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

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Why proteins need to be protected from rumen degradation in ruminants ?





- Protection of proteins is essential for productive ruminants
- Protein deficiency can inhibit ruminant productivity and even reduce weight, especially in animals that are growing, pregnant and lactating
- Provision of protein in ruminant rations is very crucial given the low protein content in tropical forages

Beef cattle ration





Konsentrate

Feed supplement





What is gambier leaf residue (GLR)?



Materials and methods

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

Ingradiant	Gambier leaf residue, %DM						
Ingreulent	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			
Таріоса	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			

Rumen in vitro digestion





RESULTS AND DISCUSSION

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

	Ga	Gambier leaf residue, %DM					
Parameters	0	2.5	5.0	7.5	SEIVI	value	
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	68.80ª	2.34	< 0.01	
TCA-soluble N, mg/100 ml	319.14 ^b	345.46 ^b	465.20ª	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	
рН	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).

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

Incubation (hours)	b ₁	b ₂	a	R ²	Р	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 = estimated value of protein degradation based on quadratic equation

 R^2 = coefficient of determination

 $\mathbf{b}_1 \& \mathbf{b}_2 =$ regression coefficient

a = intercept

 $\mathbf{D} = \mathbf{b}_1 \mathbf{x}^2 + \mathbf{b}_2 \mathbf{x} + \mathbf{a}$

 $\mathbf{x} =$ level of GLR

P = the significance of the regression equation

Deg.min. = the minimum degradation rate of protein

Conclusion

- The addition of GLR can reduce the degradation of protein, increase RUDP, increase TCA-soluble N and higher Peptide will be available for post-rumen digestion.
- The optimum level of addition of GLR is 4.64 to 5.12%

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

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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, CO₂ 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 NH₃-N, so the peptide can be determined after correction with NH₃-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.

т. н. <i>с</i>		Gambier leaf residue, %DM							
Ingredient	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					

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

Result and Discussion

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

				-							
Deremators	(ambier leaf	SEM	D voluo							
Farameters	0 2.5 5.0 7.5		7.5	SEIVI	P-value						
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					
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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	Different superscripts on the same line significantly different ($P < 0.05$)										

Different superscripts on the same line significantly different (F < 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. NH₃-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 (NH₃) 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 NH₃ as evidenced by high NH₃ concentration (5.57-7.73 mM) in this period. Hydrolysis of urea to NH₃ 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.

Incubation	b ₁	b ₂	а	\mathbb{R}^2	Р	GLR	Deg.min.
(hours)						Optimum (%)	(%/jam)
3	0.0021	-0.0178	0.1873	0.1873 0.991 <0.01 4.6		4.64	14.99
6	0.001	-0.0086 0.094 0.994 <0.01 4.7		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

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

D = nilai dugaan degradasi protein berdasarkan persamaan kuadratik R^2 = koefisien determinasi

 $\mathbf{D} = \mathbf{b}_1 \mathbf{x}^2 + \mathbf{b}_2 \mathbf{x} + \mathbf{a}$

 $b_1 \& b_2 =$ koefisien regresi

a = intersep

P = signifikansi persamaan regresi

Deg.min = protein degradation minimun

x = taraf ADG

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 (R² 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

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Conference Programme & Abstract

ASIC 2018 **AGRIFOOD SYSTEM INTERNATIONAL CONFERENCE**

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SCHEDULE

Agrifood System International Conference (ASIC) Faculty of Agriculture, Universitas Andalas Kyriad Hotel Bumi Minang, 4 -- 6 September 2018

First day agenda Tuesday, Sentember 4th

Tuesday, September 4th 2018

Time	Session	Venue/ Person in charge
08.00 - 08.30	Registration	
	Opening ceremony	Ball room
08.30 - 08.35	Opening by MC	2 MCs (English and Indonesian)
08.35 - 08.50	National Anthem: 'Indonesia Raya'	UKS - FPUA
08.50 - 09.00	Do'a	Forstudi - FPUA
09.00 - 09.10	Report from organizing committe	Prof. Ir. Yonariza, M.Sc, Ph.D
09.00 - 09.20	Welcoming remarks 1. Dean 2. Rector 3. Governor	Dr. Ir. Munzir Busniah, M.Si Prof. Tafdil Husni, SE, MBA, Ph.D Prof. Dr. IrwanPrayitno, S.Psi, M.Sc (Gong striking)
09.20 - 09.35	Minangkabau Dance Performance	UKS - FPUA
09.35 - 09.50	Coffee break	Dwi Evaliza
	Keynote speech (1 st session)	Conventional Room
09.50 - 10.35	1. Prof. Kouichi Ikegami (Digital agrifood system in era 4.0)	Moderator : Prof. Dr. Helmi
10.35 - 11.20	2. Prof. lin Handayani (Education to support agrifood system in era 4.0)	Moderator : Dr. Agustian
11.20 - 12.05	3. Dr. Daniel Ruiz de Garibay Ponce (Family farming in era 4.0)	Moderator : Prof. Dr. Dian Fiantis
12.05 - 12.20	Appreciation and photo session	Afrianingsih
12.20 - 13.30	Lunch Break	Dwi Evaliza

LIST OF PARTICIPANTS

ORAL / POSTER	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral
TITLE	System of Rice Intensification: Characteristics of Dry Matter Production in Rice (Oryza sativa L.) Cultivar Ciherang in the Use of Organic Compared with Inorganic Fertilizers	Tofu Liquid Waste and Organic-Household-Waste Compost Affecting The Yield of Cucumber (Cucumis sativus L.)	EFFECT OF BIO-PRIMING ON DETERIORATED SEED VIABILITY AND VIGOR, GROWTH AND YIELD OF SOYBEAN (Glycine max (L.) Merrill) IN ACIDIC SOIL.	The Effect of Three Different Mulches on Weed Presence and Zinnia Growth	THE EFFECT OF ISOLATE OF INDIGENUS RHIZOBAKTERI AND SILVER BLACK PLASTIC MULCH ON GROWTH AND YIELD OF POTATOES (Solanum tuberosum L.)	Technology Of Soyabean's Seed Management Unit In Jambi Province	Response of Edamame Soybean (<i>Glycine max</i> (L.)Merr.)and Sorghum (<i>Sorghum bicolor</i> (L.) Moench) on various spacing of Soybean and NPK dose in intercropping pattern	Character Components the Onion (Allium ascalonicum L.) and Selection of Callus Using NaCl and PEG In Vitro	Soil Organic Carbon Fractions in Oil Palm Management Systems	Exploration of Local Rice Germplasm from Rokan Hilir Regency Based on Morphology and Agronomy Character	Isolation the Distal Promoter of NPR1 Gene from Chili Pepper (Capsicum annuum L.)
INSTITUTION	Plant Ecology- Departement of Biology ITS	Universitas Andalas	Universitas Andalas	Murray State University	Universitas Andalas	BPTP Jambi	Universitas Andalas	Universitas Cokroaminoto Palopo	Murray State University	Riau University	Universitas Andalas
NAME	Mukhammad Muryono	Sannaida Simbolon, Irawati Chaniago, Irfan Suliansyah	P.K. Dewi Hayati dan Lutfiyah Rahmi	Steven M. Still, Anmar Muttaleb, lin Handayani	Warnita, Ardi, Yopi Zulfa	Ratna Rubiana, Yardha	Metty Rasminasari, Indra Dwipa, Irawati	Rahman Hairuddin	IP Handayani, H Widiastuti, MS Coyne, S Widawati	Ngatiman	Maythesya Oktaviani
CODE	CPPB-01	CPPB-02	CPPB-03	CPPB-04	CPPB-05	CPPB-06	CPPB-07	CPPB-08	СРРВ- 09	CPPB-10	CPPB-11

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Oral	Poster	Poster	Poster	Poster	poster	Poster	Poster	Poster	Poster	Oral
Production Analysis of Smallholding Arabica Coffee Farms in District of Solok	Factors Influencing Coffee Growers to Produce Parchment Coffee in Northern Thailand	The Partnership Models of Small Industry in Cluster Perspective to Promoting Regional Development (case study on small industries in Jambi city)	AGRICULTURE AS SOURCES OF REVENUES OF PALM OIL REPLANTING IN LEISURE LEAVES LEFT DISTRICT DISTRICT	FACTORS AFFECTING FARMERS INCOME IN TAPPING OF SAP PINE IN AKABILURU SUDISTRICT LIMAPULUH KOTA	Factors Affecting Consumer Decisions in Purchasing Agroindustry-based Snacks as Souvenirs of the City of Padang	Local Wisdom On Sago Farming In The Village Of Tanjung Peranap, Tebing Tinggi District, Meranti Island District	The Analysis of Women Empowerment in Developing Household Industry Made of Sago in East Tebing Tinggi Sub- district	FARMERS PERCEPTIONS AGAINST SUCCESS LEVEL INTEGRATED FARM ROGRAM IN VILLAGE AGROWISATA RUMBAI DISTRICT PEKANBARU CITY	Attitudes of farmers to the Rice Farming Insurance program in Tanah Datar regency	The addition of Gambier leaf residu to protect protein in the feed supplement from rumen degradation
Universitas Andalas	Thailand	Jambi University	Lancang Kuning University	Universitas Andalas	Universitas Andalas	Universitas Riau	Universitas Riau	Lancang Kuning University	Universitas Andalas	Agricultural Polytechnic of Payakumbuh
Yusmarni, Afrianingsih Putri, Cindy Paloma, Zelfi Zakir	Prathanthip KRAMOL	Erfit	Latifa Siswati, Rini Nizat, Enny Insusanty	Afrianingsih Putri,Syahyana Raesi,Dwi Evaliza, Rika Hariance	Lora Triana, Zelfi Zakir and Cipta Budiman	Eri Sayamar	Rosnita	Asgami Putri, Amalia	Siska Handayani, Rudi Febriamansyah and Mahdi	Ramaiyulis
AFF-27	AFF-28	AFF-29	AFF-30	AFF-31	AFF-32	AFF-33	AFF-34	AFF-35	AFF-36	OTR-01

OTR-01

The addition of Gambier leaf residu to protect protein in the feed supplement from rumen degradation

Ramaiyulis

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Protection of proteins is essential for productive ruminants, 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 to 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 (R² = 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 mi 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 residue; tannin; protein degradation

OTR-02

Effect Of Waretha® Probiotic On Total Colonies Of Bacillus Amyloliquefaciens, *Lactobacillus* sp, Ph And Cellulose Enzyme Activity In The Small Intestine Of Domestic Chickens

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This study aims to determine the effect of the addition of Waretha® probiotic in drinking water on the total colonies of bacteria Bacillus amyloliquefaciens, Lactobacillus sp, pH, and cellulase enzyme activity in the small intestine of domestic chickens in starter period. This study was used 200 day-old-chicks of domestic chickens CP 808 kept for 8 weeks. Type of cage used 20 units cage box measuring 85 cm x 70cm x 30 cm which each unit contains 10 chickens. This study used the Completely Randomized Design (CRD) experimental method with 4 treatments and 5 replications. Treatment is the addition of Waretha® probiotic in drinking water which is W0 (no addition of Waretha® probiotic), W1 (45 x 109 CFU/ml), W2(65 x 1010 CFU /ml), and W3 (43 x 1012 CFU /ml). The parameters measured were total bacterial colonies of Bacillus amyloliquefaciens, Lactobacillus sp, pH, and cellulose enzyme activity in the small intestine of domestic chickens. The results showed that the use of Waretha® probiotic in drinking water had asignificantly different effect (P <0.01) on the total colonies of Bacillus amyloliquefaciens, Lactobacillus sp, and cellulose enzyme activity, but not significantly different (P> 0.05) on pH in the small intestine of domestic chickens. The DMRT test showed that the total colonies of Bacillus amyloliquefaciens, Lactobacillus sp, and cellulase enzyme activity up to 43 × 1012 CFU/ml (W3) were very significant (P<0.01) higher than the treatment of W0. The conclusion of Waretha® probiotics were added to 43 x 1012CFU/ml in drinking water increased total colonies of Bacillus amyloliquefaciens, Lactobacillus sp, andcellulase enzyme activity in the small intestine, but did

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