treatment D (30% *Gliricidia* supplementation) had the lowest acetate/propionate ratio of 1.33 and treatment A (without *Gliricidia* supplementation) had the highest acetate/propionate ratio of 2.14. The low ratio of acetate/propionate in treatment D showed that supplementation of 30% *Gliricidia sepium* in a complete ration with ammoniated rice straw is beneficial because of the feed efficiency and higher relative energy use compared to those in other treatments. Decreasing the ratio of acetate propionate can also inhibit H_2 transfer and reduce the rate of methanogenesis, thereby reducing methane emissions and increasing energy utilization [47].

IV. CONCLUSION

Supplementation of *Gliricidia sepium* in a complete ration of ammoniated rice straw can increase digestibility, characteristics of fermentation, and reduce the production of methane gas in vitro. Future research can be conducted to determine the optimal effect of supplementation of 30% *Gliricidia sepium* on ruminant livestock production.

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REFERENCES

[1] Malik, K., Tokkas J., Anand R. C. and Kumari N., "Pretreated Rice Straw as An Improved Fodder for Ruminants-An Overview." Journal of Applied and Natural Science 7 (1): 514 – 520, 2015.

[2] Oladosu , Y., Raffi M. Y., Abdullah N., Magaji U., Hussin G., Ramli A., Miah G., "Fermentation Quality and Additives: A Case of Rice Straw Silage." Review Article. BioMed Research International. Article ID 7985167.

[3] Zain, M. N. Jamarun, Nurhaita, "Effect of Sulfur Supplementation on in vitro Fermentability and Degradability of Ammoniated Rice Straw." Pakistan Journal of Nutrition 9 (5): 413-415, 2010.

[4] Suryani NN, Budiasa IKM, Astawa IPA., "Rumen Fermentation and Microbial Protein Synthesis of Ettawah Grade (PE) Goat Fed Various Composition of Forage and Different Level of Concentrate." Majalah Ilmiah Peternakan. 17(2), 2014.

[5] Aung M., Thein S.M., Phaw N.C, Phyo T.H., Phyo Y.P., Phyo N.Y., Bik L.V., Bo Bo A., Maw H., Maw A.M., Maw S.Y., Moe P.P.P., Lwin D.S., Myint K.S., Kyawt Y.Y., Mu K.S., "In vitro Fermentation of Grass Based Diet Supplemented With Two Different Tree Legume Forages in Ruminant." Adv. Anim. Vet. Sci. 7(4): 272-279, 2019.

[6] Pi˜neiro-V´azquez, Angel T., Jim´enez-Ferrer, Guillermo O., Chay-Canul, Alfonso J.,Casanova-Lugo, Fernando, D´ıaz-Echeverr´ ıa, V´ıctor F., Ayala-Burgos, Armin J., Solorio-S´anchez, Francisco J., Aguilar-P´erez, Carlos F., Ku-Vera, and Juan C., "Intake, Digestibility, Nitrogen Balance and Energy Utilization in Heifers Fed Low-Quality Forage and *Leucaena leucocephala*." Animal Feed Science and Technology.

[7] Moiforay S. K., Kallon S., Sesay A. R., Sonda T. S., "Acceptability of Gliricidia sepium as Protein Supplement to Panicum maximum by West African Dwarf (WAD) goats in Sierra Leone." International Journal of Science and Research (IJSR). Volume 6 Issue 2.

[8] Jayanegara, A. Ayinda R. S. K., and Laconi E. B., "Urea Treatment of Rice Straw at Elevated Temperature and Pressure: Effects on Fiber Content, Rumen Fermentation and Digestibility." J. Indonesian Trop. Anim. Agric. 42(2):81-87.

[9] Patra, A. and S. Jyotisna. "Éxploitation of Dietary Tannins to Improve Rumen Metabolism and Ruminant Nutrition". J. Sci Food Agric; 91: 24-37. 2011.

[10] Molina-Botero, I. C., Arroyave-Jaramilloa, J., Valencia-Salazarb, S., Barahona-Rosalesc, R., Aguilar-Péreza, C. F., Burgosa, A. A., Arangod, J., Ku-Veraa, J. C., "Effects of tannins and saponins contained in foliage of Gliricidia sepium and pods of Enterolobium cyclocarpum on fermentation, methane emissions and rumen microbial population in crossbred heifers."Animal Feed and Science Technology Vol 251, 2019.

[11] Tilley, J. M. A. And R. A. Terry, “A two stage technique for the in vitro digestion of forage crops.” Journal of British Grassland Society, 18: 104 – 111, 1963.
[12] AOAC. “Official methods of analysis.” 15th Ed., Association of Official Analytical Chemists, Arlington, Virginia, 1990.
[13] Georing, H. K. and P. J. Van shoest, “Forage fiber analysis (apparatus, reagents, procedures and some applications).” Agricultural handbook No. 379. ARS, USDA, Washington, DC, 1970.
[14] Van Ranst G, Fievez V, Vandewalle M, Van Waes C, De Riek J and Van Bockstaele E., “Influence of Damaging and Wilting Red Clover on Lipid Metabolism During Ensiling and In Vitro Rumen Incubation.” Animal. Vol 4 Issue 9. Page 1528–1540, 2010.
[15] Conway, E. J. dan E. O’Malley, “Microdiffusion methods: ammonia and urea using buffered absorbents (revised methods for ranges greater than 10 µg N).” Biochemistry Journal. 36: 655-66, 1942.
[16] Diaz, A., Avendro M. and Escobar A., “Evaluation of sapindus saponaria as a defaunating agent and its effects on different ruminal digestion parameters.” Livestock research for Rural Development, Vol. 5, Number 2, 1993.
[17] Tavendale, M.H., M.P. Meagher, D. Pacheho, N. Walker, G.T. Attwood, and S. Sivakumaran, “Methane production from in vitro incubations with Lotus pnduculatus and Medigo sativa, and effects of extractable condesed tannin fractions on methanogenesis.” Animal Feed Sci. Tech, 123: 403-419, 2005.
[18] SPSS, “IBM SPSS Statistics for Windows, Version 21.0.” Armonk, NY: IBM Corp, 2012.
[19] McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., and Morgan, C.A, “Animal Nutrition. 6th Ed.” Prentice hall, London, 2002.
[20] Galiando, J., N. Gonzales, Y. Marrero, A. Sosa, T. Ruiz, G. Febles, V. Torres, A. I. Aldana, G. Achang, O. Moreira, L. Sarduy and A. C., “Noda. Effect of Tropical Plant Foliege on The Control of Methane Production and In Vitro Ruminal Protozoa Population.” Cuban Journal of Agricultural Science, Volume 48, No 4, 2014.
[21] Suryani, H., M. Zain, R.W.S. Ningrat and N. Jamarun, “Effect of Diertary supplementation Based on Ammoniated Palm Frond with Direct Fed Microbials and Virgin Coconut Oil on the Growth Perfomance and Methane Production of Bali Cattle,” Pakistan Journal of Nutrition, ISSN 1680-5194 DOI: 10.3923/pjn.2017.599.604, 2017.
[22] Galindo, J., González, N., Aldama, A.I. & Marrero, Y., “Effect of Enterolobium cyclocarpum on rumen microbial population and its activity under in vitro conditions.” Cuban J. Agric. Sci. 35:229, 2001a.
[23] Galindo, J., González, N., Aldama & A.I., Marrero, Y., “Effect of Gliricidia sepium on rumen protozoa population and cellulolytic organisms.” Cuban J. Agric. Sci. 35:223, 2001b.
[24] Hess, H. D., Kreuser, M., Diaz T. E., Lascano, C. E., Carulla, J. E., Soliva, C. R and Machmu, A.” Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid.” Anim Feed Sci Technol. 109: 79 – 94, 2003.
[25] Cheeke, P. R, “Actual and Potential applications of Yucca Shidigera and Quillaia saponaria Saponins in human and animal nutrition.” Recent Adv. Anim Nutr Aust: 13. 115- 126, 2001.
[26] Miah, M. Y., Rahman, M. S., Islam M. K and Monir, M. M., “Effects of saponin and L-carnitine on the performance and reproductive fitness of male broiler.” Int J Poult Sci. 3: 530 - 533, 2004.
[27] Anantasook, N., Wanapat M., Gunun P., and Cherdthong A., “Reducing methane production by supplementation of Terminalia chebula RETZ. containing tannins and saponins.” Animal Science Journal. Volume 87 Page: 783–790, 2016.
[28] Jayanegara, A., Sabhan, T., Takyi, A. K., Salih, A. O., and Hoffmann, E. M.,” Ruminal Fermentation Kinetics of Moringa and Peltiphyllum Supplement during early incubation Period in the In-vitro Reading Pressure Technique.” J. Indonesian Trop. Anim. Agric. 35, 165- 171, 2010.
[29] Naumann, H. D., L. O. Tedeschi, W. E. Zeller, & N. F. Huntley, “The role of condensed tannins in ruminant animal production: advances, limitations and future directions.” R. Bras. Zootec. 46: 929-949. http://dx.doi.org/10.1590/ s1806-92902017001200009, 2017.
[30] Puniya, A. K., Singh R., and Kamra D. N., “Rumen Microbiology: From Evolution to Revolution.” Springer New Delhi Heidelberg New York Dordrecht London, 2015.
[31] Owens F.N., Basalan M. “Ruminal Fermentation." In: Millen D., De Beni Arrigoni M., Lauritano Pacheco R. (eds) Rumenology. Springer, Cham, 2016.
[32] Makkar, H.P.S., “In vitro gas method for evaluation of feed containing phychemicals.” Anim. Feed. Sci. Technol. 123: 291, 2005.
[33] Aregheore, F.M., D. Perera, M.S. Yahaya, “Effect of nutritive value of batiki grass (ischaemum aristatum var. Indicum) supplemented by leaves of browses (gliricidia seoium and leucaena leucocephala) on the perfomance of goats.” Scientia agri. Bohemica. (2):63-70, 2002.
[34] Addisu, S., “Effect of Dietary Tannin Source Feeds on Ruminal Fermentation and Production of Cattle; A Review.” Online J. Anim. Feed Res., 6(2): 45-56, 2016.
[35] Trisnadewi, A.A.A.S., I.G.L.O.Cakra, I.W.Wirawan, I Made Mudita, dan N.L.G. Sumardani, “The Substitution of Gliricidia (*Gliricidia sepium*) with Calliandra (*Calliandra calothyrsus*) In Ration to In-Vitro Rumen Degradation.” Pastura Vol. 3 No. 2: 106-109, 2014.
[36] Putri E.M., Zain, M., Warly, L., Hermon, H., “In vitro evaluation of ruminant feed from West Sumatera based on chemical composition and content of rumen degradable and rumen undegradable proteins.” Veterinary World, 12(9): 1478-1483, 2019.
[37] Jayanegara, A., dan A. Sofyan. “Determination of the Biological Activity of Some Forages in Vitro Using the 'Hohenheim Gas Test' with Polyethylene Glycol as a Determinant.” Media Peternakan Vol. 31 No. 1. Bogor. Institut Pertanian Bogor, 2008.
[38] Costa, N. D., “Substitution Of Concencrate With Silage Of Cassava Leaf (Manihot Esculenta,Crantz) And Gliricidia (Gliricidia Sepium, Jacq.) Mixtures on Feed Consumption and Average Daily Gain of Goat.” IOSR Journal of Pharmacy. Volume 7, Issue 10 Version 1. PP. 34-38, 2017.
[39] Oliveiraa, A.P.D., Bagaldob A.R., Louresb D.R.S., Bezerrac L.R., Moraesd S.A., Yamamotoe S.M., Araújob F.L., Cirnef L.G., and Oliveira R.L. “Effect of Ensiling Gliricidia with Cassava on Silage Quality, Growth Performance, Digestibility, Ingestive Behavior and Carcass Traits in Lambs.” Animal Feed Science and Technology. Volume 24. Page 198-209, 2018.
[40] Orden. E. A., S. A. Abdulrazak, E. M. Cruz, M. E. M. Orden, T. Ichnohe and T. Fujihara, “Leucaena leucocephala and Gliricidia sepium Supplementation in Sheep Fed with Ammonia Treated Rice Straw: Effect on Intake, Digestibility, Microbial Protein Yield and Live-Weight Changes.” Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No: 12: 1659-1666, 2000.
[41] Jusoh, S., Alimon, A.R. and Iman, M.N. “Effect of Gliricidia Sepium Leaves and Molasses Inclusion on Aerobic Stability, Value and Digestibility of Napier Grass Silage.” Mal. J. Anim. Sci. 19(1): 33-42, 2016.
[42] Valente, T. N. P., Lima E. D. S., Santos W. B. R. D., Cesário A. S., Tavares C. J., Fernandes Í. L., and Freitas M. A. M. D. “Ruminal Microorganism Consideration and Protein Used in The Metabolism of The Ruminants: A review.” African Journal of Microbiology Research. Vol. 10 (14), pp. 456-464. DOI: 10.5897/AJMR2016.7627, 2016.
[43] Zain, M., R.W.S.Ningrat, Erpomen, E., and H. Suryani., “Effect of doses and different sources of tannins on in vitro ruminal methane, vollatile fatty acids production and on bacteria and protozoa populations.” Asian journal of animal science.11 (1): 47-53, 2017.
[44] Jayanegara, A., Goel, G., Makkar, H.P.S., Becker, K., “Divergence between Purified Hydrolysable and Condensed Tannin Effects on Methane Emission, Rumen Fermentation and Microbial Population In Vitro.” Animal Feed Science and Technology.
[45] González, N. “Evaluación de morera (Morus albaLin) en la fermentación y control de la metanogénesis ruminal de búfalos de río (Bubalus bubalis).” PhD. Thesis. Instituto de Ciencia Animal, Mayabeque, Cuba, 2010.
[46] Muchals, M., Kusmartono dan Marjuki, “Effect of addition of tree leaves on VFA and in vitro digestibility of cassava-based rations.” Jurnal ilmu-ilmu peternakan 24 (2): 8-19, 2014.
[47] Naumann, H. D., L. O. Tedeschi, W. E. Zeller, & N. F. Huntley, “The role of condensed tannins in ruminant animal production: advances, limitations and future directions.” R. Bras. Zootec. 46: 929-949.