Nig. J. Anim. Prod. 2018, 45(1): 325 - 334
©Nigerian Society for Animal Production
Nigerian Journal of Animal Production

Effect of dietary inclusion of cashew nut shell liquid on *in vitro* and *in vivo* protein digestibility and utilization in West African dwarf goats

*Aderinboye, R. Y., Busari, A. A., Olaoye, O. N., Adelusi, O. O., Adebayo, K. O. and Onwuka, C. F. I.

Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria,
**Correspondence: aderinboyery@funaab.edu.ng; +234 903 379 5787*

Abstract

Effect of cashew nut shell liquid (CNSL) in diets for West African dwarf (WAD) goats on protein digestibility and utilization was evaluated in vitro and in vivo. Four diets consisting of Panicum maximum supplemented with concentrate pellets containing 0, 5, 10 and 15 mL/kg of CNSL were used. Panicum and pellets were combined in ratio 70: 30 of required dry matter (DM). For in vitro experiment, 500 mg (n=8 per diet) of diet samples (DM basis) were incubated at 39°C for 48 h following the procedure of Menke and Steingass (1988). Dry matter and crude protein digestibility, ammonia nitrogen (NH_3-N) and bacteria count were determined after 24 and 48 h incubation by recovering feed residues and rumen liquor. For in vivo experiment, twenty-four WAD goats were divided into four groups of six animals each. Each group was fed one of the four diets at 5% body weight (DM basis). Dry matter and crude protein intake, weight gain, protein efficiency ratio, rumen NH_3-N, bacteria count and crude protein digestibility were measured during 98 days of feeding and digestibility trial. Experiments were arranged in a completely randomized design and data analyzed using one way analysis of variance procedure of SAS (1999). Results showed that 5 – 15 mL/kg CNSL in supplemental pellets reduced ($P < 0.05$) protein digestibility in vitro but increased ($P < 0.05$) total-tract protein digestibility in vivo. In vitro rumen NH_3-N decreased ($P < 0.05$) with 5 – 15 ml CNSL inclusion after 24 and 48 h. At 30 and 60 days post-feeding, 10 – 15 mL CNSL reduced ($P < 0.05$) rumen NH_3-N in goats Protein efficiency ratio was higher ($P < 0.05$) with CNSL and goats fed 15 mL CNSL had the highest ($P < 0.05$) protein efficiency ratio. Rumen bacteria population in vitro and in vivo decreased ($P < 0.05$) with 5 – 15 mL CNSL in supplemental pellets. In conclusion, the reduced in vitro protein digestibility with reduced NH_3-Nproduction and bacteria population in vitro and in vivo suggests an inhibitory effect of CNSL on rumen proteolysis. Cashew nut shell liquid in supplemental pellet for WAD goats up to 15 mL/kg therefore inhibited dietary protein breakdown in the rumen with consequent improvement in protein digestibility and utilization at the lower tract.

Keywords: Cashew nut shell liquid, Protein digestibility, Bacteria, Protein efficiency ratio

Introduction

Rumen microorganisms degrade feed nutrients to produce volatile fatty acids and synthesize microbial protein as an energy and protein supply for the ruminant. This fermentation process incurs losses in form of methane and ammonia nitrogen resulting in energy and protein inefficiencies, respectively that may limit production performance and contribute to the release of pollutants to the environment (Calsamiglia *et al*., 2007). Bacteria are the most abundant microorganisms in the rumen, of which 40 percent or more have proteolytic activity (Andrade-Montemayor *et al*., 2009). The proteolytic bacteria ferment dietary proteins into peptides and amino acids which are then further degraded to produce ammonia. Excessive ammonia production in the rumen is a major nutritional

inefficiency in ruminant animals (Eschenlauer *et al*., 2002) and if dietary proteins can be protected from ruminal deamination, ammonia declines and the animal has more amino acids for its nutrition (Russell and Mantovani, 2002). Several strategies have been employed to manipulate the rumen in ways that would reduce losses of dietary nutrients. The use of ionophores has proven effective in reducing ruminal degradation of peptides and amino acids, thereby increasing the flow of protein of dietary origin to the small intestine (McGuffey *et al*., 2001). According to Tedeschi *et al*. (2003), monensinin the diets of ruminants may decrease protein degradation in the rumen and may increase feed protein utilization by an average of 3.5 percentage units. However, ionophores such as monensin are classified as antibiotics (Hersom and Thrift, 2012) and the use of antibiotics has been restricted in animal feeds for fears of appearance of antibiotic residue in animal products and risk to human health (Landers *et al*., 2012; Yang *et al*., 2015 and Beyene, 2016). Recent interests in ruminant nutrition has focused on evaluating other alternatives to antibiotics and the addition of some plant extracts to the rumen have shown inhibitory effect on deamination resulting in lower ammonia nitrogen (Calsamiglia *et al*., 2007).Cashew nut shell liquid (CNSL) is a by-product from the cashew processing industry with reported antimicrobial activities (Watanabe *et al*., 2010 and Danielsson *et al*., 2014). This study was carried out to evaluate the effect of cashew nut shell liquid onprotein digestibility and utilization*in vitro* and *in vivo* in West African dwarf goats.

Materials and methods

Experimental site

This study was carried out at the Animal nutrition laboratory and the small ruminant research farm of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta. The region is located in the savannah vegetation zone of Southwest Nigeriaand lies between latitude 7°5.5'- 7°8'N and longitude 3°11.2' - 3°23.5'E. The annual temperature and humidity are 34.7°C and 82%, respectively.

Dietary treatments

In this study, *Panicum maximum* was supplemented with concentrate pellets containing CNSL at varying inclusion levels of 0, 5, 10 and 15 mL/kg to make four different dietary treatments. Diet 1 (control diet) included *Panicum maximum* supplemented with concentrate pellet containing 0 ml CNSL, Diet 2 included *Panicum maximum* supplemented with concentrate pellet containing 5 ml CNSL, Diet 3 included *Panicum maximum* supplemented with concentrate pellet containing 10 ml CNSL and Diet 4 included *Panicum maximum* supplemented with concentrate pellet containing 15 ml CNSL. For *in vitro* and *in vivo* studies, *Panicum maximum* was supplemented with the concentrate pellets in the ratio 70: 30, respectively of the required dry matter. The *Panicum maximum* grass used was cut after 6 weeks of growth at 15 cm above ground level from an established pasture. The concentrate supplement was compounded using wheat offal, rice bran, groundnut cake, brewers' dried grain, cassava flour, bone meal and salt as ingredients. The ingredients were properly mixed to ensure homogeneity. Thereafter, the complete concentrate mixture was incorporated with cashew nut shell liquid at the varying levels of inclusion. Solvent-extracted cashew nut shell liquid with a density of 0.95 was used in this study. The concentrate mixture according to the different levels of CNSL inclusion was thoroughly mixed and then

processed into pellets of 9 mm diameter size to give the four different concentrate pellets. Aliquot samples of *Panicum maximum* and the different concentrate pellets were collected and oven-dried at 60°C to constant weight for determination of dry matter. The oven-dried samples were the milled for subsequent determination of proximate, fibre and *in vitro*analysis.

In vitro digestibility measurement

Oven-dried samples of *Panicum maximum* and the four concentrate pellets were milled through a 1-mm sieve. Approximately 500 mg (n = 8 per dietary treatment) of each diet sample according to the specified ratio of *Panicum maximum* and pellets in the dietary treatments were weighed into small dacron filter bags of known weights, tied-up and inserted into 100 mL glass syringes and incubated *in vitro* following the procedure of Menke and Steingass (1988). A medium (mL) containing 400 distilled water, 0.1 micro-element solution, 200 buffer solution, 200 macro-element solution, 1 resazurin and 40 reduction solution added in this orderwas mixed with rumen liquor in ratio 2:1 (v/v), respectively. The rumen liquor was collected by suction method (Babayemi and Bamikole, 2006) prior to morning feeding from three West African dwarf goats fed with grass (60%) and concentrate (40%) diet. The rumen liquoron collection was immediately transferred to the laboratory in a pre-warmed thermo-flask at 39°C. It was then strained through a four-layered cheese cloth prior to mixing with the medium. Thirty milliliters (30 ml) of the medium-rumen liquor mixture was introduced into each syringe and incubated at 39±0.5°C for 48h. Two empty Dacron bags were inserted into separate syringes containing 30 mL of medium-rumen liquor mixture and included in the run to serve as blanks. Dry matter and crude protein digestibility were determined at 24 and 48 h after incubation by recovering the residues. Digestibility of dry matter and crude protein were calculated as difference in sample dry matter and crude protein content, respectively before and after incubation with corrections for deposits contributed from the digestion medium made with the blanks.

In vitro ammonia nitrogen determination and bacteria count

Samples of post-incubation digestion liquid were recovered from each syringe for ammonia nitrogen determination and bacterial count. Approximately 10 mL of post-incubation rumen liquor was sampled for ammonia nitrogen determination at the end of 24 and 48 h while about 5 ml was collected for bacteria analysis at the end of 24 h.

In vivo protein utilization and digestibility measurement

Twenty-four growing West African dwarf goats (8 ± 2 kg initial body weight, below 12 months of age) were used for this study. The animals were allowed an adaptation period of one month prior to commencement of the study. After the adaptation period, animals were weighed individually and then divided into four groups of six animals each on weight equalization basis. Each group of animals was allotted to one of the four dietary treatments. Animals were fed at 5% of their body weight on dry matter basis. *Panicum maximum* was fed at 70% of individual animals' daily dry matter requirement while the supplemental pellet made up the remaining 30%. Animals were fed for a period of 84 days during which dry matter intake was estimated on daily basis and weight changes monitored weekly. Protein intake was determined from the daily dry matter intake in order to determine protein efficiency ratio which was calculated by

dividing the weight gain (g/day) in animals by the protein intake (g/day). At the end of the 84 days of feeding, animals were transferred into metabolic crates with provision for separate collection of faeces and urine. Total faeces voided from individual animals were collected and weighed daily during a seven-day collection period which followed a 5-day adjustment period. About 5% of total faeces voided per day from each animal were subsampled and oven-dried at 65°C for 48 h until constant weights for dry matter determination. The faeces subsamples for the seven days were then pooled by animal and stored for subsequent analysisof crude protein content. Digestibility of dry matter and crude protein were calculated using the formula for total collection technique (Khan *et al*. 2003).

In vivo ammonia nitrogen determination and bacteria count

On days 30 and 60 of the feeding period, samples of rumen liquor were collected three hours post-feeding from individual animals via suction method and immediately filtered through four-layered cheese cloth. Approximately 10 mL of the filtrate was used for ammonia nitrogen determination. About 5 mL portion of unfiltered rumen liquorwhich was thoroughly mixed to ensure homogeneity in the liquid and solid phases was sub-sampled on day 60 at three hours post-feeding for bacteria count.

Laboratory analyses

The proximate composition of feed and faeces samples were determined by the methods of A. O. A. C. (2000) with crude protein content calculated from the nitrogen (N) content value as N×6.25. Ammonia nitrogen in *in vitro* and *in vivo* rumen liquor samples were analyzed using the micro-kjeldahl method (A. O. A. C., 2000). The conventional roll-tube technique (Hungate, 1969) was used for culturing and isolation of bacteria from rumen fluid samples. Cultured bacteria was then identified according to Cowan and Steel (1993) and total bacteria count was done according to Baker and Breach (1995) method of total viable counting.

Statistical analysis

Data collected were subjected to one-way analysis of variance using the analysis of variance procedure of SAS (1999). Significant differences between means were compared at 5% probability level using the Duncan multiple range test of SAS (1999). The statistical model used was $Yij = \mu + Ti + \epsilon ij$ where Yij is the observation. μ is the population mean, Ti is the effect of CNSL inclusion (I = 1 – 4) in the diet and ϵij is the residual error.

Results

The nutrient composition of the experimental diets as presented in Table 1. *Panicum maximum* had crude protein content of 90 g/kg DM while the crude protein content of the concentrate pellets ranged between 140 and 142.1 g/kg DM. The inclusion of CNSL in the concentrate pellets did not (P>0.05) alter the protein content of the diet. Ether extract and metabolizable energy contents were however, higher with increasing levels of CNSL in the pellet diets.

The *in vitro* protein digestibility, ammonia production and bacteria population in response to dietary inclusion of CNSL is presented in Table 2. At 24 and 48 h post-incubation, CNSL caused a significant ($P < 0.05$) reduction in protein digestibility at 5, 10 and 15 mL of inclusion relative to the control. At 24 h, it was observed that higher doses of 10 -15 mL did not (P>0.05) vary in their effect compared with 5 ml inclusion level but at 48 h, there was a further reduction in protein digestibility at 10 – 15

Table 1: Gross and nutrient composition (g/kg) of experimental diets

	Concentrate pellet containing varying levels of cashew nut shell liquid					
Gross composition	0 ml/kg	5 ml/kg	10 ml/kg	15 ml/kg		
Wheat offal	40	40	40	40		
Rice bran	14	14	14	14		
Soya bean meal	5	5	5	5		
Brewers' dried grain	18	18	18	18		
Cassava flour	20	20	20	20		
Bone meal	2	2	2	2		
Salt	1	1	1	1		
Cashew nut shell liquid[1]	-	+	++	+++		
Total	**1000 g**	**1000 g**	**1000 g**	**1000 g**		
Nutrient composition[2]					**SEM**	*Panicum maximum*
Dry matter (as fed)	958.0	965.2	967.0	963.4	3.9	407.0
Crude protein	140.2	141.1	140.0	142.1	2.2	90.0
Ether extract	83.8	106.2	111.5	115.2	4.2	32.49
Organic matter	893.0	894.5	894.0	895.0	1,5	928.8
Neutral detergent fibre	471.0	468.8	465.3	464.1	1.8	485.6
Acid detergent fibre	203.0	198.2	197.3	190.7	2.4	309.2
Acid detergent lignin	91.0	92.4	91.3	92.8	1.5	100.5
Metabolizable energy (kcal/kg)	3194[b]	3296[a]	3318[a]	3344[a]	19.19	2330

[a,b]Mean within the same row with different superscript are significantly different (P < 0.05)
SEM: standard error of mean; [1]Cashew nut shell liquid included as feed additive to a complete concentrate diet at 0 ml/kg (-), 5 ml/kg (+), 10 ml/kg (++) and 15 ml/kg (+++); [2]Nutrient composition in g/kg dry matter

mL inclusion relative to the digestibility values at 5 mL inclusion. *In vitro* ammonia production reduced significantly (P < 0.05) with inclusion of 5 – 15 mL of CNSL at 24 and 48 h post-incubation. At both incubation hours, diets without CNSL produced the highest (P < 0.05) concentration of ammonia while diets containing 15 ml CNSL produced the lowest (P < 0.05) concentration of ammonia. When bacteria population was determined at 24 h post incubation period, there was a decreased (P < 0.05) in bacteria population in rumen fluid with 5 – 15 mL of CNSL inclusion relative to the control.

Table 2: *In vitro* microbial protein degradation, ammonia production and bacteria population in response to diets containing cashew nut shell liquid

	Levels of dietary cashew nut shell liquid inclusion				
Parameter	**0 mg/kg DM**	**5 mg/kg DM**	**10 mg/kg DM**	**15 mg/kg DM**	**SEM**
In vitro protein degradation, %:					
24 h post incubation	64.67[a]	60.61[b]	58.89[b]	58.80[b]	0.71
48 h post incubation	73.39[a]	70.66[b]	67.74[c]	65.83[c]	0.53
In vitro ammonia production, mg/100 mL:					
24 h post incubation	20.83[a]	16.53[b]	14.92[bc]	11.88[c]	1.00
48 h post incubation	25.90[a]	19.40[b]	17.33[bc]	14.17[c]	1.32
Bacteria population (CFU/ml)[1]	2.16[a]	1.22[b]	1.18[b]	1.05[b]	0.14

[a,b,c]Mean within the same row with different superscript are significantly different (P< 0.05); SEM: standard error of mean; [1]Bacteria population was determined at 24 h post incubation

Table 3 shows the protein digestibility, protein efficiency ratio, rumen ammonia nitrogen concentration and bacteria population in West African dwarf goats fed supplemental pellets containing varying levels of CNSL. Protein digestibility was higher ($P < 0.05$) in goats fed pellets containing 10 – 15 mL of CNSL inclusion relative to the digestibility value in goats fed the control diet. Protein efficiency ratio was higher ($P < 0.05$) with increasing levels of CNSL with the highest ($P < 0.05$) ratio observed in goats fed pellets containing 15 ml CNSL. Similar to the *in vitro* results, the inclusion of CNSL in the diet of goats caused a reduction ($P < 0.05$) in the concentration of ammonia nitrogen in the rumen. At 30 days of feeding, rumen ammonia nitrogen reduced ($P < 0.05$) from 30.38 mg/100 mL to between 13.27 and 25.11 mg/100 mL while at 60 days of feeding, concentration reduced ($P < 0.05$) from 38.92 mg/100 mL to between 18.06 and 26.26 mg/100 mL. Bacteria count in the rumen of goats revealed a decrease ($P < 0.05$) when pellets containing CNSL were fed.

Table 3: *In vivo* protein utilization, digestibility, rumen ammonia production and bacteria count in West African dwarf goats in response to dietary cashew nut shell liquid inclusion

	Levels of dietary cashew nut shell liquid inclusion				
Parameter	**0 mg/kg DM**	**5 mg/kg DM**	**10 mg/kg DM**	**15 mg/kg DM**	**SEM**
Protein intake, g/d	61.67^{a}	54.95^{b}	51.00^{c}	49.42^{d}	0.30
Animal weight gain, g/d	23.4^{c}	29.2^{b}	30.1^{ab}	31.0^{a}	0.62
Protein efficiency ratio	0.38^{c}	0.53^{b}	0.60^{b}	0.64^{a}	0.24
Protein digestibility, %	67.38^{b}	68.76^{ab}	69.39^{a}	70.12^{a}	0.33
In vivo rumen ammonia production, mg/100 mL[1]:					
30 days post feeding	30.38^{a}	25.11^{ab}	16.08^{bc}	13.27^{c}	2.82
60 days post feeding	38.92^{a}	26.26^{b}	20.11^{b}	18.06^{b}	3.20
Bacteria population (CFU/ml)[2]	2.15^{a}	1.80^{b}	1.68^{b}	1.61^{b}	0.07

^{a,b,c}Mean within the same row with different superscript are significantly different ($P< 0.05$); SEM: standard error of mean; [1]1 mg/100 mL is equivalent to 1 mg/dL or 10 mg/L; [2]Bacteria population determined in the rumen at 3 h after feeding on day 60

Discussion

The similarity in crude protein content of the pellet diets implies that CNSL inclusion at 5- 15 mL/kg inclusion did not alter the protein content of the diet and would therefore not bind dietary proteins at these inclusion levels. The variation in ether extract however was attributed to extra contributions of oil from CNSL which was also thought to consequently increase the metabolizable energy content of the pellet diets. The reduction effect of CNSL on protein digestibility *in vitro* suggests that CNSL reduced substrate colonization by proteolytic microbes thereby reducing degradation. Although studies with cashew nut shell liquid with goats are scarce, certain plant oils have similarly been associated with reduction of proteins digestion in the rumen (McIntosh *et al*., 2003; Salamatazar *et al*., 2011). According to Hart *et al.* (2008), the main effects of plant oils in the rumen include reduced protein and starch degradation and an inhibition of amino acid degradation. One of the modes of action suggested by these authors for plant oils was on inhibition of hyper-ammonia producing bacteria.The similarity in the effect of 5 – 15 mL CNSL at 24 h and the difference in the effect of 10 -15 mL CNSL inclusion at 48 h compared with 5 mL inclusion level indicates that the effect of

CNSL on protein digestibility is dependent on the level of inclusion and retention time in the rumen. A longer retention time would possibly be required at higher inclusion levels.

The reduction in *in vitro* ammonia production at 24 and 48 h with 5 – 15 mL of CNSL inclusion was assumed as the consequent effect of reduced *in vitro* protein digestibility. The similar effect of CNSL observed on ammonia production *in vivo* confirms CNSL to have an inhibitory effect on rumen protein degradation. The reduced ammonia production suggests an increase in the supply of dietary protein to the lower tract and decreased supply of ammonia for microbial growth. The increased *in vivo* protein digestibility and improved protein efficiency ratio with CNSL substantiates possibility for increased flow of proteins to the lower tract allowing for improved utilization of dietary proteins. On the other hand however, the reduction in bacteria population observed in our study *in vitro* and *in vivo* could confirm a reduction in the availability of ammonia for microbial synthesis. According to Leng (1991), on a forage based diet, rumen ammonia level should be above 200 mg nitrogen/L. Kanjanapruthipong and Leng (1998) reported the level of rumen ammonia nitrogen required for optimum microbial synthesis to be between 50 – 238 mg/L while a minimum of 5 mg/dL (50 mg/L) of NH_3-N concentration in the rumen is considered adequate for microbial protein production (NRC., 1985). Concentration of ammonia in the rumen with and without CNSL in this study was well above 50 mg/L indicating its adequacy for optimum microbial synthesis. The reduced bacteria population with inclusion of CNSL at 5 – 15 mL could therefore, not pose any detrimental effect on rumen bacteria survival and normal rumen function but only probably saved excessive utilization of dietary protein in the rumen. Similar to our results, when CNSL was administered to non-lactating (Shinkai *et al*., 2012) and lactating cows (Branco *et al*., 2015), there was a reported decrease in bacteria population. The reduction in bacterial population in the rumen could be due to the antibacterial activity of the active compounds in CNSL. Cashew nut shell liquid contains some active compounds which have been reported to exhibit antibacterial properties (Watanabe *et al*., 2010; Parasa *et al*., 2011 and Danielsson *et al*., 2014). Although bacteria species related to proteolysis were not identified in this study, the reduction in bacteria population, the concomitant reduction in protein degradation *in vitro* and reduced *in vitro* and *in vivo* ammonia production could be an evidence of decreased proteolytic bacteria.

Conclusion

The study showed that cashew nut shell liquid inclusion in supplemental pellets for West African dwarf goats at 5 – 15 mL/kg reduced *in vitro* protein digestibility, *in vitro* and *in vivo* ammonia nitrogen production and rumen bacteria population. However, *in vivo* protein digestibility at the lower tract and protein efficiency ratio was higher with CNSL inclusion. The effect of CNSL in supplemental pellets for goats reflects inhibition of protein breakdown in the rumen and possible increase in the flow of un-degradable proteins to the lower tract for better utilization particularly at 15 mL/kg of inclusion. The reduction effect of CNSL on rumen ammonia production is however, not likely threaten the survival of bacteria in the rumen at the level of inclusion as rumen ammonia concentrations were well above the minimum level for microbial growth.

Acknowledgement
The authors acknowledge the Tertiary Education Trust Fund (TETFund), Nigeria for the funding of this study under the 2013/2014 TETFund Institution-based Research Intervention Allocation for a project titled, "Utilization of cashew nut shell liquid as a dietary strategy to manipulate rumen fermentation for improved nutritional performance of ruminants".

References

Andrade-Montemayor, H., García, G. T. and Kawas, J. 2009. Ruminal fermentation modification of protein and carbohydrate by means of roasted and estimation of microbial protein synthesis. *Revista Brasileira de Zootecnia*, *38*(spe): 277 - 291. https://dx.doi.org/10.1590/S1516-35982009001300028

A. O. A. C., 2000. Official methods of analysis of AOAC. *International 17th edition; Gaithersburg, MD, USA.*

Babayemi, O. J. and Bankole, M. A. 2006. Effects of *Tephrosia candida* DC Leaf and its Mixtures with Guinea Grass on *in vitro* Fermentation Changes as Feed for Ruminants in Nigeria. *Pakistan Journal of Nutrition 5 (1): 14 – 18.*

Baker, F. J. and Breach, M. R. 1995. Total viable counting. Medical microbiology techniques. Butterworth Publishers, 420 - 424.

Beyene, T. 2016. Veterinary drug residues in food-animal products: its risk factors and potential effects on public health. *Journal of Veterinary Science and Technology* 7: 285. DOI:10.4172/2157-7579.1000285

Branco, A. F., • Giallongo, F., • Frederick, T., Weeks, H., Oh, J. and Hristov, A. N., 2015. Effect of technical cashew nut shell liquid on rumen methane emission and lactation performance of dairy cows. *Journal of Dairy Science 98 (6): 4030–4040.*

Calsamiglia, S., Busquet, M., Cardozo, P. W., Castillejos, L. and Ferret, A. 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. *Journal of Dairy Science* 90: 2580-2595.

Cowan, S. T. and Steel, K. J. 1993. Enterobacteriacea. In: G. I. Barrow, R. K. A. Felthan, (Eds). Manual for identification of medical bacteria (3rd edition), Cambridge University press, United Kingdom, 213 - 218.

Danielsson, R., Werner-Omazic, A., Ramin, M., Schnürer, A., Griinari, M., Dicksved, J. and Bertilsson, J. 2014. Effects on enteric methane production and bacterial and archaeal communities by the addition of cashew nut shell extract or glycerol: An *in vitro* evaluation. *Journal of Dairy Science 97: 5729–5741.* http://dx.doi.org/10.3168/jds.2014-7929

Eschenlauer, S. C. P., McKain, N., Walker, N. D., , N. R., Newbold, C. J. and Wallace, R. J. 2002. Ammonia production by ruminal microorganisms and enumeration, isolation, and characterization of bacteria capable of growth on peptides and amino acids from the sheep rumen. *Applied and Environmental Microbiology 68(10): 4925–4931.* DOI: 10.1128/AEM.68.10.4925-4931.2002

Hart, K. J., Yáñez-Ruiz, D. R., Duval, S. M., McEwan, N. R. and Newbold, C. J. 2008. Plant extracts to manipulate rumen fermentation. *Animal Feed Science and Technology 147 (1–3): 8–35* https://doi.org/10.1016/j.anifeedsci.2007.09.007

Hersom, M. and Thrift, T. 2012. Application of Ionophores in Cattle Diets. University of Florida/Institute of Food and Agricultural Sciences Extension services. http://edis.ifas.ufl.edu/pdffiles/AN/AN28500.pdf

Hungate, R. E. 1969. A roll tube method for cultivation of strict anaerobes. In: Norris, J. R., Ribbons, D. W. (eds.): Methods in microbiology. Academic Press, London and New York, 3B, 117 -132.

Kamalak, A., Canbolat, O., Gurbuz, Y. and Ozay, O. 2005. Protected Protein and Amino Acids in Ruminant Nutrition**.** *Journal of Science and Engineering 8(2): 84 - 87*

Kanjanapruthipong, J. and Leng, R. A. 1998. The effects of dietary urea on microbial population in the rumen of sheep. *Asian Journal of Animal Science 11 (6): 661 – 672.*

Khan, M. A., Mahr-Un-Nisa and Sarwar, M., 2003. Techniques measuring digestibility for the nutritional evaluation of feeds. *International Journal of Agriculture and Biology, 5, 91–94*

Landers, T. F., Cohen, B., Wittum, T. E. and Larson, E. L. 2012. A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. Public Health Report 127(1): 4–22. DOI: 10.1177/003335491212700103

Leng, R. A. 1991. Application of biotechnology to nutrition of animals in developing countries. FAO Animal Production and Health Paper. Food and Agriculture Organization of the United Nations, Romehttp://www.fao.org/docrep/004/T0423E/T0423E03.htm#ch3.3

McGuffey, R. K., Richardson, L. F. and Wilkinson, J. I. D. 2001. Ionophores for dairy cattle: current status and future outlook. *Journal of Dairy Science* 84 (supplement): E194 - E203 DOI: http://dx.doi.org/10.3168/jds.S0022-0302(01)70218-4

McIntosh, F. M., Williams, P., Losa, R., Wallace, R. J., Beever, D. A. and Newbold, C. J. 2003. Effects of essential oils on ruminal microorganisms and their protein metabolism. *Applied and Environmental Microbiology 69 (8): 5011–5014*

Menke, K. H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Animal Research and Development 28: 7–55.*

NRC. 1985. Ruminant Nitrogen Usage. National Academy Press, Washington, D. C., USA. p. 138.

Parasa, L. S., Tumati, S. R., Kumar, L. C. A., Chigurupati, S. P. and Rao, G. S. 2011. In vitro antimicrobial activity of cashew (*Anacaardium occidentale, L.*) nuts shell liquid against methicillin resistant *Staphylococcus aureus* clinical isolates. *International Journal of*

Pharmacy and Pharmaceutical Sciences 3 (4): 436 - 440

Russell, J. B and Mantovani, H. C. 2002. The bacteriocins of ruminal bacteria and their potential as an alternative to antibiotics. *Journal of Molecular Microbiology and Biotechnology 4(4): 347–355.*

Salamatazar, M., Salamatdoust-nobar, R., Sis, N. M., Shabestari, A. N., Najafyar, S., Khodaparast, B., Amini, P. N. and Rezayi, N. 2011. Effects of Thyme methanolic Extract on Ruminal Protein Degradation of Soybean Meal using Nylon Bag Technique. *Current Research Journal of Biological Sciences 3(6): 612-615, 2011*

SAS, 1999. Statistical Analysis Systems user's guide (Statistics, Version.8). SAS Institute Inc, Cary.

Shinkai, T., Enishi, O., Mitsumori, M., Higuchi, K., Kobayashi, Y., Takenaka, A., Nagashima, K., Mochizuki, M. and Kobayashi, Y. 2012. Mitigation of methane production from cattle by feeding cashew nut shell liquid. *Journal of Dairy Science 95: 5308-5316*

Tedeschi, L. O., . and Tylutki, T. P. 2003. **Potential environmental benefits of ionophores in ruminant diets.** *Journal of Environmental Quality 32(5): 591-602.*

Watanabe, Y., Suzuki, R., Koike, S., Nagashima, K., Mochizuki, M., Forster, R. J. and Kobayashi, Y. 2010. In vitro evaluation of cashew nut shell liquid as a methane-inhibiting and propionate-enhancing agent for ruminants. *Journal of Dairy Science*93: 5258-5267.

Yang, C., Chowdhury, M. A. K., Hou, Y. and Gong, J. 2015. Phytogenic compounds as alternatives to in-feed antibiotics: potentials and challenges in application. *Pathogens* 4, 137–156. DOI: 10.3390/pathogens4010137.

Received: 14th November, 2017
Accepted: 20th February, 2018