African Journal of Agricultural Research Vol. 7(13), pp. 1997-2003, 5 April, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.1347
ISSN 1991-637X © 2012 Academic Journals

Full Length Research Paper

Effect of eucalyptus (*Camaldulensis*) leaf meal powder on rumen fermentation characteristics in cattle fed on rice straw

N. S. Manh[1], M. Wanapat[2]*, S. Uriyapongson[2], P. Khejornsart[2] and V. Chanthakhoun[2]

[1]National Institute Animal Science, Ha Noi, Vietnam.
[2]Department of Animal Science, Faculty of Agriculture, Tropical Feed Resources Research and Development Center (TROFREC), Khon Kaen University, Khon Kaen 40002, Thailand.

Accepted 7 March, 2012

Three, Holstein Friesian, non-lactation crossbred dairy cows were used to evaluate the effect of Eucalyptus (*Camaldulensis*) leaf meal powder (EUCAP) supplementation on feed intake, digestibility and rumen fermentation. The animals were randomly assigned according to a 3 × 3 Latin square design using three levels of EUCAP supplementation (0, 100 and 200 g/hd/d) and offered rice straw *ad libitum*, together with concentrate at 0.5% body weight. The results revealed that voluntary feed intake (kg/hd/d) was significantly decreased when EUCAP was supplemented at 200 g/hd/d. Digestibility coefficient (%) of DM, OM, CP, NDF and ADF were similar among treatments. Ruminal temperature and pH were not affected by EUCAP supplementation. However, NH_3-N and BUN concentrations were decreased when supplementation of EUCAP at 200 g/hd/d. Ruminal fungal zoospores were not significantly different among treatments, while protozoa, bacteria population by direct counts were significantly reduced with increasing supplementation levels of EUCAP. In addition, viable total bacteria, proteolytic bacteria and cellulolytic bacteria were decreased when EUCAP were supplemented at 100 and 200 g/hd/d while amylolytic bacteria was not different among treatments (P>0.05). Furthermore, total volatile fatty acid concentrations, proportion of acetate, acetate to propionate ratio were reduced with increasing level of supplementation, while, proportion of propionate increased. Methane production was reduced in supplemented treatments at 100 g/hd/d. Supplementation of EUCAP at 100 g/day for ruminants could be on alternative feed enhancer which reduces rumen methane gas production in cattle, while nutrient digestibilities were unchanged.

Key words: Eucalyptus, rumen fermentation, rice straw, rumen manipulation, cows.

INTRODUCTION

In livestock production systems, antibiotics are commonly fed to animals to prevent diseases and metabolic disorders, as well as to improve feed efficiency. A number of chemical feed additives such as 'antibiotics', 'ionophores', 'methane inhibitors' and 'defaunating' agents have been introduced in the ruminant nutrition to improve rumen fermentation with an aim to enhance the efficiency of ruminant production (Patra and Saxena, 2009). However, most of these additives are not used routinely because of the toxicity problems to the host animals and residues of these chemicals in the animal derived foods and bacterial resistance to antibiotics as results of increased use in the feeds. Consequently, considerable effort has been devoted towards developing alternatives to antibiotics. Currently, numerous studies have attempted to exploit these plant secondary metabolites as natural feed additives to improve the efficiency of rumen fermentation such as enhancing protein metabolism, decreasing methane production (McIntosh et al., 2003). Recently, many reviews have been published on plant extracts such as saponins, tannins and essential oils (EOs) as rumen modifiers

*Corresponding author. E-mail: metha@kku.ac.th.

Table 1. Ingredient and chemical composition of experimental diets.

Item	Concentrates	Rice straw	EUCAP
Ingredients (%)			
Cassava chip	61		
Rice bran	10		
Coconut meal	12		
Palm meal	12		
Urea	3		
Molasses	0.5		
Sulfur	0.5		
Premix mineral	0.5		
Salt	0.5		
Chemical composition, % of DM			
DM	93.7	95.6	93.7
OM	93.2	87.5	94.5
Ash	6.8	12.5	5.5
CP	14.1	3.2	9.5
NDF	24.9	76.2	34.3
ADF	15.6	47.2	22.0

DM = Dry matter, CP = crude protein, EE = ether extract, OM = organic matter, NDF = neutral detergent fiber, ADF = acid detergent fiber.

(Calsamiglia et al., 2007). Plant derived EOs may be a useful means to improve efficiency of nutrient utilization in ruminants and reduce the impact of their production on the environment (Benchaar et al., 2008). The Eucalyptus is a tall evergreen tree native with many species available and these can be found in many parts of the world, which produce a wide variety of oils.

According to Akin et al. (2010) who reported that the major components of *Eucalyptus camaldulensis* were ethanone (13.73%), eucalyptol (25.36%), caryophyllene (11.55%). Eucalyptol (1.8- cineole) is the main active ingredient in eucalyptus oil (EuO) from *E. Camaldulensis* (Sallam et al., 2009). Recently, the *in vitro* studies have demonstrated that EOs or their components have the potential to favorably alter rumen metabolism (Busquet et al., 2006). However, there are few experimental data on effects of the Eucalyptus on rumen digestion and rumen ecology. Therefore, the objective of this study was to investigate Eucalyptus (*Camaldulensis*) leaf powder (EUCAP) on rumen fermentation efficiency and rumen ecology.

MATERIALS AND METHODS

Animals, treatments and experimental design

Three Holstein Friesian non- lactating crossbred dairy cows with average live weight of 380 ± 15 kg were randomly assigned according to a 3 × 3 Latin square design to investigate effect of EUCAP supplementation on feed intake, digestibility and rumen ecology. The dietary treatments were supplemented at: 0 (control), 100 and 200 g/hd/d of EUCAP, respectively. Animals were kept in individual pens (4 × 6 m) with free access to mineral block and water. The experiment were conducted for three periods, and each period lasted for 21 days, all animals were fed on respective diets with concentrate supplementation (14.1% CP) at 0.5% of BW (DM), twice daily at 07.00 and 16.00 h, and rice straw *ad libitum*. The EUCAP were collected from fresh leave and were sun-dried and ground into powder form. Ground EUCAP was mixed with the concentrate before feeding according to respective treatments. Chemical composition of concentrates, rice straw and EUCAP are shown in Table 1.

Sample collection and chemical analysis

Roughage, concentrate and refusals were randomly collected for chemical composition analysis. Fecal samples were taken by rectal sampling from each individual cattle during the last 7 days of each period. The composited samples were dried at 60°C and ground (1-mm screen using Cyclotech Mill, Teactor, Sweden) and then analyzed for DM, ether extract, ash, CP content (AOAC, 1990), NDF, ADF (Van Soest, 1994), and acid-insoluble ash (AIA) by Van Keulen and Young (1977). Rumen fluid and blood samples were collected at 0, 2, 4 and 6 h post-feeding in the last day of each period. Approximately 200 ml of rumen fluid was taken by stomach tube connected with vacuum pump at each time at the end of each period. Rumen fluid was immediately measured in terms of pH and temperature using a portable pH and temperature meter (HANNA instrument HI 8424 microcomputer, Singapore). Rumen fluid samples were filtered through four layers of cheesecloth. Rumen fluid was divided into three portions: the first portion was used for VFA and NH_3–N analysis where 5 ml of H_2SO_4 solution (1 M) was

Table 2. Effect of EUCAP supplementation on feed intakes and nutrient digestibility.

Items	EUCAP supplementation, g/hd/d			SEM	Contrast	
	0	100	200		L	Q
Rice straw DM intake						
kg/day	5.5[a]	5.0[ab]	4.8[b]	0.29	*	NS
% BW	1.6[a]	1.4[ab]	1.2[b]	0.12	*	NS
Total DM intake						
kg/day	7.4[a]	6.9[ab]	6.7[b]	0.26	*	NS
% BW	2.1[a]	1.9[ab]	1.7[b]	0.15	*	NS
Digestion coefficient, %						
DM	63.9	61.3	59.8	2.35	NS	NS
OM	67.3	65.6	65.0	1.97	NS	NS
CP	63.8	59.1	56.6	3.95	NS	NS
NDF	54.5	52.2	51.6	1.69	NS	NS
ADF	61.8	58.9	58.2	2.41	NS	NS

[a, b] Values within the row of a different superscript are significantly different (P<0.05), * = P < 0.05, ns = non-significant (P>0.05), L = linear, Q = quadratic.

added to 45 ml of rumen fluid. The mixture was centrifuged at 16,000 ×g for 15 min and supernatant was stored at –20°C prior to NH_3–N analysis was done by using the micro-Kjeldahl methods (AOAC, 1990). Second portion was fixed with 10% formalin solution in sterilized 0.9% saline solution for total direct count of protozoa, and fungal zoospores using the methods of Galyean (1989) based on the use of a hemacytometer (Hausser Scientific, Horsham, PA). Third portion was taken to study cultured groups of viable bacteria using roll-tube technique (Hungate, 1969) for identifying rumen bacterial group (cellulolytic, proteolytic, amylolytic and total viable bacteria).

The blood sample (about 10 ml) drawn from the jugular vein into EDTA containing tubes was separated by centrifugation at 500 ×g for 10 min at 4°C to sequent blood which was stored at -20°C until analysis of blood urea N according to the method of Crocker (1967).

Statistical analysis

Statistical analyses were performed using the GLM procedure of SAS (1998). Difference between treatment means were determined by Duncan's new multiple range test (DMRT) (Steel and Torrie, 1980) with P<0.05 were accepted as representing statistically significant differences.

RESULTS

Feed intakes and digestion coefficients

Intake of roughage, concentrate, total DMI and digestion coefficient are presented in Table 2. Total intakes of three treatments were reduced by EUCAP supplementation. However, digestion coefficient (%) of DM, OM, CP, NDF and NDF were not significantly different among treatments.

Rumen fermentation characteristics

Rumen ecology parameters for pH, temperature, NH_3-N and VFAs are shown in Table 3. The rumen temperatures were quite stable at 37.9 to 38.7°C. Rumen pH was in the range from 6.6 to 7.0. Ruminal NH_3-N concentrations were significantly decreased by treatments at each hour of sampling. This was noted that, as an effect of EUCAP supplemented on treatment. BUN concentrations were significantly reduced by treatments. Total VFA concentrations in the rumen were significantly different among treatments. Under this study all supplemented groups had significantly higher C_3. However, the control group had significantly higher C_2 than those of supplemented groups. The C_2/C_3 ratio were significantly different (P<0.05) among treatments. Methane production in the rumen was affected by animals receiving different levels of EUCAP supplementation as compared with the control.

Rumen microorganism population

The effect of EUCAP supplementation on rumen microorganisms are presented in Table 4. It was found that supplementation of EUCAP decreased population of total 'protozoa' as compared with control. However, the population of fungi was not changed among three treatments (P>0.05) when supplementation of EUCAP. Amylolytic bacteria was not affected by supplemented EUCAP after 4 h (P>0.05). Nevertheless, total vaiable bacteria proteolytic bacteria and 'cellulolytic bacterial' population were significantly different among treatments in animals receiving at 100 and 200 g/hd/d of EUCAP

Table 3. Effect of EUCAP supplementation on ruminal pH, temperature, NH_3-N and volatile fatty acids VFAs concentration.

Items	EUCAP supplementation, g/hd/d			SEM	Contrast	
	0	100	200		L	Q
Rumen parameter						
pH	6.9	6.7	6.7	0.17	NS	NS
Temperature, ℃	38.5	38.5	38.2	0.21	NS	NS
NH_3-N, mg/dL	14.8^a	10.6^b	10.0^b	2.23	*	NS
BUN, mg/dl	9.3^a	9.0^{ab}	8.2^b	0.53	*	*
Total VFA, mmol/L	120.7^a	103.0^b	92.8^b	5.21	*	NS
VFA, mol/100 mol						
Acetate, C2	67.7^a	66.6^{ab}	65.8^b	0.86	*	NS
Propionate, C3	20.7^a	21.3^{ab}	22.2^b	0.48	*	NS
Butyrate, C4	11.6	12.0	12.1	0.53	NS	NS
C2:C3 ration	3.3^a	3.2^{ab}	3.0^b	0.13	*	NS
CH_4, mmol/L**	35.5^a	29.9^b	26.3^b	1.89	*	NS

$^{a, b}$ Values within the row of a different superscript are significantly different ($P<0.05$), * = $P < 0.05$, ns = non-significant ($P>0.05$), L= linear, Q = quadratic, ** Calaculated according to Moss et al. (2000) CH_4 production = 0.45 (C_2)-0.275(C_3) + 0.4 (C_4).

Table 4. Effect of concentration of EUCAP supplementation in concentrate on ruminal microbes and viable bacterial counts in cattle.

Items	EUCAP supplementation, g/hd/d			SEM	Contrast	
	0	100	200		L	Q
Ruminal microbes, cells/ml						
Protozoa, x 10^5	2.7^a	2.3^{ab}	2.1^b	0.25	*	NS
Fungal zoospore, x 10^5	3.6	2.9	2.6	0.56	0.06	NS
Viable bacteria, CFU/ml						
Total, x 10^8	3.7^a	3.4^{ab}	2.9^b	0.21	*	NS
Amylolytic , x 10^6	9.3	9.3	8.5	0.62	NS	NS
Proteolytic, x 10^6	2.4^a	2.2^{ab}	2.1^b	0.13	*	NS
Cellulolytic, x 10^7	7.5^a	7.3^{ab}	7.2^b	0.11	*	NS

$^{a, b}$ Values within the row of a different superscript are significantly different ($P<0.05$). * = $P < 0.05$, NS = non-significant ($P>0.05$), L = linear, Q = quadratic, CFU = colony forming unit.

($P<0.05$).

DISCUSSION

Chemical composition of feeds

The concentrate was formulated using simple and locally available feed ingredients with the 14% (CP) content of experimental diet and was recommended for maintenance of crossbred cattle (Kearl, 1982). The proximate analysis of EUCAP in this study was closed with Sallam et al. (2010) who found that Eucalyptus fresh leaves had CP: 7.64%, NDF 61.62% and ADF 50.4%. Salem et al. (2006) reported the chemical of Eucalyptus contained, CP, NDF, ADF were 15.4, 61.5 and 54.2 respectively, especially EOs with 15.5 ml/kg DM. Moreover, Brooker and Kleinig (2006) suggested that the chemical composition of Eucalyptus depends upon the type and nature of the constituents and their individual concentration with varies of species, season, location, climate, soil type, age of the leaves, fertility regime, the method used for drying the plant material.

Effect on feed intake and digestibility

The data indicated that EUCAP affected on feed intake in cattle but not on digestion coefficients of DM, OM, CP, NDF and ADF. These results were similar to previous work of Sallam et al. (2010) who suggested that EOs are the volatile components responsible for some of the characteristic aroma of foliage species, may also have negative effects on DM intake. In general, these results

are consistent with previously worked. Salem et al. (2000) reported that secondary compounds, particularly phenolics could act by lowering foliage palatability by their negative effects in the mouth, such as by astringent bitterness binding to salivary proteins in the mouth or by negative effects on gustative receptors. The results from this study indicated that, EUCAP probably could inhibit rumen microorganisms attached on particle of roughage. However, the results in this study was found that EUCAP had no effect on nutrient digestibilities of DM, OM, NDF and ADF even with increasing levels of EUCAP ($P>0.05$).

Correspondingly to Sallam et al. (2009) who reported that supplementation EuO, the dry mater and organic matter digestibility was slightly decreased at the high levels but it did not differ significantly in comparison with the control.

Characteristics of ruminal fermentation and blood metabolite in cattle

Rumen temperature and pH were similar in all treatments. The rumen pH in cattle was found in the optimal pH range (6.6 ± 0.5) to maintain normal cellulolytic organisms (Van Soest, 1994). EUCAP supplementation significantly decreased on ruminal ammonia nitrogen (NH_3-N) concentration ($P<0.05$) and tended to decrease with enhancing levels of EUCAP supplementation. This result was lower than those reports by Wanapat and Pimpa (1999) (15 to 30 mg/dl). However, Preston and Leng (1987) reported that the crucial level for ammonia has been variously reported as 5 to 25 mg of NH_3-N/dl. Borchers (1965) who showed that the addition of EOs to ruminal fluid (1 g/l) containing 'casein' resulted in an accumulation of amino acid (AA) and a decrease in ammonia N (NH_3–N) concentration, suggesting inhibition of AA deamination by ruminal bacteria. Decreasing in ammonia production was associated with a reduction in a number of group bacteria called hyper-ammonia producing (HAP) bacteria. Collectively, results of the studies by Newbold et al. (2004) suggest that effects of EOs on ruminal protein metabolism are on AA degradation and these effects are likely due to inhibition of HAP bacteria. Wallace et al. (2002) suggested that the main mechanism of activity of EOs was the inhibition of bacterial attachment to feed particles, and subsequently, NH_3 production (deamination) from AA was decrease. These were suggested that the microbial species are affected by the EOs.

McIntosh et al. (2003) and Newbold et al. (2004) reported that EOs can impair protein metabolism in the rumen through two additive mechanisms: i) by reducing protein degradation to peptides and ii) by specifically inhibiting some microbes. Under this study, BUN concentrations were significantly different between supplemented groups and the control group. The differences in BUN concentrations among treatments have been related directly to N levels of diet intake and effect of EUCAP inhibited on NH_3–N. Preston (1996) clearly suggested that the concentration of ammonia absorbed from the rumen was reflected in circulating BUN. The results revealed that control groups had shown higher ($P<0.05$) total VFA concentration and C_2 proportion than in supplemented treatments. However, the control group had significantly higher acetate proportion than in other treatments. All supplemented groups were higher in propionate proportion ($P<0.05$), supplementation at 200 g/hd/d of EUCAP given the highest propionate proportion. This study did not show any difference in butyrate proportion ($P>0.05$). In addition, this study agreed with Kumar et al. (2009) who expressed that the effect of inclusion of EuO at the levels of 0.66, 1.0, 1.33 and 1.66 µl/ml of incubation medium reduced in total volatile fatty acids ($P<0.05$). Moreover, Busquet et al. (2006) noted that effects of EOs on ruminal fermentation at 24 h batch culture increased total VFA concentration, while the highest concentration in most treatments decreased total VFA concentration. Similar to previous observations, Evans and Martin (2000) observed that 'thymol', a primary component of some EOs, modified the concentration of volatile fatty acids *in vitro* incubations of ruminal fluid, when 'thymol' was added to ruminal fluid at the level of 400 µg/ml, concentration of methane, acetate and propionate were decreased.

The result under this study was noted that, the C_2:C_3 ratios were significantly different ($P<0.05$) between control group and the supplemented groups. These results were in agreement with Castillejos et al. (2007) who stated that Rosemary oil (*Rosmarinus officinalis*) containing (1.8-cineole, α-pinene, γ-terpineol and verbenone) had effects on rumen fermentation at 500 mg of EOs/l, leading increasing propionate proportion but reducing acetate and butyrate proportions, as well as the acetate and propionate propositions. This result was related to the work by Pierre (2008) who found that the major constituents of Eucalyptus (α-pinene: 12.13%; β-cymene: 14.59%; γ-terpinene: 14.80%; 1.8-cineole: 51.12%), thus EUCAP supplementation might affect rumen fermentation, especially acetate: propionate proportions. Calculation of ruminal methane (CH_4) production using VFAs concentration according to equation of Moss et al. (2000) showed that methane gas production tended to be reduced in supplementation with 100 and 200 g/day of EUCAP. This result was consistent with several previous studies using Eucalyptus and its compounds in *in vitro* fermentation. Sallam et al. (2010) reported that Eucalyptus fresh leave and residue leaves had abundance in total phenols (TP) and total tannins (TT) but with negligible content of condensed tannins (CT). There was significantly reduction of 53% of methane emission (ml/g DM) in cumulative gas production.

In addition, Sallam et al. (2009) suggested that EuO concentration of the EuO at 25, 50, 100 and 150 µl linearly decreased CH_4 production by 26.0, 46.8, 77.3 and 85.3%, respectively.

Rumen microorganism population

These results showed that the effects of EUCAP changed population of rumen microorganism. Many EOs have dose-dependent effects on bacteria, protozoa and fungi (Greathead, 2003). In general, gram-positive bacteria appeared to be more susceptible to inhibition by plant essential oil compounds than gram-negative bacteria (Davidson and Naidu, 2000). The activity of EO affects electron transport, ion gradients, protein translocation, phosphorylation steps, and other enzyme-dependent reactions causing the affected bacteria to loose chemiosmotic control (Ultee et al., 1999). In addition, *Ruminobacter amylophilus* and *Prevotella* spp. as hyper-NH_3-producing bacteria was inhibited by EOs concentrations of at least 200 mg/L (McIntosh et al., 2003). In our study, the bacteria populations were decreased by 100 and 200 g/d of EUCAP. Moreover, cellulolytic bacterial and proteolylic bacteria populations were decreased with increasing concentration of EUCAP. This result was similar to Delaquis et al. (2002) who reported that crude oils distilled fractions of Eucalyptus (*Camaldulensis*) against some common Gram-positive and Gram-negative. It is therefore possible that variation in composition between batches of EOs is sufficient to cause variability in the degree of susceptibility of Gram-negative and Gram-positive bacteria (Burt, 2004). Russell and Strobel (1989) found that effect of EOs to the inhibition of ruminal cellulolytic bacteria such as *Cellulotytic ruminococci* and *Butyrivibrio fibrisolvens*. Protozoa populations were decreased relating to increasing concentration of EUCAP in the diets ($P<0.05$). In agreement with these observations, Sallam et al. (2009) found that protozoa counts were reduced by 29.0, 38.7, 62.9 and 64.5%, respectively by adding 25, 50, 100 and 150 µl of EuO compared to the control.

Kumar et al. (2009) suggested that EuO at the levels of 0, 0.33, 0.66, 1.0, 1.33 and 1.66 µl/ml, the numbers of 'holotrichs' and 'spirotrichs' decreased ($P<0.05$) by increasing level of EOs. The results indicated that EOs had a potential to inhibit methane production when protozoa population decreased. Tatsuoka et al. (2008) suggested that protozoa counts numerical decreases with EOs addition about 4.5, 5.2 and 6.6%, respectively when compared with control and that indicate the reduction possibility of methane production in the rumen of dairy cattle with EOs addition based on Eucalyptus, Methol and Mint oils due to 9 to 25% of methane production in the rumen could be attributed to protozoa associated methanogenic bacteria. Eucalyptus leaves contain flavonoids and volatile oils, hence inhibitory interactions between 'terpenes', as well as other plant secondary compounds may inhibit the activity of rumen protozoa and methanogenic bacteria (Sallam et al., 2009).

Conclusions

The results from this study suggested that EUCAP supplied at 100 g/hd/d could be an alternative rumen enhancer in reducing rumen methane gas production. Based on this study, EUCAP could be a potential feed source to reduce rumen methane production however further research are still required.

ACKNOWLEDGMENTS

The authors would like to express our sincere thanks to the Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand and the NUFU project for providing financial support for the research and the use of the research facilities.

REFERENCES

Akin M, Aktumsek A, Nostro A (2010). Antibacterial activity and composition of the essential oils of Eucalyptus *Camaldulensis* Dehn. and *Myrtus communis* L. growing in Northern Cyprus. Afr. J. Biotechnol., 9: 531-535.

AOAC, (1990). Official methods of analysis, 5th edn. Association of Official Analytical Chemists, Arlington, VA.

Benchaar C, Calsamiglia A, Chaves AV, Fraser GR, Colombatto D, McAllister TA, Beauchemin KA (2008). A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed Sci. Technol., 145: 209-228.

Borchers R (1965). Proteolytic activity of rumen fluid *in vitro*. J. Anim. Sci., 24: 1033-1038.

Brooker MIH, Kleinig DA (2006). Field Guide to Eucalyptus. Vol.1. South-eastern Australia, Third edition. Bloomings, Melbourne.

Burt S (2004). Essential oils: Their antibacterial properties and potential applications in foods-a review. Int. J. Food Microb., 94: 223-253.

Busquet M, Calsamiglia S, Ferret A, Kamel C (2006). Plant extracts affect *in vitro* rumen microbial fermentation. J. Dairy Sci., 89: 761-771.

Calsamiglia S, Busquet M, Cardozo PW, Castillejos L, Ferret A (2007). Invited review: Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci., 90: 2580–2595.

Castillejos L, Calsamiglia S, Mart´ın-Tereso J, Ter Wijlen H (2007). *In vitro* evaluation of effects of ten essential oils at three doses on ruminal fermentation of high concentrate feedlot-type diets. Anim. Feed Sci. Technol., 145: 259-270.

Crocker CL (1967). Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am. J. Med. Technol., 33: 361-365.

Davidson PM, Naidu AS (2000). Phyto-phenols. In: Natural Food Antimicrobial Systems. A.S., Naidu, ed. CRC Press, Boca Raton, FL. pp. 265-293.

Delaquis RJ, Stanich K, Girard B, Massa G (2002). Antimicrobial activity of individual and mixed fractions of *dill, cilantro, coriander* and Eucalyptus essential oils. Int. J. Food Microbiol., 74: 101-109.

Evans JD, Martin SA (2000). Effects of *thymol* on ruminal micro-organisms. Curr Microbiol., 41: 336-340.

Galyean M (1989). Laboratory Procedure in Animal Nutrition Research. Department of Animal and Life Science, New Mexico State University, U.S.A., pp. 107-122.

Greathead H (2003). Plant and plant extracts for improving animal productivity. Proc. Nutr. Soc., 62: 279-290.

Hungate RE (1969). A roll tube method for cultivation of strict anaerobes. In: Methods in Microbiology. (Eds., Norris, J.R., and Ribbons, D.W). Academic Press, New York, USA., pp. 117-313.
Kearl LC (1982). Nutrition requirements of ruminants in developing countries. Utah. USA: pp. 71-87.
Kumar R, Kamra DN, Agrawal N, Chaudhary LC (2009). Effect of Eucalyptus (*Eucalyptus globulus*) oil on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. Anim. Nutr. Feed Technol., 9: 237-243.
McIntosh FM, Williams P, Losa R, Wallace RJ, Beever DA, Newbold CJ (2003). Effects of essential oils on ruminal microorganisms and their protein metabolism. Appl. Environ. Microbiol., 69: 5011-5014.
Moss AR, Jouany JP, Newbold J (2000). Methane production by ruminants: Its contribution to global warming. Ann. Zootech., 49: 231-253.
Newbold CJ, McIntosh FM, Williams P, Losa R, Wallace RJ (2004). Effects of a specific blend of essential oil compounds on rumen fermentation. Anim. Feed Sci. Technol., 114: 105-112.
Patra AK, Saxena J (2009). Dietary phytochemicals as rumen modifiers: a review of the effects on microbial populations. Antonie van Leeuwenhoek, 96: 363-375.
Pierre MJD (2008). Antifungal Potential of Eucalyptus *Saligna* and Eucalyptus *Camaldulensis* essential Oils from *Cameroon* against *Phaeoramularia Angolensis*. Eur. J. Sci. Res., 24: 348-357.
Preston RL (1996). Protein requirements for growing-finishing cattle and lambs. J. Nutr., 90: 157-160.
Preston TP, Leng RA (1987). Manipulation of feeding and the rumen ecology system. Anonymous. Matching ruminant production system with available resources in the Tropics and Sub-tropics. Penambul books, Armidale, Australia, pp. 83-92.
Russell JB, Strobel HJ (1989). Effect of ionophores on ruminal fermentation. Appl. Environ. Microbiol., pp. 55: 1-6.
Salem AZM, Gonz´alez JS, L´opez S, Ranilla MJ (2000). The effect of feeding alfalfa treated with *quebracho* on parotid salivation in sheep. In: Van Arendonk, J.A.M., (Ed.), Proceedings of the 51st Annual Meeting of the European Association for Animal Production (EAAP), Session N5.17. Wageningen Press, The Hague, the Netherlands: p. 152.
Salem AZM, Salem MZM, El-Adawy MM, Robinson PH (2006). Nutritive evaluations of some browse tree foliages during the dry season: Secondary compounds, feed intake and *in vivo* digestibility in sheep and goats. Anim. Feed Sci. Technol., 127: 251-267.
Sallam SMA, Bueno ICS, Brigide P, Godoy PB, Vitti DMSS, Abdalla AL (2009). Efficacy of Eucalyptus oil on *in vitro* ruminal fermentation and methane production. Options Mediterraneennes - Nutritional and foraging ecology of sheep and goats. 85: 267-272.
Sallam SMA, Bueno ICS, Nasser MEA, Abdalla AL (2010). Effect of eucalyptus (Eucalyptus *citriodora*) fresh or residue leaves on methane emission *in vitro*. Ital. J. Anim. Sci., 9: 299-303.
SAS, (1998). SAS/STAT User's Guid 6. 12th ed. SAS Institue Inc., Cary, Nort Carolina.
Steel RGD, Torrie J (1980). Principles and Procedures of Statistics. McGraw Hill Book Co., New York, NY, USA.
Tatsuoka N, Hara K, Mlkuni K, Hara K, Hashimoto H, Itabashi H (2008). Effects of the essention oil *cylodextrin* complexes on ruminal methane production *in vitro*. Anima. Sci. J., 79: 68-75.
Ultee A, Kets EPW, Smid EJ (1999). Mechanism of action of *carvacrol* on the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol., 65: 4606-4610.
Van Keulen J, Young BA (1977). Evaluation of acid insoluble ash as a neutral marker in ruminant digestibility studies. J. Anim. Sci., 44: 282-287.
Van Soest PJ (1994). Nutrition ecology of the ruminant. 2nd ed Comstock publishing associates a division of Cornell University Press, Ithaca and London.
Wallace RJ, McEwan NR, McIntosh FM, Teferedegne B, Newbold CJ (2002). Natural products as manipulators of rumen fermentation. Asian-Aust. J. Anim. Sci., 15: 10-21.
Wanapat M, Pimpa O (1999). Effect of ruminal NH_3-N levels on ruminal fermentation, purine derivatives, digest digestibility and rice straw intake in swamp buffaloes. Asian-Aust. J. Anim. Sci., 12: 904-907.