ISSN 1819-1878

Asian Journal of Animal Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS Asian Journal of Animal Sciences

ISSN 1819-1878
DOI: 10.3923/ajas.2017.47.53

Research Article
Effects of Doses and Different Sources of Tannins on *in vitro* Ruminal Methane, Volatile Fatty Acids Production and on Bacteria and Protozoa Populations

R.W.S. Ningrat, Mardiati Zain, Erpomen and Heny Suryani

Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Kampus Limau Manis, 25163 Padang, Indonesia

Abstract
Background and Objective: Tannins have the ability to bind protein and can act to increase animal productivity by rendering protein inactive in the rumen and releasing it post-rumen for use by the animal for meat or milk production.This study was to determine the effect of supplementation of tannin from two different sources at different doses to ammoniated oil palm frond on *in vitro* rumen fermentation characteristics. Source of tannin is Gambier Leaves Waste (GLW) from Payakumbuh and Painan, two different districts in West Sumatera province. Doses of GLW on dry matter bases. **Materials and Methods:** The experiment was arranged in a block randomized design with seven treatments and three replications. The treatments were A = oil palm frond was treated with 4% urea, B1 = A+10% GLW Payakumbuh, B2 = A+ 15% GLW Payakumbuh, B3 = A+20% GLW Payakumbuh, C1 = A+10% GLW Painan, C2 = A+ 15% GLW Painan and C3 = A+20% GLW Painan. Variables measured were methane production, DM, OM, NDF and ADF digestibility, concentration of NH_3-N, partial VFAs, microbial protein synthesis, population of rumen bacteria and protozoa and ruminal fluid pH. Data was analyzed using analysis of variance (ANOVA) and differences among means were tested using Duncan. **Results:** The results showed that the supplementation of GLW were significantly ($p<0.05$) increased degradability, fermentability and reduce methane gas production. The treatment had no significant effect ($p>0.05$) on ruminal fluid pH, propionate production and population of bacteria. The DM digestibility increased from 48.45 (Treatment A)-52.95% (Treatment B2) and OM digestibility 51.34 (Treatment A)-57.30% (Treatment B2). The concentration of VFAs increased from 71.00-95.78 mM. Molar proportion of VFAs was shifted from acetate to propionate production and reduced the ratio of acetate to propionate. The rumen pH with supplementation of tannins is relatively more stable. Methane production decreased from 27.22 (Treatment A)-12.67 mM (Treatment B2) and to 15.13 mM (Treatment C1). The methane production reduced to 53% (Treatment B2) and 45% (Treatment C1) compare control. **Conclusion:** These results showed that 15% GLW Payakumbuh and 10% GLW Painan was suitable to be used as doses and source of tannins but the supplementation of 15% GLW Payakumbuh give the best results on digestibility and in reducing methane gas production.

Key words: Ammoniated oil palm frond, gambier leaves waste, methane, *in vitro* digestibility

Received: October 15, 2016 Accepted: November 16, 2016 Published: December 15, 2016

Citation: R.W.S. Ningrat, Mardiati Zain, Erpomen and Heny Suryani, 2017. 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. Anim. Sci., 11: 47-53.

Corresponding Author: Mardiati Zain, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Kampus Limau Manis, 25163 Padang, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oil palm frond can be used as source of fiber feed in ruminant ration after supplemented with additive that stimulates rumen microbial growth as reported by Zain *et al*.[1]. The use of low-quality feed such as oil palm frond in ruminant also led to higher production of methane in the rumen as discussed by Suryani *et al*.[2]. Methane production of ruminant livestock contributes to 95% of the total methane emissions produced by livestock and humans and approximately 18% of the total releases of greenhouse gases in the atmosphere as reported by Kreuzer and Soliva[3].To reduce the emission of methane from ruminants, it is necessary to apply a strategic feeding system for more efficient utilization of feed. The method should be promoted by nutritional manipulation process in ruminants which leads to a decrease in methane production in the rumen, while optimizing productivity so that the self sufficiency of the national beef cattle program can be achieved and the environment stay healthy.

Tannins are anti-nutritive compounds which has a phenol group. Tannins form a complex bond with protein, carbohydrates (cellulose, hemicellulose and pectin), minerals, vitamins and enzymes in the rumen microbes as reported by McSweeney *et al*.[4]. Complex tannins bond to proteins can be released in the low pH in the abomasum so that proteins can be degraded by the enzyme pepsin and amino acids they contain can be utilized by livestock as discussed by Jayanegara *et al*.[5]. Tannins can be used as to reduce the emission of methane in the rumen because it is plays role as an agent to reduce the population of protozoa as reported by Makkar[6].

Different sources of tannins have been shown to have differing impacts on CH_4 production, likely due to tannin composition and type. Tannins have the ability to bind protein and can act to increase animal productivity by rendering protein inactive in the rumen and releasing it post-rumen for use by the animal for meat or milk production. This study was conducted to evaluate the effect of doses from different source of tannins to reduce methane in diet based on ammoniated oil palm frond.

MATERIALS AND METHODS

This study was conducted in the Laboratory of Ruminant Nutrition, Faculty of Animal Science, Andalas University. This study was to determine the effect of supplementation of tannin from two different sources at different doses to ammoniated oil palm frond on *in vitro* rumen fermentation characteristics. Source of tannin is Gambier Leaves Waste (GLW) from Payakumbuh and Painan, two different districts in West Sumatera province.

Experimental design: This experiment carried out randomized block design with seven treatments and three groups as replications. The treatments were A = oil palm frond that treated with 4% urea (control) previously as reported by Zain *et al*.[7], B1 = A+10% GLW Payakumbuh, B2 = A+15% GLW Payakumbuh, B3 = A+20% GLW Payakumbuh, C1 = A+10% GLW Painan, C2 = A+15% GLW Painan, C3 = A+20% GLW Painan. The GLW was residue from gambier extract. Doses of GLW on dry matter bases. In West Sumatera, gambier extract was prepared using a traditional method. Gambier leaves and stems were boiled for 1.5 h and then pressed to obtain the extract as reported by Anggraini *et al*.[8]. The GLW Payakumbuh and GLW Painan contain total tannin concentration 12.5 and 15.6% dry matter, respectively. The oil palm frond ammoniated and GLW used in experiments were ground to pass through a 1 mm screen. Oil palm frond ammoniated chemical composition was as follows; Organic Matter (OM), 922; Crude Protein (CP), 79; Crude Fibre (CF), 294; NDF, 474 and ADF, 323 g kg^{-1} Dry Matter (DM). Ruminal fluid was obtained from a cannulated steer. The *in vitro* digestibility measurement was conducted according to Tilley and Terry[9]. Fermentation tubes contained of 50 mL of rumen fluid and 200 mL McDougall buffer solution. Substrate for fermentation consists of ammoniated oil palm frond and GLW according to treatments. Three fermentation tubes that did not contain substrate were also incubated and used as blanks. Tubes were incubated for 48 h at a temperature of 39°C in a shaker water bath. After incubation for 48 h, fermentation activity was stopped by immersion in iced water to stop the activities of microbial activity. Tubes were then centrifuged at 1500 rpm for 30 min and the supernatant was removed. Samples residue were oven dried at 60°C for 24 h and stored for later chemical analysis according to AOAC[10], for Dry Matter (DM), Organic Matter (OM), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF). Supernatants were used to analyze the concentration of Total Volatile Fatty Acids (TVFAs), partial VFA and NH_3-N rumen fluid. Population of bacteria and rumen protozoa were counted by Ogimoto and Imai[11] method. Calculating of methane gas production based on VFAs production according to Moss *et al*.[12].

Statistical analysis: Data were analyzed by ANOVA using a block randomized design with subsampling.

Differences among means were tested using Duncan Multiple Range Test (DMRT) according to Steel and Torrie[13].

RESULT AND DISCUSSION

Digestibility of nutrient: Supplementation of several doses and two sources of tannin on rumen digestibility of ammoniated oil palm frond were showed in Table 1. Analysis of variance showed that the treatments were significantly different ($p<0.05$) on DM, OM, NDF and ADF digestibility. The result showed that DM and OM digestibility of ammoniated oil palm frond without GLW (A) was significantly different ($p<0.05$) with treatment B2 but not different with other treatments, however it appears that the addition of GLW tends to give the higher values. This result indicated that supplementation of tannin could increased the nutrient digestibility. This increase was likely due to the role of GLW in reducing methane production, hence energy used more to digest DM and OM. In treatment with GLW supplementation showed that increased doses of tannin source more than 15% was found to decrease nutrient digestibility. This is supported by Jayanegara *et al.*[14] stated that *in vitro* ruminal OM digestibility decreased with increasing dietary tannin levels. On the other side it has also been demonstrated that feed intake, protein and DM digestibility, BW gain, milk yield and wool growth can be affected negatively by tannins as discussed elsewhere[15-17].

Supplementation of GLW on digestibility of NDF and ADF was showed that NDF and ADF digestibility of ammoniated palm frond without GLW (A) were significantly different ($p<0.05$) from palm frond with GLW supplementation. Supplementation GLW increased digestibility of NDF and ADF up to 15% supplementation. Increasing doses of more than 15% reduced NDF and ADF digestibility. This was likely to do the role of tannin in decreasing the degradation of nutrients in the rumen which then may be degraded in the hindgut. Patra[17] stated that *in vitro* studies are useful to screen different tannin products for their effects on methane and identify the most effective ones. Special attention should be given to indicators of rumen fermentation and digestibility, because the supplementation of tannins often decreases digestibility e.g., of Crude Protein (CP), Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) and tends to decrease total VFA production due to ability to interact with other macro-molecules such as protein and carbohydrate.

Supplementation doses 15% GLW Payakumbuh and 10 GLW Painan on ammoniated palm frond (B2 and C1)) was significantly different ($p<0.05$) compared to not GLW supplementation (Treatment A). This indicated that theses doses of GLW could increase DM, OM, NDF and ADF digestibility. Fiber and protein digestibility as well as intake and performance can be affected when tannin concentration is higher than 50 g kg^{-1} feed as reported by Mueller-Harvey[18] which is the reason why, in some situations, the use of tannin binders are proposed to reduce the anti-nutritive effects of tropical forages[19,20].

Microbial Protein Syntesis (MPS): Supplementation of GLW on microbial protein synthesis could be seen in Table 1. Supplementation of GLW were significantly different ($p<0.05$) (compared to control (A and B2). Although, B1, B3, C1, C2 and C3 were not signifficantly different but there was a tendency of higher value of MPS on supplementation of GLW both of Payakumbuh and Painan. This indicated that GLW as tannin source could increased MPS of ammoniated oil palm frond. Higher production of MPS due to availability of NH_3 to growing of rumen bacteria. Production of NH_3-N in rumen was indicative of protein degradation process in the rumen. Doses of GLW more than 15% from Payakumbuh and 10% from Painan reduced the MPS. It was indicated that the tannins in this treatment had negative effect of protein degradation in rumen that showed lower NH_3-N production so the process of protein synthesis by rumen microbes was reduced. Makkar *et al.*[21] reported that microbial protein synthesis was enhanced

Table 1: Effect of treatments on nutrient digestibility and rumen microbial synthesis protein

	Digestibility				
Treatments	DM	OM	NDF	ADF	Microbial protein synthesis (mg mL^{-1})
A	48.45^{b}	51.34^{b}	32.17^{c}	22.73^{c}	138.64^{b}
B1	51.59^{ab}	54.17^{ab}	42.27^{b}	34.23^{b}	144.95^{b}
B2	52.09^{a}	57.30^{a}	52.22^{a}	45.08^{a}	175.10^{c}
B3	50.93^{ab}	53.15^{ab}	47.23^{b}	43.46^{ab}	141.40^{b}
C1	51.08^{ab}	54.16^{ab}	47.96^{b}	43.46^{a}	157.57^{bc}
C2	50.69^{ab}	52.83^{ab}	46.03^{b}	40.41^{b}	147.28^{b}
C3	48.65^{b}	51.04^{b}	46.24^{b}	42.07^{ab}	149.48^{b}
SE	1.12	1.87	0.60	0.56	1.70

Different superscripts in the same row show highly significant effect ($p<0.05$). DM: Dry matter, OM: Organic matter, NDF: Neutral detergent fiber, ADF: Acid detergent fiber

Table 2: Effect of treatments on VFA production in rumen

Treatments	Acetate	Propionate	Butyrate	Valerate+isovalerate+isobutirate	VFA total	Acetate: Propionate ratio
A	43.84^{ab}	18.11	7.61	2.89^{cd}	71.00^{b}	3.98^{a}
B1	46.81^{ab}	23.44	9.06	4.35^{a}	83.70^{ab}	2.70^{c}
B2	58.91^{a}	23.66	9.75	3.76^{ab}	95.78^{a}	3.52^{ab}
B31	31.42^{b}	16.42	5.47	2.62^{cd}	65.94^{b}	3.38^{ab}
C1	39.95^{ab}	21.54	9.07	4.18^{ab}	75.49^{ab}	2.58^{b}
C2	48.08^{ab}	20.13	7.07	3.02^{cd}	79.40^{ab}	3.65^{a}
C3	35.71^{b}	18.13	7.32	2.28^{d}	62.44^{b}	3.40^{b}
SE	6.50	1.70	1.64	0.45	3.50	0.30

Different superscripts in the same row show highly significant effect ($p<0.05$)

in the presence of tannins because tannin is able to reduce the number of protozoa that are predators of rumen bacteria.

Concentration of total VFA and partial VFA: Effect of GLW supplementation on ammoniated oil palm frond to total VFA concentration as shown in Table 2. Table 2 indicated that ammoniated palm frond without GLW (A) had significantly different compared with ammoniated palm frond with GLW supplementation (B2). The VFAs was a major energy source for ruminants. The mechanism whereby tannins decreased total and partial VFA is similar as explained above since VFAs is also an end product of rumen microbial fermentation as discussed by Jayanegara *et al.*[22].

Concentration of partial VFA was affected by feed composition in ransum. Production of acetate, propionate and butyrate depend on carbohydrate fermentation and small part of feed protein fermentation. Treatment supplementation of GLW as tannin source showed increasing propionate production compared to control. Proportion of low propionate caused substrate content low starch and beside bacterial species evolve in rumen as discussed by Russel and Rychlik[23]. This study on B2 treatment, proportion of acetace was higher than control. This is suspected that bacteria which produce acetate better evolve with doses and source GLW that be given. Many things influence VFA composition, one of these is composition of rumen microbial population. Proportion of propionic acid tend to increased on feed that be given tannin supplementation compared to control. This is evidenced with decrease ratio of acetate acid:propionic acid. Wina *et al.*[24] stated that the main influence supplementation of tannin on rumen fermentation was convertion pattern of short chain fatty acid that is increasing proportion of propionate and decreasing ratio of acetate:propionate.

The mean of propionate production on Table 2 showed that the highest mean was found in treatment 15% GLW Payakumbuh (B2) compared to the other treatments. Propionate production was affected by tannin supplementation. In contrast to other substances such as monensin, saponins and essential oils, the effects of tannins on methanogenesis are often not associated with an increased propionate production[14,17]. An inhibition on the growth of cellulolytic bacteria in the presence of tannins is apparently a causal factor of such VFA shift thus lower the acetate production as a major VFA resulted from cellulolytic bacteria fermentation as reported by Jayanegara *et al.*[14]. Although, propionate in general was decreased due to purified tannin additions, the magnitude of decrease was relatively small as compared to acetate decrease. The decrease of acetate to propionate ratio due to purified tannin additions is favourable towards mitigation of methanogenesis since fermentation of glucose to acetate yields H_2, a main substrate of the methane formation and on the contrary, the fermentation of glucose to propionate consumes H_2[25,26]. Decreased acetate production on the treatment (B3 and C3) indicated depression of *in vitro* fermentation, which was in line with the decreased DM and OM degradation as reported by McSweeney *et al.*[4].

Table 2 also showed that butyrate production had no different among treatments but for valerate, iso-valerate and iso-butirate production had significant different among treatments. It is showed that supplementation of tannins till 20% on dry matter not interfered the study of rumen microbes to digest feed carbohydrates.

NH_3-N and pH rumen fluid: Concentration of NH_3-N of ammoniated oil palm frond without GLW (A) was not significant effect ($p>0.05$) as compared with GLW supplementation (Table 3). Supplementation of GLW tends to decreased NH_3-N concentration. Concentration of NH_3-N indicated the feed protein degradation process in rumen. It is well known that tannins complex with proteins, thereby reducing their degradation in the rumen. Decreasing the degradation of CP was reduced for all tannin treatments. This was in agreement with the lower NH_3-N production in more of 10% GLW supplementation because higher tannins in treatments. NH_3-N generated in this study ranged from 8.50-10.93 mM. The results were considered as normal. The optimum range of NH_3-N in the rumen according to Roffler and Satter[27] for maximum microbial growth was 3.75-15.00 mM.

Table 3: Effect of treatments on fermentation (pH, bacteria and protozoa population, methane and NH_3-N production) in the rumen

Treatments	pH	NH_3-N (mM)	Total bacteria (cell mL^{-1})	Total protozoa (cell mL^{-1})	Methane (CH_4) production (mM)
A	6.87	10.93	1.36×10^{9}	11.3×10^{4a}	27.22^{a}
B1	6.80	9.54	1.53×10^{9}	2.3×10^{4c}	23.64^{ab}
B2	6.82	9.36	1.53×10^{9}	1.4×10^{4c}	12.67^{c}
B3	6.75	8.64	1.23×10^{9}	4.8×10^{4b}	13.14^{c}
C1	6.78	9.88	1.46×10^{9}	4.7×10^{4a}	15.13^{c}
C2	6.79	9.54	1.43×10^{9}	9.3×10^{4b}	17.22^{c}
C3	6.82	8.50	1.33×10^{9}	8.8×10^{4a}	21.90^{b}
SE	0.03	0.08	0.03	0.21	2.98

Different superscripts in the same row show highly significant effect ($p<0.05$)

The result showed on Table 3 indicated that GLW supplementation not effects on rumen pH. Rumen pH was relatively stable for all treatments. The range of pH values generated in this study ranged from 6.75-6.87. This value is already adequate to ensure optimal rumen microbial activity, where the normal rumen pH for microbial activity is 6.0-7.0 as reported by Grant and Mertens[28].

Population of bacteria, protozoa and methane gas production: Supplementation of GLW on bacteria, protozoa population and methane production could be seen on Table 3. The population of protozoa in the incubation medium decreased significantly ($p<0.05$) due to supplementation tannins, according to Makkar *et al.*[21] the use of tannins were added to the diet can reduce the population of rumen protozoa. Protozoa population was directly propotional with methane gas production. Methanogen bacteria did symbiosis with protozoa in the rumen. Supplementation of 15% GLW Payakumbuh was significantly different ($p>0.05$) compared to control (A). This was indicated that GLW containing tannins decreased protozoa population. This was in line with the mean value of methane gas production which also decrease (12.67 mM) compared the other treatment. It had also increased bacteria population. Decreasing of protozoa population had an effect on increased population of bacteria because protozoa was predator that prey bacteria to meet the needs of protein. Furthermore, Kurihara *et al.*[29] stated that partial elimination of protozoa in rumen cause increased population of rumen bacteria.

Supplementation 15% GLW Payakumbuh and 10% GLW Painan also could decreased methane gas production till 53 and 45% compare to control, respectively. Tannins are known to decrease protozoa population as reported by Bhatta *et al.*[30] in which part of the methanogens are living together as discussed by Carulla *et al.*[19] and contribute to the lower methane emission. The higher decreasing methane in this study due to tannins in the present study was higher than the previous study of Jayanegara *et al.*[14]. It is may be caused the different source and doses tannins that using. The inhibition of methanogenesis by tannins was probably the result of a suppression of fibre degradation. The mechanisms in which tannins are able to mitigate ruminal methane emissions, according to Travendale *et al.*[31] were indirectly, through reduction in fibre digestion which in turn decreases H2 production as a precursor of methane and directly, through inhibition of the growth or activity of methanogens. Reducing CH_4 production with additional tannins appears to be attributable to a concomitant decline in digestibility of nutrients. The latter is the result of complexes formed by tannins with carbohydrates and proteins and under rumen pH conditions[16,4,6,18].

CONCLUSION

The results of these experiments can be concluded that the supplementation of GLW on ammoniated palm frond *in vitro* can increase degradability, fermentability and reduce methane gas production compared with no supplementation. Supplementation of 15% GLW Payakumbuh and 10% GLW Painan were suitable to be used as the optimal doses in conjunction to the source of tannins but the supplementation of 15% GLW Payakumbuh give the best results on digestibility and reduced methane gas production. Different dose of these two sources tannins was because they had different tannin concentration.

SIGNIFICANCE STATEMENT

- Exploration of tannin compound of local agricultural waste to improve livestock productivity
- Usage of local feed ingredients to increase bioprocess in the rumen thereby increasing the VFA (Main ruminal product) and the microbial population increases in order to stimulate the production of cattle
- Improving the usage of low quality forage in the rumen through a combination of fortification technology using tannin to control microbial growth, so it can be multinutrient feed supplement in livestock rations

- New opportunities for feed industry to produce feed supplement based on agricultural waste to get the feed affordable by the farmers

ACKNOWLEDGMENTS

This study was supported by Professor Research Grant Cluster by BOPTN Andalas University, contract No. 81/UN-16/HKRGB/LPPM/2016. The study would not have been possible without the cooperation of my graduated student and technical assistance of Laboratory of Ruminant Nutrition, Faculty of Animal Science of Andalas University.

REFERENCES

1. Zain, M., J. Rahman, Khasrad and Erpomen, 2016. Supplementation of *Saccharomyces cerevisiae* and *Sapindus rarak* in diet based of Oil Palm Frond (OPF) on nutrient digestibility and daily weight gain of goat. Asian J. Anim. Vet. Adv., 11: 314-318.
2. Suryani, H., M. Zain, R.W.S. Ningrat and N. Jamarun, 2016. Supplementation of Virgin Coconut oil (VC) on *in vitro* fermentability, degradability and methane production of ammoniated palm frond. (In Process).
3. Kreuzer, M. and C.R. Soliva, 2008. Nutrition: Key to methane mitigation in ruminants. Proc. Soc. Nutr. Physiol., 17: 168-171.
4. McSweeney, C.S., B. Palmer, D.M. McNeill and D.O. Krause, 2001. Microbial interactions with tannins: Nutritional consequences for ruminants. Anim. Feed Sci. Technol., 91: 83-93.
5. Jayanegara, A., I. Ikhsan and T. Toharmat, 2013. Assessment of methane estimation from volatile fatty acid stoichiometry in the rumen *in vitro*. J. Indonesian Trop. Anim. Agric., 38: 103-108.
6. 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 Rumin. Res., 49: 241-256.
7. Zain, M., T. Sutardi, Suryahadi and N. Ramli, 2008. Effect of defaunation and supplementation methionine hydroxy analogue and branched chain amino acid in growing sheep diet based on palm press fiber ammoniated. Pak. J. Nutr., 7: 813-816.
8. Anggraini, T., A. Tai, T. Yoshino and T. Itani, 2011. Antioxidative activity and catechin content of four kinds of *Uncaria gambir* extracts from West Sumatra, Indonesia. Afr. J. Biochem. Res., 5: 33-38.
9. Tilley, J.M.A. and R.A. Terry, 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass Forage Sci., 18: 104-111.
10. AOAC., 2007. Official Method of Analysis. 18th Edn., Association of Official Analytical Chemist, Gaithersburg, MD., USA.
11. Ogimoto, K. and S. Imai, 1981. Atlas of Rumen Microbiology. Japan Scientific Societies Press, Tokyo.
12. Moss, A.R., J.P. Jouany and J. Newbold, 2000. Methane production by ruminants: Its contribution to global warming. Annales Zootechnie, 49: 231-253.
13. Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw Hill Book Co., New York, USA., ISBN-13: 9780070609266, Pages: 633.
14. 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. J. Anim. Physiol. Anim. Nutr., 96: 365-375.
15. Kumar, R. and M. Singh, 1984. Tannins: Their adverse role in ruminant nutrition. J. Agric. Food Chem., 32: 447-453.
16. Reed, J.D., 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci., 73: 1516-1528.
17. Patra, A.K., 2010. Meta analyses of effects of phytochemicals on digestibility and rumen fermentation characteristics associated with methanogenesis. J. Sci. Food Agric., 90: 2700-2708.
18. Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric., 86: 2010-2037.
19. Carulla, J.E., M. Kreuzer, A. Machmller and H.D. Hess, 2005. Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Aust. J. Agric. Res., 56: 961-970.
20. McGinn, S.M., Y.H. Chung, K.A. Beauchemin, A.D. Iwaasa and C. Grainger, 2009. Use of corn distillers' dried grains to reduce enteric methane loss from beef cattle. Can. J. Anim. Sci., 89: 409-413.
21. Makkar, H.P.S., M. Blummel and K. Becker, 1995. *In vitro* effects of and interactions between tannins and saponins and fate of tannins in the rumen. J. Sci. Food. Agric., 69: 481-493.
22. Jayanegara, A., N. Togtokhbayar, H.P.S. Makkar and K. Becker, 2009. 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.
23. Russell, J.B. and J.L. Rychlik, 2001. Factors that alter rumen microbial ecology. Science, 292: 1119-1122.
24. Wina, E., S. Muetzel and K. Becker, 2005. The impact of saponins or saponin-containing plant materials on ruminant production-A review. J. Agric. Food Chem., 53: 8093-8105.
25. Morgavi, D.P., E. Forano, C. Martin and C.J. Newbold, 2010. Microbial ecosystem and methanogenesis in ruminants. Animal, 4: 1024-1036.

26. Hristov, A.N., J. Oh, J.L. Firkins, J. Dijkstra and E. Kebreab *et al*., 2013. SPECIAL TOPICS-Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci., 91: 5045-5069.
27. Roffler, R.E. and L.D. Satter, 1975. Relationship between ruminal ammonia and nonprotein nitrogen utilization by ruminants. I. Development of a model for predicting nonprotein nitrogen utilization by cattle. J. Dairy Sci., 58: 1880-1888.
28. Grant, R.J. and D.R. Mertens, 1992. Influence of buffer pH and raw corn starch addition on *in vitro* fiber digestion kinetics. J. Dairy Sci., 75: 2762-2768.
29. Kurihara, Y., T. Takechi and F. Shibata, 1978. Relationship between bacteria and ciliate protozoa in the rumen of sheep fed on a purified diet. J. Agric. Sci., 90: 373-381.
30. Bhatta, R., Y. Uyeno, K. Tajima, A. Takenaka and Y. Yabumoto *et al*., 2009. Difference in the nature of tannins on *in vitro* ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. J. Dairy Sci., 92: 5512-5522.
31. Tavendale, M.H., L.P. Meagher, D. Pacheco, N. Walker, G.T. Attwood and S. Sivakumaran, 2005. Methane production from *in vitro* rumen incubations with *Lotus pedunculatus* and *Medicago sativa* and effects of extractable condensed tannin fractions on methanogenesis. Anim. Feed Sci. Technol., 123-124: 403-419.