The Addition of the Gambier Leaf Residue to Protect Protein in the Cattle Feed Supplement from Rumen Degradation

Ramaiyulis[#], Madiati Zain*, Rusmana Wijaya Setia Ningrat*, Lili Warly*

[#] *Agricultural Polytechnic Payakumbuh, Tanjung Pati, Limapuluh Kota,26271 Indonesia*
E-mail: ramaiyulis@gmail.com

*[2]*Department Animal Science, Andalas University, Kampus Limau Manis, Padang 25163 Indonesia*
E-mail: mardiatiz@yahoo.com, oz.ningrat@gmail.com, liliwarly_uapdg@yahoo.co.id

ABSTRACT

This study aimed to obtain optimal gambier leaf residue (GLR) that can protect the protein of cattle feed supplement (CFS) from rumen microbial degradation. Gambier (*Uncaria gambir* Roxb.) leaf residue containing 9.96% condensed tannin was added in CFS containing 29% crude protein with a mixture of brown sugar, soybean meal, coconut cake, tapioca, urea, and minerals at level 0 (control), 2.5%, 5.0%, and 7.5%, so obtained condensed tannin content in CFS: 0%, 0.68%, 1.17% and 1.60% then which were tested in rumen digestion *in vitro*. Degradation of crude protein in the rumen decreased linearly with an increase in tannin level and ratio of tannin: protein in CFS (R^2 = 0.905; P <0.05) with degradation of 58.18%-69.87% so that *rumen undegraded dietary protein* (RUDP), TCA-soluble N and peptide N was obtained respectively in the range (49.91-74.07; 319.14-465.20; 304.40-451.25) mg / 100 ml rumen fluid, the lowest at control and highest at level 5% GLR. NH3-N concentration decreased linearly with an increase in GLR level while the rumen pH was neutral and not significant with GLR level. The estimated value of optimum GLR addition was obtained at the range of 4.64%-5.12% with the rate of protein degradation 1.05-14.99%/hour with the lowest rate at 3 hours and the highest at 48 hours of incubation.

Keywords: cattle feed supplement, gambier leaf residu, tannin, protein degradation

INTRODUCTION

Protein is a nutritional element that is needed in the growth of animal body tissues. Protein deficiency can inhibit livestock productivity and even reduce weight, especially in animals that are growing, pregnant and lactating (Lazzarini et al. 2013). Provision of protein in ruminant rations is very crucial given the low protein content in tropical forages (Evitayani et al., 2004; Ramaiyulis et al., 2018) and concentrates, especially on small farms, therefore cattle feed supplements (CFS) are made which can supply protein feed for livestock and support microbial protein synthesis in the rumen.

This paper is presented at ASIC 2018, Padang

In ruminants, have a uniqueness in protein metabolism by rumen microbial activity. Dietary protein in the rumen will be degraded to polypeptides and amino acids and then deaminated to ammonia (NH3) which is needed for microbial protein synthesis (Krehbiel, 2014). The rate of degradation is an indicator of the amount of protein from ration available for ruminant (Orskov et al., 1982). Protein degradation in the rumen will reduce the biological value of feed protein (Owens and Sapienza, 2014), therefore proteins need to be protected so that more amino acids are available in post-rumen digestion. Protein protection needs to consider the supply of ammonia to microbes (Griswold et al. 2003, Patra and Aschenbach, 2018) because microbial proteins are the main protein source for ruminant (Garg et al., 2013; Owen and Sapienza, 2014).

Gambier leaf residue (GLR) is a waste from the extraction of gambir leaves (*Uncaria gambier* Roxb.) Containing 9.96% condensed tannins and potentially added to CFS for protecting proteins from microbial degradation in the rumen. Tannins are polyphenol compounds that can form complexes with proteins that are resistant to microbial degradation at neutral pH in the rumen (Bunglavan and Dutta, 2013). GLR is abundantly available from gambier industries considering that Indonesia is the main gambier producer in the world, especially West Sumatra (Ferdinal, 2014). This study aims to obtain the optimal level of GLR which results in a minimum degradation of the CFS protein in the rumen.

MATERIALS AND METHODS

Treatment ration: Cattle feed supplement made with the composition in Table 1 with 4 levels of GLR addition, namely 0% (control), 2.5%, 5.0% and 7.5% (DM) with iso-protein and energy. GLR was taken from the gambier industrial center in Limapuluh Kota, West Sumatra, Indonesia and then dried in an oven at 60 °C for 24 hours and then ground into flour. Brown sugar plus 37.50% water, boiled until all the sugar is melted, then added the soybean meal, coconut meal, and GLR then stirred and then with all the other CFS ingredients. The dough is forming with a pellet machine and dried in an oven at 60 °C for 24 hours.

In vitro procedure: In vitro rumen digestion was carried out following the first stage procedure of Tilley and Terry (1969), using different rumen cattle fluids on 4 replications. Rumen fluid is taken immediately after the cattle are cut by squeezing the rumen contents using 4 layers of gauze and accommodated with a thermos that has been warmed previously by filling warm water at 40 °C for 2 minutes. Rumen liquid was mixed with a McDougall (1948) buffer at ratio 1: 4 and then poured 50 ml into a fermenter tube containing 0.5 g of CFS sample and without CFS as blank. To create an anaerobic condition, CO_2 gas is sprayed for 30 seconds into the fermenter tube and immediately installed a rubber cap that has been equipped with a fermentation gas exhaust valve. Incubation was carried out in a shaker water bath at a temperature of 39 °C with a horizontal stirring of 20 swings per minute for 3, 6, 12, 24 and 48 hours of incubation. At the end of the incubation, the fermenter tube is soaked in cold water 4 °C to stop the fermentation process.

Sample analysis: The ruminal pH in the fermenter tube was measured with Hanna pH meter (Hi9807-phep) then the fermenter tube content was centrifuged at 3,000 rpm for 5 minutes at 4 °C. The supernatant was analyzed to determine the NH3-N concentration in rumen fluid using Conway micro diffusion method (AOAC, 1990) while the residue was washed with distilling water 3 times with centrifuges as before and then filtered with Whatman#41 filter paper, dried and weighed. Dry residues were analyzed to determine crude protein by the Kjeldahl method (AOAC, 1990). Rumen undegraded dietary protein (RUDP) is the difference between residual protein and the blank, whereas protein degradation is the percentage of difference sample protein incubated with RUDP.

TCA-soluble N is determined by following the procedure of Griswold et al. (2003), rumen fluid is homogenized using stirred for 2 minutes, then pour 10 ml into a centrifuge tube and add 20 ml mixture of Trichloroacetic acid (TCA) 20% and Sulfosalicylic acid (SSA) 2% and leave it for 1 hour. Then centrifuged 3,000 rpm 20 minutes 4 °C and the residue obtained was analyzed using the Kjeldahl method (AOAC, 1990). TCA-soluble N is estimated to contain nitrogen from peptides, amino acids, and NH_3-N, so the peptide can be determined after correction with NH_3-N.

Data analysis: Data were analyzed statistically using variance analysis (ANOVA) from randomized block design then continued with Duncan's test to determine the significant differences between treatments (Steel and Torrie, 1980). Regression analysis was used to determine the optimum level of GLR in CFS and to estimate the minimum rate of protein degradation in the rumen.

Table 1. Cattle feed supplement composition with the addition of gambier leaf residue

Ingredient	**Gambier leaf residue, %DM**			
	0	**2.5**	**5.0**	**7.5**
Brown sugar	15	15	15	15
Bran	29	28	27	26
Coconut meal	15	14	12	11
Soybean meal	15	15	15	15
Tapioca	15	15	15	15
Urea	5	5	5	5
Salt	3	2.5	3	2.5
Mineral	3	3	3	3
Gambier leaf residue	0	2.5	5	7.5
Chemical composition				
Organic matter, %	84.32	84.51	85.95	85.96
Crude protein (CP), %	29.30	29.36	29.64	29.90
Tannin, %	0	0.68	1.17	1.60
Tannin: CP non-urea	0	62,42	31,21	20,81

Result and Discussion

Table 2. In vitro rumen digestibility of cattle feed supplement with addition gambier leaf residue.

Parameters	Gambier leaf residue, %DM				SEM	P-value
	0	2.5	5.0	7.5		
Sample protein, mg / 100 ml	165.66	168.14	169.75	170.96		
Crude protein degradation, %	69.87^{a}	58.18^{b}	56.37^{b}	59.76^{b}	2.96	0.046
RUDP, mg/100 ml	49.91^{b}	70.31^{a}	74.07^{a}	68.80^{a}	2.34	<0.01
TCA-soluble N, mg/100 ml	319.14^{b}	345.46^{b}	465.20^{a}	433.16^{a}	26.01	0.010
NH_3-N, mg/100 ml	15.17^{a}	14.75^{b}	13.95^{b}	13.11^{b}	0.33	0.049
Peptide N, mg/100 ml	304.40^{b}	330.29^{b}	451.25^{a}	420.75^{a}	25.82	0.026
pH	6.85	6.95	6.93	6.95	0.03	0.088

Different superscripts on the same line significantly different (P <0.05)
RUDP = rumen undegraded dietary protein; TCA = trichloroacetic acid

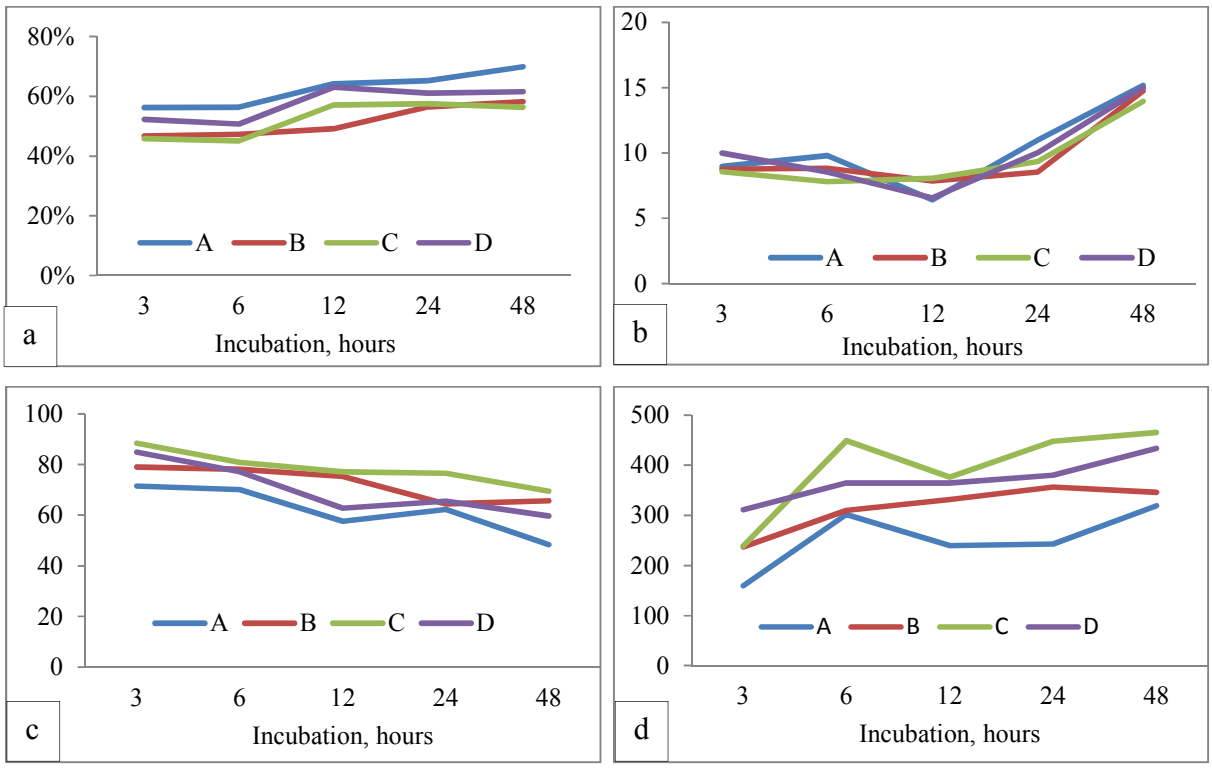

Figure 1. Effect of gambier leaf residue addition (A = 0%; B = 2.5%; C = 5.0%; D = 7.5%) to cattle feed supplement against kinetics of: a. protein degradation (%), b. NH_3-N concentration (mg/100 ml of rumen fluid), c. Rumen undegraded dietary protein (mg/100 ml rumen fluid) and d. TCA-soluble N (mg /100 ml of rumen fluid).

CFS samples with various levels of GLR addition in this study were incubated 165.66-170.96 mg /100 ml of rumen fluid and produced in vitro digestibility as shown in Table 2. Crude protein degradation decreased from control due to the addition of GLR (P = 0.046) with a degradation value of 69.87% in the control and 56.37% -58.18% with GLR addition. Multiple regression analysis showed the degradation of crude protein in the rumen was significant (P <0.05) decreased linearly with an increase in tannin levels and the ratio of tannin with crude protein in CFS (Table 1) with R^2 = 0.905. Therefore, degradation of crude protein CFS in the rumen occurs due of the addition of GLR that containing condensed tannin compounds in which these compounds are able to bind proteins to form a tannin-protein complex that is resistant to proteolytic enzymes from rumen microbes (Getachew et al ., 2000).

Figure 1 shows the degradation of protein with the addition of ADG is always lower than the control at each incubation time. The percentage of CFS protein degraded at 3 hours incubation ranged from 45.76% -56.24%, the lowest was at the level of 5% ADG and the highest was in the control, this figure was relatively constant until the incubation was 6 hours. Then at 12 hours incubation, there was an increase to 49.15% -64.13%, the lowest was at the level of 2.5% ADG and the highest was in the control and relatively constant until 48 hours incubation. In the rumen, more than 70% of the soluble protein is degraded to ammonia (NH_3) after amino acid deamination (Barry and Manley, 1984). The crude protein in CFS consisted of 15,46% feed protein and non-protein nitrogen (urea) equivalent to 14.38% CP. High degradation of crude protein at 3 and 6 hours of incubation was hydrolysis of urea to NH_3 as evidenced by high NH_3 concentration (5.57-7.73 mM) in this period. Hydrolysis of urea to NH_3 in the rumen lasts 1-8 hours of incubation with a peak at 2 hours of incubation (Chizzotti et al. 2008; Kardaya et al. 2009).

The addition of GLR in CFS increased TCA-soluble N concentrations in all incubation periods (P <0.05). TCA-soluble N is a combination of nitrogen derived from feed proteins that escape degradation and protein from microbes in the form of peptides, amino acids, and ammonia (Griswold et al., 2003). The increase in TCA-soluble N occurs due to the increase in protein feed that escapes degradation (RUDP). In Figure 1d, it is seen that 5.0% and 7.5% GLR levels always produce higher TCA-soluble N than lower GLR levels.

Table 3. The estimated value of the optimum GLR level to obtain the minimum protein degradation rate in the incubation period.

Incubation (hours)	b_1	b_2	a	R^2	P	GLR Optimum (%)	Deg.min. (%/jam)
3	0.0021	-0.0178	0.1873	0.991	<0.01	4.64	14.99
6	0.001	-0.0086	0.094	0.994	<0.01	4.71	7.57
12	0.0007	-0.0051	0.0524	0.782	0.091	4.89	4.42
24	0.0002	-0.0017	0.027	0.947	0.032	5.02	2.35
48	0.0001	-0.0013	0.0145	0.961	0.021	5.12	1.05

D = nilai dugaan degradasi protein berdasarkan persamaan kuadratik
$D = b_1x^2+b_2x+ a$
b_1 & b_2 = koefisien regresi
a = intersep
x = taraf ADG
R^2 = koefisien determinasi
P = signifikansi persamaan regresi
Deg.min = protein degradation minimun

The pattern of CFS crude protein degradation in the rumen with the addition of GLR can be estimated through the quadratic regression equation D = b1x2 + b2x + a with a high correlation (R^2 ranged 0.782-0.994; P <0.05) that shown in Table 3. Results of regression analysis showed that optimum GLR addition ranged 4.64% -5.12% of CFS dry matter (P <0.05). Other researchers also reported optimal levels of 0.50% condensed tannins in coconut cake (Zamsari et al. 2012) and 0.6% condensed tannins in soybean meal (Subrata et al., 2005).

CONCLUSION

The addition of GLR in CFS can reduce the degradation of protein in the rumen so that increasing RUDP which results in an increase in TCA-soluble N with a higher Peptide N will be available for post-rumen digestion. The content of tannin and the ratio of tannins/ protein in CFS significantly correlated with protein degradation in the rumen which had a positive impact in increasing the utilization of protein from CFS for cattle. The optimum level of addition of GLR is 4.87% of CFS dry matter that gets the minimum protein degradation rate.

ACKNOWLEDGMENTS

We thanks to the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for the funding this research. Then for the laboratory of Agricultural Polytechnic of Payakumbuh and laboratory ruminant nutrition of Andalas University which has facilitated this research.

REFERENCES

AOAC. 1990. Official Methods of Analysis Association of Official Agriculture Chemist. Agricultural Chemical. Association of Official Agriculture Chemists, Inc. Virginia.

Barry TN, Manley TR, 1984. The role of condensed tannins in the nutritional value of Lotus pedunculatus for sheep. 2. Quantitative digestion of carbohydrates and proteins. Br. J. Nutr. 51:493–504.

Bunglavan dan Duta. 2013. Use of Tannins as Organic Protectants of Proteins in Digestion of Ruminants. J. of Livestock Sci. 4:67-77.

Chizzotti, F.H.M., O.G. Pereira, L.O. Tedeschi, S.C.V. Filho, M.L. Chizzotti, M.I. Leao and D.H. Pereira. 2008. Effects of dietary nonprotein nitrogen on performance, digestibility, ruminal characteristics, and microbial efficiency in crossbred steers. J. Anim. Sci. 86: 1173–1181.

Evitayani, L. Warly, A. Fariani, T. Ichinohe and T. Fujihara. 2004. Study on Nutritive Value of Tropical Forages in North Sumatra, Indonesia. Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 11 : 1518-1523.

Ferdinal, N. 2014. A Simple purification method of catechin from gambier. IJASEIT. 4(6): 53-55.

Garg, M. R., Sherasia, P. L., Bhanderi, B. M., Phondba, B. T., Shelke, S. K., Makkar, H.P.S. 2013. Effects of feeding nutritionally balanced rations on animal productivity, feed conversion efficiency, feed nitrogen use efficiency, rumen microbial protein supply, parasitic load, immunity and enteric methane emissions of milking animals under field conditions. Anim. Feed Sci. and Technol. 179: 24– 35

Getachew G, Makkar HPS, Becker K, 2000. Tannins in tropical browses: effects on in vitro microbial fermentation and microbial protein synthesis in media containing different amounts of nitrogen. J Agric Food Chem 84: 3581–3588.

Griswold, K.E., G.A. Apgar, J.Bouton, and J.L.Firkins. 2003. Effects of urea infusion and ruminal degradable protein concentration on microbial growth digestibility and fermentation in continuous culture. J. Anim. Sci. 81(1): 329-336.

Jones GA, McAllister TA, Muir AD, Cheng KJ. 1994 Effects of sainfoin (*Onobrychis viciifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacterium. Appl Environ Microbiol 60:1374–1378.

Kardaya, D., K.G. Wiryawan, A. Parakkasi dan H.M. Winugroho. 2009. In vitro slow-release urea contained in rice straw-based diets to increase the efficiency of rumen microbial protein synthesis. JITV. 15(2):105-117.

Komolong MK, Barber DG, McNeill DM, 2001. Post-ruminal protein supply and N retention of weaner sheep fed on a basal diet of lucerne hay (*Medicago sativa*) with increasing levels of quebracho tannins. Animal Feed Science and Technology. 92: 59–72.

Krehbiel, C.R. 2014. Applied nutrition of ruminants: Fermentation and digestive physiology. Professional Animal Scientists. 30: 129-139.

Kumar R, Singh M, 1984. Tannins: their adverse role in ruminant nutrition. J Agr Food Chem 32: 447-453.

Lazzarini I, Detmann E, Paulino MF, Valadares Filho SC, Valadares RFD, Oliveira FA, et al. Nutritional performance of cattle grazing on low-quality tropical forage supplemented with nitrogenous compounds and/or starch. Rev Bras Zootec. 2013;42:664–74. doi:10.1590/S1516-35982013000900009.

McDougall, EI. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. The Biochemical Journal 43, 99–109.

McSweeney CS, Palmer B, Bunch R, Krause DO. 2001. Effect of the tropical forage calliandra on microbial protein synthesis and ecology in the rumen. J Appl Microbiol 90:78–88

Min, B. R., Attwood, G. T., McNabb, W. C., Molan, A. L., & Barry, T. N. 2005. The effect of condensed tannins from *Lotus corniculatus* on the proteolytic activities and growth of rumen bacteria. Anim. Feed Sci. and Techno. 121(1-2), 45-58.

Orskov, E.R. 1982. Protein Nutrition Ruminants. 2nd edition. Academic Press Limited, London.

Owens, F.N. and D.A. Sapienza. 2014. Applied protein nutrition of ruminants-Current status and future directions. Professional Animal Scientists. 30: 150-179.

Patra, A. K. and J.R. Aschenbach. 2018. Ureases in the gastrointestinal tracts of ruminant and monogastric animals and their implication in urea-N/ammonia metabolism: A review. J. Adv. Res. https://doi.org/10.1016/j.jare.2018.02.005

Perez-Maldonado RA, Norton BW, 1996. The effects of condensed tannins from *Desmodium intortum* and *Calliandra calothyrsus* on protein and carbohydrate digestion in sheep and goats. Bri. J. Nutr. 76: 515 – 533.

Preston, T.R. and R.A. Leng. 1987. Matching Ruminant Production System with Available Resources in the Tropics. Penambul Books, Armidale. 245 p.

Ramaiyulis, M. Zain, RWS. Ningrat, L. Warly. 2018. Interaction Effects of Cattle Feed Supplement and Concentrate on Rumen Fermentability and Fiber Fraction Degradability in Low-Quality Forage. Sch. J. Agric. Vet. Sci. Sch. 5(6): 337-342. doi:10.21276/ sjavs.2018.5.6.9

Satter, L.D. and L.L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Brit. J. Nutr. 32: 199-208.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedures of Statistics. Mc Graw-Hill Inc.

Subrata A., L.M. Yusiati, dan A. Agus. 2005. Utilization of tea pulp tannins on the effects of defaunation, parameters of rumen fermentation and microbial protein synthesis by in vitro. Agrosains. 18 (4) : http://i-lib.ugm.ac.id/jurnal/detail.php?dataId=714

Tilley, J.M.A and R.A. Terry. 1963. A two-stage technique for the in vitro digestion of forage. J. British Grassland Society. 18: 104–111.

Waghorn GC, Ulyat MJ, John A, Fisher MT. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. Br. J. Nutr. 57: 115-126.

Waghorn, G. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. Animal Feed Science and Technology, 147(1-3), 116-139.

Wang, Y., Douglas G.B., Waghorn G.C., Barry T.N. and Foote A.G. 1996. Effect of condensed tannins upon the performance of lambs grazing *Lotus corniculatus* and lucerne. Journal of Agricultural Science, Cambridge 126: 87-98.

Wina, E., dan D. Abdurohman. 2005. The formation of ruminal bypass protein (in vitro) by adding tannins isolated from *Calliandra colothyrsus* leaves or formaldehyde. JITV. 10(4): 274-280.

Wischer, G., J. Boguhn, H. Steingab, M. Schollenberger, M. Rodehutscord. 2013. Effects of different tannin-rich extracts and rapeseed tannin monomers on methane formation and microbial protein synthesis in vitro. Animal. 7(11) : 1796-1805

Zamsari, M. Sunarso, and Sutrisno. 2012. The use of natural tannins in protecting coconut cake protein in terms of protein fermentability in vitro**.** Animal Agriculture J. 1(1): 406-41.