UNIVERSITI PUTRA MALAYSIA

EFFECTS OF CONDENSED TANNIN FRACTIONS FROM LEUCAENA LEUCOCEPHALA (LAM.) DE WIT HYBRID ON METHANE MITIGATION, RUMEN FERMENTATION AND DIVERSITY OF METHANOGENS, PROTOZOA AND BACTERIA IN VITRO

SAMINATHAN MOOKIAH

IB 2015 13

EFFECTS OF CONDENSED TANNIN FRACTIONS FROM *LEUCAENA LEUCOCEPHALA* (LAM.) DE WIT HYBRID ON METHANE MITIGATION, RUMEN FERMENTATION AND DIVERSITY OF METHANOGENS, PROTOZOA AND BACTERIA *IN VITRO*

By

SAMINATHAN MOOKIAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Dgree of Doctor of Philosophy

December 2015

DEDICATION

This thesis is dedicated to my parents and brothers
for their love, endless support,
and encouragement

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

EFFECTS OF CONDENSED TANNIN FRACTIONS FROM *LEUCAENA LEUCOCEPHALA* (LAM.) DE WIT HYBRID ON METHANE MITIGATION, RUMEN FERMENTATION AND DIVERSITY OF METHANOGENS, PROTOZOA AND BACTERIA *IN VITRO*

By

SAMINATHAN MOOKIAH

December 2015

Chairman: Professor Norhani Abdullah, PhD
Faculty : Institute of Bioscience

Methane (CH_4) emission is a primary environmental concern due to its contribution to global warming and climate change. Methane gas released from livestock, in particular the ruminants accounts to about one-third of global anthropogenic CH_4 emission. Condensed tannins (CTs) are secondary plant metabolites that have shown methanogenic toxicity, resulting in reduced CH_4 formation in ruminants. Condensed tannins are also known to bind proteins. The CTs produced by plants vary in molecular weights (MWs). The effects of CTs on protein-binding affinity and rumen methanogens may be dependent on the size of the CTs molecules. At the moment, it is not clearly understood whether CTs of different MWs would exert these effects differently. Thus, it was hypothesised that higher MWs, would be more efficient in binding protein and mitigating CH_4 than CTs with lower MWs. Therefore, the objectives of the present study were to determine the effects of CT fractions of different MWs from a *Leucaena leucocephala* hybrid-Rendang (LLR) on protein binding affinity and CH_4 mitigation by rumen microbes *in vitro*. In conjunction to these, the effects of CTs of different MWs on rumen microbial fermentation activities and microbial species were also determined. Condensed tannins were extracted from LLR and fractionated into five fractions (F1–F5) using size exclusion chromatography procedure. The degrees of polymerization (DP) of the CT fractions were measured by a modified vanillin assay, the MWs of the fractions were determined by Q-TOF LC/MS, and their structures were investigated using ^{13}C-NMR. The protein-binding affinities of CT fractions were measured using a protein precipitation assay. The *in vitro* gas production test was used to investigate the effects of CT fractions on CH_4 production, rumen microbial fermentation and populations (methanogens, protozoa and bacteria) *in vitro*. Based on the vanillin assay, it was found that the DP of the five CT fractions (fractions F1–F5) ranged from 4.86 to 1.56. The number-average MWs (M_n) of the different fractions were 1265.8, 1028.6, 652.2, 562.2, and 469.6 for fractions F1, F2, F3, F4, and F5, respectively. The ^{13}C-NMR results showed that the CT fractions possessed monomer unit structural heterogeneity. The *b* values representing the CT quantities needed to bind half of the maximum precipitable bovine serum albumin

increased with decreasing MWs from fraction F1 to fraction F5, with values of 0.216, 0.295, 0.359, 0.425, and 0.460, respectively. This indicated that higher MWs fractions had higher protein-binding affinity. The total gas [ml/g dry matter (DM)] and CH_4 (ml/g DM) productions decreased significantly ($P < 0.05$) with increasing MWs of the CT fractions, with no significant reduction in DM digestibility. However, the *in vitro* nitrogen disappearance decreased significantly ($P < 0.05$) with the inclusion of CT fraction F1 (highest MW) when compared with the control (without CTs) and other fractions (F2–F5). The inclusion of CT fraction F1 also significantly ($P < 0.05$) decreased total volatile fatty acid, acetic acid concentrations and acetic/propionic acid ratio when compared with that of the control. The real-time PCR assay showed that higher MWs CT fractions (fractions F1 and F2) significantly ($P < 0.05$) decreased the total methanogens and methanogens from the order *Methanobacteriales*, and total protozoa than the lower MWs CT fractions (fractions F3-F5). Inclusion of higher MWs CT fractions F1 and F2 significantly ($P < 0.05$) increased the *Fibrobacter succinogens* population compared to CT fractions F3–F5. Whereas, inclusion of CT fractions (F1–F5) significantly ($P < 0.05$) decreased the *Ruminococcus flavefaciens* population compared with that of the control. Amplification of archaeal V3 regions of 16S rRNA genes using Illumina MiSeq sequencer showed that the relative abundance of the predominant unclassified *Thermoplasmata*-associated group (VadinCA11 gut group) increased significantly ($P < 0.05$), corresponding with increasing MWs of the CT fractions, whereas the predominant methanogen genus *Methanobrevibacter* was significantly ($P < 0.05$) decreased. The partial 18S rRNA gene analysis of the rumen protozoa using Illumina sequencer showed that the relative abundance of the predominant genus *Entodinium* significantly ($P < 0.05$) decreased with inclusion of CT fractions F1, F2 and F3 as compared with the control. In contrast, significant ($P < 0.05$) increases in second predominant rumen protozoa genus, *Anoplodinium-Diplodinium* were observed with CT fractions F1–F4 than that of the control. Illumina MiSeq sequencing of the V3 region of the bacterial 16S rRNA genes illustrated that the relative abundance of predominant genus *Prevotella* and unclassified *Clostridiales* were significantly ($P < 0.05$) decreased, corresponding with increasing MWs of CT fractions, whereas the cellulolytic bacteria *Fibrobacter* genus was significantly ($P < 0.05$) increased. In conclusion, CTs of different MWs have varying ability to bind proteins and decreased ruminal CH_4 production by altering the populations and diversities of rumen methanogens and protozoa, and the effects were more pronounced for CTs with higher-MWs. The bacterial population and fermentation activities were also influenced by CT fractions, but the changes had no adverse effect on DM degradability. The strong binding affinity of higher MWs CTs to proteins may be beneficial in reducing degradation of feed protein by rumen microbes, thus enhancing bypass protein in ruminants. Moreover, higher MWs CTs could be potential methanogen inhibitors, which can be incorporated in ruminant diet to mitigate the CH_4 emission, thus improving the feed efficiency and animal productivity, and at the same time reducing the contribution of ruminant livestock to global CH_4 inventory.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KESAN PECAHAN TANIN TERKONDENSASI DARIPADA *LEUCAENA LEUCOCEPHALA* (LAM.) DE WIT HIBRID TERHADAP MITIGASI METANA, FERMENTASI RUMEN SERTA DIVERSITI METHANOGEN, PROTOZOA DAN BAKTERIA *IN VITRO*

Oleh

SAMINATHAN MOOKIAH

Disember 2015

Pengerusi: Profesor Norhani Abdullah, PhD
Fakulti : Institut Biosains

Pengeluaran gas metana (CH_4) adalah salah satu keprihatinan utama terhadap alam sekitar disebabkan sumbanganya kepada pemanasan global dan perubahan iklim. Pengeluaran CH_4 daripada ruminan adalah lebih kurang satu pertiga pengeluaran CH_4 antropogen global. Tanin terkondensasi (CTs) adalah metabolik sekunder tumbuhan, menunjukkan ketoksikan methanogenic, mengakibatkan kekurangan pembentukan CH_4 dalam ruminan. Tanin terkondensasi juga dapat mengikat protein. Tanin terkondensasi dihasilkan oleh tumbuhan adalah berbeza dari segi berat molekul (MWs). Kesan CTs terhadap afiniti ikatan-protein dan methanogens rumen bergantung kepada saiz molekul. Ketika ini, adalah tidak dapat ditentukan sama ada CTs yang berbeza MWs akan menunjukkan pengaruh ke atas kesan-kesan yang berlainan. Justeru, ia dihipothesiskan bahawa CTs yang mempunyai MWs lebih tinggi akan lebih cekap mengikat protein dan mengurangkan CH_4 daripada CTs yang mempunyai MWs lebih rendah. Justeru, kajian ini bertujuan menyiasat kesan pecahan-pecahan CT yang mempunyai MW yang berbeza daripada *Leucaena leucocephala* hybrid-Rendang (LLR) dalam afiniti ikatan-protein dan pengurangan CH_4 oleh mikrob rumen *in vitro*. Rentetan itu, kesan CTs yang mempunyai MWs berbeza dalam fermentasi mikrob rumen aktiviti dan spesies mikrob juga ditentukan. Tanin terkondensasi telah diekstrak daripada LLR, ditulen dan dipemeringkatkan kepada lima pecahan dengan menggunakan teknik kromatografi penyisihan saiz. Darjah pempolimeran (DP) pecahan-pecahan CT diukur dengan ujian vanilin yang telah diubah suai, MWs pecahan CT telah ditentukan melalui Q-TOF LC/MS, dan struktur-struktur pecahan CT disiasat menggunakan ^{13}C-NMR. Afiniti ikatan-protein bagi pecahan-pecahan CT ditentukan dengan menggunakan ujian pemendakan protein. Ujian pengeluaran gas *in vitro* digunakan untuk menyiasat kesan pecahan-pecahan CT dalam pengeluaran CH_4, fermentasi rumen, serta populasi dan diversiti methanogens, protozoa dan bakteria *in vitro*. Kajian berdasarkan ujian vanilin mendapati bahawa DP lima pecahan CT (pecahan F1–F5) adalah antara 4.86 hingga 1.56. Nombor purata berat molekul (M_n) bagi pecahan-pecahan CT ialah 1265.8, 1028.6, 652.2, 562.2 dan 469.6, masing-masing untuk pecahan F1, F2, F3, F4 dan F5. Keputusan ^{13}C-NMR menunjukkan bahawa

pecahan-pecahan CT berbeza antara satu sama lain dengan unit konstituen yang berlainan. Nilai *b* yang mewakili kuantiti CT yang diperlukan untuk mengikat separuh daripada albumin serum bovin bertambah dengan penurunan MW daripada pecahan F1 ke pecahan F5, masing-masing dengan nilai 0.216, 0.295, 0.359, 0.425 dan 0.460. Keputusan ini menunjukkan bahawa pecahan-pecahan CT yang terdiri daripada MW lebih tinggi mempunyai afiniti ikatan-protein yang lebih tinggi. Jumlah gas [ml /g bahan kering (DM)] dan pengeluaran CH_4 (ml / g DM) menurun secara ketara (P < 0.05) dengan penambahan MW pecahan-pecahan CT, tetapi tidak ada perbezaan ketara antara pecahan-pecahan CT dalam degradasi DM. Bagaimanapun, kehilangan nitrogen *in vitro* menurun dengan ketara (P < 0.05) dengan kemasukan pecahan CT F1 berbanding dengan kawalan (tanpa CT) dan pecahan-pecahan CT yang lain (F2–F5). Kemasukan pecahan CT F1 didapati mengurangkan (P < 0.05) jumlah asid lemak meruap, konsentrasi asid asetik dan nisbah asid asetik/propionik berbanding dengan kawalan. PCR masa nyata menunjukkan bahawa pecahan-pecahan CT yang terdiri daripada MW yang lebih tinggi (pecahan F1 and F2) mengurangkan jumlah methanogen dan methanogens dalam order *Methanobacteriales* dan jumlah protozoa berbanding dengan pecahan-pecahan CT yang mempunyai MW yang lebih rendah (pecahan F3–F5). Pecahan-pecahan CT MW yang lebih tinggi F1 and F2 dapat meningkatkan (P < 0.05) populasi *Fibrobacter succinogens* dengan ketara berbanding dengan pecahan-pecahan CT F3–F5. Manakala, kemasukan pecahan-pecahan CT (F1–F5) mengurangkan (P < 0.05) populasi *Ruminococcus flavefaciens* dengan ketara apabila dibandingkan dengan kawalan. Amplifikasi rantau V3 gen 16S rRNA archaeal daripada semua sampel rumen dengan menggunakan penjujuk Illumina MiSeq menunjukkan bahawa kelimpahan relatif dominan kumpulan dikaitkan-*Thermoplasmata* yang tidak diklasifikasikan (kumpulan VadinCA11) bertambah dengan ketara (P < 0.05), sepadan dengan peningkatan MW pecahan-pecahan CT, manakala dominan methanogen genus *Methanobrevibacter* menurun dengan ketara (P < 0.05). Analisis sebahagian gen 18S rRNA protozoa menggunakan penjujuk Illumina menunjukkan bahawa kelimpahan relatif dominan genus *Entodinium* berkurangan secara ketara (P < 0.05) dengan kemasukan pecahan-pecahan CT seperti F1, F2 and F3 berbanding dengan kawalan. Bagaimanpun, peningkatan ketara (P < 0.05) dalam kelimpahan relatif protozoa rumen yang kedua dominan, iaitu *Anoplodinium Diplodinium* telah diperhatikan dengan pecahan-pecahan CT F1–F4 berbanding dengan kawalan. Penjujukan Illumina MiSeq rantau V3 gen-gen 16S rRNA bakteria menunjukkan bahawa kelimpahan relatif dominan *Prevotella* dan *Clostridiales* yang tidak diklasifikasikan berkurangan secara ketara (P < 0.05), selaras dengan penambahan MW pecahan-pecahan CT, manakala bakteria selulosa jenis *Fibrobacter* meningkat dengan ketara (P < 0.05). Secara kesimpulannya, CTs yang berbeza MWs mempunyai keupayaan berlainan dalam afiniti ikatan-protein dan mengurangkan pengeluaran CH_4 dengan mengubah populasi methanogen rumen dan protozoa, dan kesanya lebih ketara untuk CT dengan MW yang lebih tinggi. Populasi bakteria dan aktiviti fermentasi juga dipengaruhi oleh pecahan-pecahan CT, tetapi perubahannya tidak menjejaskan degradasi DM. Afiniti ikatan-protein yang kuat untuk CT yang mempunyai MWs yang tinggi mungkin bermanfaat dalam mengurangkan degradasi protein tumbuhan oleh mikrob rumen, justeru meningkatkan "bypass protein" dalam ruminan. Tambahan lagi, CTs yang mempunyai MWs yang tinggi berpotensi sebagai perencat methanogen, yang boleh digabungkan dalam diet ruminan bagi mengurangkan pemancaran CH_4, seterusnya meningkatkan kecekapan makanan dan produktiviti haiwan, dan juga mengurangkan sumbangan ternakan ruminan kepada inventori CH_4 global.

ACKNOWLEDGEMENTS

First of all, I wish to express my utmost thanks and deepest gratitude to the chairman of the supervisory committee, Professor Dr. Norhani Abdullah, for her supervision, invaluable guidance and advices, patience, endless support, and encouragement throughout this study and for her critical analysis and helpful suggestions during the preparation of the thesis. My sincere appreciation is extended to my former supervisor, the late Associate Professor Dr. Sieo Chin Chin (since January–October 2015) for her advice, support and assistance during the preparation of the thesis. I would like to express my heartfelt gratitude to my former supervisor, Professor Dr. Ho Yin Wan (Retd.) (since 2011–2014), for her kind assistance, support and encouragement that taught me to be passionate in research. I am grateful she has also given me the opportunity to pursue my doctorate degree and embark on interesting and exciting scientific research.

I would also like to express my appreciation to Professor Dr. Clemente Michael Wong Vui Ling, who is members of the supervisory committee, for their guidance, advices and kind assistance. Thanks are also extended to Dr. Emilia Abdulmalek, Faculty of Science for her help in the compound structural elucidation, and Dr. Gan Han Ming, School of Science, Monash University Malaysia, for his guidance and assistance in the bioinformatics analysis.

My heartfelt appreciations are also due to Mr. Khairul Kamar Bakri, Ms. Nadia, Madam Haw Ah Kam (Retd.) and Mr. Nagayah Muniandy (Retd.), staff of the Microbial Technology Unit, Laboratory of Vaccines and Immunotherapeutics, Institute of Bioscience, for their technical support and kind assistance throughout the course on my study.

I would like to extend my gratitude to the Malaysian Ministry of Education, for providing the financial support under the MyBrain15 (PhD) scholarship for my study.

I wish to thank my fellow labmates and friends, Helen, Fadilah, Shaufi, Shirley, Dr. Tan Hui Yin, Lily, Naveena and Sharanya for their friendship, help, encouragement and support. They have all helped me to overcome the challenges encountered, and indeed have been true friends. Thanks for making the long hours in the lab a pleasant and memorable one.

Finally, the most special thanks are extended to my parents, uncle, aunty and brothers for their continuous support, encouragement and prayers which have inspired and motivated me throughout the course of this study. I will always be grateful for their constant love and encouragement that they gave me along the way. Most of all, I thank God for all His blessings.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Norhani Abdullah, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Clemente Michael Wong Vui Ling, PhD
Professor
Biotechnology Research Institute
Universiti Malaysia Sabah
(Member)

_____

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by Graduate Student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric number: Saminathan Mookiah (GS30487)

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of
Chairman of
Supervisory
Committee: Norhani Abdullah, PhD

Signature: _____
Name of
Member of
Supervisory
Committee: Clemente Michael Wong Vui Ling, PhD

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

BLAST	- Basic Local Alignment Search Tool
Bp	- basepair
BSA	- Bovine Serum Albumin
BW	- Body Weight
Cm	- Centimeter
CH_4	- Methane
CO_2	- Carbon dioxide
CP	- Crude protein
Ct	- threshold cycle
CT	- Condensed Tannin
CTs	- Condensed Tannins
°C	- Degree Celsius
Da	- Dalton
DM	- Dry matter
DNA	- deoxyribonucleic acid
dNTP	- deoxyribonucleotide triphosphate
DP	- Degree of polymerization
Es	- amplification efficiency
F	- Fraction
FAO	- Food and Agriculture Organization
G	- gram
G	- gravity
GPC	- Gel Permeation Chromatography
H	- Hydrogen
H	- hour
HCl	- Hydrochloric acid
HPLC	- High performance liquid chromatography
H_2SO_4	- sulphuric acid
HT	- Hydrolysable Tannins
IPCC	- Intergovernmental Panel on Climate Change
IVDMD	- *In vitro* DM degradability
IVND	- *In vitro* nitrogen degradability
K	- Potassium
Kb	- kilo basepair
Kg	- Kilogramme
L	- Liter
LLB	- *Leucaena leucocephala* hybrid-Bahru
LLR	- *Leucaena leucocephala* hybrid-Rendang
M_w	- weight average molecular weight
M_n	- number average molecular weight
M	- molar / molarity

Mg	- milligram
Min	- minutes
mL	- milliliter
Mm	- millimetre
µg	- microgram
µL	- microliter
µm	- micrometre
N	- nano
N	- Nitrogen
Na	- Sodium
ND	- nitrogen degradability
NH_4	- Ammonium
Nm	- nanometre
NTC	- No-template control
OH	- Hydroxyl
PCR	- polymerase chain reaction
PDI	- Polydispersity index
PEG	- Polyethylene glycol
pH	- Puissance Hydrogen
Q-TOP LC/MS	- Liquid chromatograph-quadrupole time-of-flight mass spectrometer
R^2	- correlation coefficient
SEM	- Standard Error Mean
TAE	- Tris-acetate EDTA
U	- Unit
V	- Volt
v/v	- Volume per volume
w/v	- Weight per volume

CHAPTER 1

INTRODUCTION

In the past century, the ruminant sector has played a major role in human nutrition. This sector contributed about 30% of global meat production (equivalent to 81 million tonnes) and about 83% of global milk production (equivalent to 717 million tonnes) in 2013 (FAOSTAT, 2014). The demand for ruminant products is expected to increase due to the growth of the human population, in which the global ruminant meat and milk production is forecasted to grow at a rate of 1.2% and 1.1%, respectively, during the period 2006–2050 (FAOSTAT, 2014).

At present, the productivity of ruminants is under ever increasing pressure from the public to improve the environmental sustainability of ruminant meat production and dairy farming. Apart from maintaining the productivity, the latest challenge for ruminant nutritionist is to minimise the excretion of environmentally hazardous wastes by ruminants. Methane (CH_4) emissions from ruminants have been identified as a primary environmental concern because of the contribution to the greenhouse effect and global warming (Moss *et al*., 2000). Methane is the end-product of feed fermentation to dispose of hydrogen (H_2) produced by microbes in the rumen. It is a potent greenhouse gas (GHG) and has a heat trapping potential of 34 times more than carbon dioxide (CO_2) (IPCC, 2013). Ruminants typically lose 3 to 12% of their ingested energy as eructated CH_4 (Johnson and Johnson, 1995).

Enteric CH_4 emission from cattle is greatly influenced by the ruminant diet. Modification of rumen fermentation offers a potential approach to minimize waste excretion from animals. In ruminants, the rumen is an important site of digestion, owing to complex microbial communities including bacteria, archaea, fungi and protozoa (Zened *et al*., 2013). A better understanding of the effects of the modifiers on rumen microbial populations could maintain the animal productivity and decrease environmental pollution. Increasing the dry matter (DM) intake and the feeding of more digestible forage and legume have been reported to improve digestibility and reduce CH_4 production (Iqbal *et al*., 2008). On contrary, rumen modification approaches such as defaunation, the use of ionophores, dicarboxylic acid, oils, antibiotic and analogues are not permanent solutions to ruminal CH_4 mitigation due to the adaptation of rumen microbes (Cottle *et al*., 2011). Therefore, recent research has focused on investigating strategies at the plant and plant extract level, which might offer a long-term solution of CH_4 production and a promising approach for future research.

Forages and legumes containing condensed tannins (CTs) have been shown to mitigate enteric ruminal CH_4 emission (Hess *et al*., 2003a; Tavendale *et al*., 2005; Soltan *et al*., 2012). It has been suggested that using the CTs extract to reduce CH_4 emissions may be a better alternative than feeding tannin-rich forages (Beauchemin *et al*., 2007). Condensed tannins, also known as proanthocyanidins, are heterologous compounds that vary in structure and size, with free phenolic groups. They are complexes of oligomers

and polymers built up of flavan-3-ols (catechins) and/or flavan-3,4-diol (epigallocatechins), with molecular weights (MWs) ranging from 500 to 20,000 Daltons (Da) (Hagerman and Butler, 1991). The different combination of monomeric units and carbon-carbon bonds lead to differences in MWs and structures of CTs that could play key roles in biological activities (Rakhmani *et al*., 2005).

The mechanism by which CTs reduce CH_4 production is not well understood, although a reduction in ruminal methanogens and protozoa could be a main factor in the suppression of CH_4 production (Animut *et al*., 2008a). In many of the studies, supplementation of CTs resulted in reduced CH_4 emissions accompanied by detrimental effects on DM digestibility (Woodward *et al*., 2001; Hess *et al*., 2003b, Animut *et al*., 2008b). Condensed tannins have also been found to exhibit protein binding ability (Kumar and Horigome, 1986; Osborne and McNeill, 2001; Huang *et al*., 2010). Depending on their molecular weights, CT fractions vary in their capability to bind proteins. They are able to protect feed protein from being degraded by rumen microorganisms through formation of CT-protein complexes. The CT-protein complexes are then dissociated under the acidic condition of the abomasum releasing proteins for digestion and absorption (McNabbl *et al*., 1993), presumably increasing the N utilisation and reducing NH_3 emissions from manure (Woodward *et al*., 2009). Furthermore, CTs also reduce protein degradation in the rumen through binding to extracellular protein-degrading enzymes (Smith *et al*., 2005). A study by Khamseekhiew (2006) on *Leucaena* hybrid, which was used as a feed supplement for sheep in Malaysia, found that the CTs extracted from *Leucaena* hybrid had a strong binding affinity for proteins.

Leucaena leucocephala, a tropical scrub legume with high crude protein (CP) content ranging from 200 to 300 g/kg DM (Khamseekhiew, 2006), has been widely used as a feed additive to overcome nutrient deficiencies in ruminants in the tropics and sub-tropical regions. In Malaysia, several generations of crossing between *L. leucocephala* and *L. diversifolia* for acid tolerance resulted in two new *L. leucocephala* hybrids, namely 62-2-8 *L. leucocephala* hybrid-Bahru (LLB) and 40-1-18 *L. leucocephala* hybrid-Rendang (LLR) (Wong *et al*., 1998). These two hybrids have adaptability for high soil aluminium, are acid tolerant and resistant to psyllid attack. They also have a high content of secondary metabolite compounds, such as CTs. Khamseekhiew (2006) reported that the *L. leucocephala* hybrids exhibited lower DM digestibility and nitrogen (N) degradability than the local *L. leucocephala* in ruminants, owing to the higher content of CTs.

The MWs and chemical structures of CTs may be the primary factors determining their beneficial effects on CH_4 mitigation and improving the utilisation of feed proteins by ruminants (Aerts *et al*., 1999; Vidal *et al*., 2003). Recently, Tan *et al*. (2011a) found that the inclusion of 30–40 mg CTs/g DM of unfractionated pure CTs from LLR reduced CH_4 production and populations of methanogens and protozoa with no adverse effects on DM digestibility and nitrogen degradability *in vitro*. Later studies indicated that unfractionated pure CTs from LLR could alter the diversities of bovine rumen methanogens and protozoa (Tan *et al*., 2011b; 2103). However, the latter studies conducted by Tan *et al*. (2011a; 2011b; 2013), used a preparation containing a mixture of CTs with different MWs. At present, it is not known whether CTs of different MWs

from LLR would differ on their effects on protein-binding affinity, CH_4 production, rumen fermentation parameters and populations and diversities of rumen microorganisms.

Therefore, the present study was conducted to investigate the effects of CT fractions of different MWs from *Leucaena leucocephala* hybrid-Rendang (LLR) on protein binding affinity and rumen microbial activities. The specific objectives of this study were:

1. To extract, purify and fractionate CTs from *Leucaena leucocephala*-hybrid Rendang (LLR) and to determine their molecular weights (MWs), degree of polymerisation (DP) and structure of the CT fractions.
2. To determine the protein binding affinity of unfractionated CTs and CT fractions from LLR using a protein precipitation assay.
3. To evaluate the effects of CT fractions of different MWs from LLR on *in vitro* CH_4 production, *in vitro* DM degradability and N disappearance, and volatile fatty acid (VFA) production using the *in vitro* gas production test.
4. To determine the effects of CT fractions of different MWs from LLR on the population and diversity of bovine rumen archaea, protozoa and bacteria *in vitro* using real-time PCR and Illumina MiSeq sequencing approaches.

REFERENCES

Abdulrazak, S., Muinga, R., Thorpe, W., and Ørskov, E. (1997). Supplementation with *Gliricidia sepium* and *Leucaena leucocephala* on voluntary food intake, digestibility, rumen fermentation and live weight of crossbred steers offered Zea mays stover. *Livestock Production Science*. 49(1): 53-62.

Abreu, A., Carulla, J. E., Lascano, C. E., Diaz, T., Kreuzer, M., and Hess, H.D. (2004). Effects of fruits on ruminal fermentation and duodenal nitrogen flow of sheep fed a tropical grass diet with and without legume. *Journal of animal science*. 82(5): 1392-1400.

Aerts, R. J., Barry, T. N., and McNabb, W. C. (1999). Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. *Agriculture, Ecosystems and Environment*. *75*(1): 1-12.

Anantasook, N., Wanapat, M., Cherdthong, A., and Gunun, P. (2013). Changes of microbial population in the rumen of dairy steers as influenced by plant containing tannins and saponins and roughage to concentrate ratio. *Asian-Australasian journal of animal sciences*. 26(11): 1583.

Anbarasu, C., Dutta, N., Sharma, K., and Rawat, M. (2004). Response of goats to partial replacement of dietary protein by a leaf meal mixture containing *Leucaena leucocephala*, Morus alba and Tectona grandis. *Small Ruminant Research*. 51(1): 47-56.

Animut, G. R., Goetsch, A., Patra, A., Sahlu, T., Varel, V., and Wells, J. (2008a). Methane emission by goats consuming diets with different levels of condensed tannins from lespedeza. *Animal Feed Science and Technology*. 144(3): 212-227.

Animut, G. R., Goetsch, A., Patra, A., Sahlu, T., Varel, V., and Wells, J. (2008b). Methane emission by goats consuming different sources of condensed tannins. *Animal feed science and technology*. 144(3): 228-241.

Ansorge, W. J. (2009). Next-generation DNA sequencing techniques. *New biotechnology*. 25(4): 195-203.

Arcuri, P. B., Odenyo, A. A., Arcuri, E. F., Ribeiro, M. T., Guimarães, M. F. M., and Carneiro, J. D. C. (2011). Tannin-tolerant bacteria from crossbred Holstein x Zebu cows. *Pesquisa Agropecuária Brasileira*. 46(3): 272-279

Arndt, D., Xia, J., Liu, Y., Zhou, Y., Guo, A. C., Cruz, J. A., Sinelnikov, I., Budwill, K., Nesbø, C. L., Wishart, D. S. (2012). METAGENassist: a comprehensive web server for comparative metagenomics. *Nucleic Acids Res*, 40(Web Server issue): W88-95. doi: 10.1093/nar/gks497.

Artz, W. E., Bishop, P. D., Dunker, A. K., Schanus, E. G., and Swanson, B. G. (1987). Interaction of synthetic proanthocyanidin dimer and trimer with bovine serum

albumin and purified bean globulin fraction G-1. *Journal of agricultural and food chemistry*. 35(3): 417-421.

Ashraf-Khorassani, M., and Taylor, L. T. (2004). Sequential fractionation of grape seeds into oils, polyphenols, and procyanidins via a single system employing CO_2-based fluids. *Journal of agricultural and food chemistry*. 52(9): 2440-2444.

Asquith, T. N., and Butler, L. G. (1986). Interactions of condensed tannins with selected proteins. *Phytochemistry*. 25(7): 1591-1593.

Asquith, T. N., Uhlig, J., Mehansho, H., Putman, L., Carlson, D. M., and Butler, L. (1987). Binding of condensed tannins to salivary proline-rich glycoproteins: the role of carbohydrate. *Journal of agricultural and food chemistry*. 35(3): 331-334.

Bacon, J. R., and Rhodes, M. J. (2000). Binding affinity of hydrolyzable tannins to parotid saliva and to proline-rich proteins derived from it. *Journal of agricultural and food chemistry*. 48(3): 838-843.

Bae, H. D., McAllister, T. A., Muir, A. D., Yanke, L. J., Bassendowski, K. A., and Cheng, K. J. (1993a). Selection of a method of condensed tannin analysis for studies with rumen bacteria. *Journal of agricultural and food chemistry*. 41(8): 1256-1260.

Bae, H. D., McAllister, T. A., Yanke, J., Cheng, K.-J., and Muir, A. (1993b). Effects of condensed tannins on endoglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. *Applied Environmental Microbiology*. 59(7): 2132-2138.

Barahona, R., Sanchez, S., Lascano, C. E., Owen, E., Morris, P., and Theodorou, M. K. (2006). Effect of condensed tannins from tropical legumes on the activity of fibrolytic enzymes from the rumen fungus *Neocallimastyx hurleyensis*. *Enzyme and microbial technology*. 39(2): 281-288.

Barros-Rodríguez, M., Sandoval-Castro, C. A., Solorio-Sánchez, J., Sarmiento-Franco, L., Rojas-Herrera, R., and Klieve, A. V. (2014). *Leucaena leucocephala* in ruminant nutrition. *Tropical and Subtropical Agroecosystems*. 17(2).

Barry, T. N. (1989). Condensed tannins: their role in ruminant protein and carbohydrate digestion and possible effects upon the rumen ecosystem. The roles of protozoa and fungi in ruminant digestion. Armidale NSW. 2351: 153-169.

Barry, T. N., Manley, T., and Duncan, S. (1986). The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. *British journal of nutrition*. 55(1): 123-137.

Beauchemin, K., Kreuzer, M., O'mara, F., and McAllister, T. (2008). Nutritional management for enteric methane abatement: a review. *Animal Production Science*. 48(2): 21-27.

Beauchemin, K., McGinn, S., Martinez, T., and McAllister, T. (2007). Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. *Journal of animal science*. 85(8): 1990-1996.

Behrens, A., Maie, N., Knicker, H., and Kögel-Knabner, I. (2003). MALDI-TOF mass spectrometry and PSD fragmentation as means for the analysis of condensed tannins in plant leaves and needles. *Phytochemistry*. 62(7): 1159-1170.

Ben Salem, H., Nefzaoui, A., Ben Salem, L., and Tisserand, J. (1997). Effect of *Acacia cyanophylla* Lindl. foliage supply on intake and digestion by sheep fed lucerne hay-based diets. *Animal Feed Science and Technology*. 68(1-2): 101-113.

Benchaar, C., McAllister, T., and Chouinard, P. (2008). Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or *Yucca schidigera* saponin extracts. *Journal of Dairy Science*. 91(12): 4765-4777.

Bennick, A. (2002). Interaction of plant polyphenols with salivary proteins. *Critical Reviews in Oral Biology andMedicine*. 13(2): 184-196.

Bhatta, R., Uyeno, Y., Tajima, K., Takenaka, A., Yabumoto, Y., Nonaka, I., and Kurihara, M. (2009). Difference in the nature of tannins on *in vitro* ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. *Journal of Dairy Science*. 92(11): 5512-5522.

Bravo, L., Abia, R., and Saura-Calixto, F. (1994). Polyphenols as dietary fiber associated compounds. Comparative study on *in vivo* and *in vitro* properties. *Journal of agricultural and food chemistry*. 42(7): 1481-1487.

Brewbaker, J. (1986). Leguminous trees and shrubs for Southeast Asia and the South Pacific. In *Forages in Southeast Asian and South Pacific Agriculture*, Proceedings of the ACIAR, Canberra, Australia, Dates (Days Months Year); Blair, G.J., Ivory, D.A., Evans, T.R., Eds.; Publisher: Location (city, state, country), Volume 12, pp. 43–50.

Brewbaker, J. L. (1987). *Leucaena*: a multipurpose tree genus for tropical agroforestry. In: Steppler, H.A., Ramachandran Nair, P.K. (Eds.), Agroforestry: A Decade of Development. International Council for Research in Agroforestry, Nairobi, Kenya, 289-323.

Brooker, J. D., O'Donovan, L., Skene, I., and Sellick, G. (2000). Mechanisms of tannin resistance and detoxification in the rumen. In 'Tannins in Livestock and Human Nutrition: Proceedings of an International Workshop, Adelaide, Australia. ACIAR Proceedings No. 92'. Adelaide (Ed. JD Brooker) pp. 117-122. (Australian Centre for International Agricultural Research: Adelaide, Australia).

Brunet, S., Jackson, F., and Hoste, H. (2008). Effects of sainfoin (*Onobrychis viciifolia*) extract and monomers of condensed tannins on the association of abomasal nematode larvae with fundic explants. *International Journal for Parasitology*. 38(7): 783-790.

Bryant, M. (1979). Microbial methane production—theoretical aspects. *Journal of animal science*. 48(1): 193-201.

Butler, L. G., Riedl, D. J., Lebryk, D., and Blytt, H. (1984). Interaction of proteins with sorghum tannin: mechanism, specificity and significance. *Journal of the American Oil Chemists' Society*. 61(5): 916-920.

Butter, N., Dawson, J., Wakelin, D., and Buttery, P. (2000). Effect of dietary tannin and protein concentration on nematode infection (*Trichostrongylus colubriformis*) in lambs. *The Journal of Agricultural Science*. 134(01): 89-99.

Cai, Y.-Z., Sun, M., Xing, J., Luo, Q., and Corke, H. (2006). Structure–radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life sciences*. 78(25): 2872-2888.

Carulla, J., Kreuzer, M., Machmüller, A., and Hess, H. (2005). Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Crop and Pasture Science*. 56(9): 961-970.

Casamayor, E. O., Massana, R., Benlloch, S., Øvreås, L., Díez, B., Goddard, V. J., and Pedrós-Alió, C. (2002). Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. *Environmental Microbiology*. 4(6): 338-348.

Castillo, A., Cuyugan, O., Fogarty, S., and Shelton, H. (1997). Growth, psyllid resistance and forage quality of *Leucaena leucocephala*, *L. pallida*, *L. diversifolia* and the F1 hybrid of *L. leucocephala* x *L. pallida*. *Tropical Grasslands*. 31: 188-200.

Cenci, F., Louvandini, H., McManus, C., Dell'Porto, A., Costa, D., Araújo, S. D., and Abdalla, A. (2007). Effects of condensed tannin from *Acacia mearnsii* on sheep infected naturally with gastrointestinal helminthes. *Veterinary parasitology*. 144(1): 132-137.

Chadhokar, P. (1982). *Gliricidia maculata*: a promising legume fodder plant [in Sri Lanka]. *World animal review*.

Chai, W.-M., Shi, Y., Feng, H.-L., Qiu, L., Zhou, H.-C., Deng, Z.-W., Yan, C. L., and Chen, Q.-X. (2012). NMR, HPLC-ESI-MS, and MALDI-TOF MS analysis of condensed tannins from *Delonix regia* (Bojer ex Hook.) Raf. and their bioactivities. *Journal of agricultural and food chemistry*. 60(19): 5013-5022.

Chanjula, P., Wanapat, M., Wachirapakorn, C., and Rowlinson, P. (2004). Effects of various levels of cassava hay on rumen ecology and digestibility in swamp buffaloes. *Asian Australasian Journal Of Animal Sciences*. 17(5): 663-669.

Chanthakhoun, V., and Wanapat, M. (2010). Effect of legume (*Phaseolus calcaratus*) hay supplementation on rumen cellulolytic bacterial populations in swamp buffaloes investigated by the real-time PCR technique. *Journal of Animal Veterinary Advances*. 9(11): 1654-1659.

Chanthakhoun, V., Wanapat, M., Kongmun, P., and Cherdthong, A. (2012). Comparison of ruminal fermentation characteristics and microbial population in swamp buffalo and cattle. *Livestock Science*. 143(2): 172-176.

Chiquette, J., Cheng, K.J., Costerton, J., and Milligan, L. (1988). Effect of tannins on the digestibility of two isosynthetic strains of birdsfoot trefoil (*Lotus corniculatus* L.) using *in vitro* and *in sacco* techniques. *Canadian Journal of Animal Science*. 68(3): 751-760.

Chollot, B., Chapon, L., and Urion, E. (1962). Polyphenolisehe und proteinisehe VorNiufer der Oxydationstriibung. (Nancy, Versuchslab. d. Brauerei-Hochschule.). *Brauwiss*. 15: 321- 329.

Chung, K.T., Lu, Z., and Chou, M. (1998). Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. *Food and Chemical Toxicology*. 36(12): 1053-1060.

Cieslak, A., Zmora, P., Pers-Kamczyc, E., and Szumacher-Strabel, M. (2012). Effects of tannins source (*Vaccinium vitis idaea* L.) on rumen microbial fermentation *in vivo*. *Animal Feed Science and Technology*. 176(1): 102-106.

Clausen, T. P., Provenza, F. D., Burritt, E. A., Reichardt, P. B., and Bryant, J. (1990). Ecological implications of condensed tannin structure: a case study. *Journal of Chemical Ecology*. 16(8): 2381-2392.

Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M., and Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*. 21(18): 3674-3676.

Coombridge, B. A. (1997). *Pinus radiata* bark extractives: flavonoids and tannins. University of Waikato.

Cortés, J. E., Moreno, B., Pabón, M. L., Avila, P., Kreuzer, M., Hess, H. D., and Carulla, J. E. (2009). Effects of purified condensed tannins extracted from *Calliandra*, *Flemingia* and *Leucaena* on ruminal and postruminal degradation of soybean meal as estimated *in vitro*. *Animal Feed Science and Technology*. 151(3): 194-204.

Cottle, D., Nolan, J., and Wiedemann, S. (2011). Ruminant enteric methane mitigation: a review. *Animal Production Science*. 51(6): 491-514.

Czerkawski, J. (1986). Degradation of solid feeds in the rumen: spatial distribution of microbial activity and its consequences. Paper presented at the proceedings of 6th International Symposium on Ruminant Physiology, Banff (Canada). 10-14 Sep 1984.

Czochanska, Z., Foo, L. Y., Newman, R. H., and Porter, L. J. (1980). Polymeric proanthocyanidins. Stereochemistry, structural units, and molecular weight. *Journal of the Chemical Society, Perkin Transactions*. 1: 2278-2286.

Dalzell, S. A., and Kerven, G. L. (1998). A rapid method for the measurement of *Leucaena* spp proanthocyanidins by the proanthocyanidin (butanol/HCl) assay. *Journal of the Science of Food and Agriculture*. 78(3): 405-416.

Dawra, R. K., Makkar, H. P., and Singh, B. (1988). Protein-binding capacity of microquantities of tannins. *Analytical Biochemistry*. 170(1): 50-53.

De Bruyne, T., Pieters, L., Deelstra, H., and Vlietinck, A. (1999). Condensed vegetable tannins: biodiversity in structure and biological activities. *Biochemical Systematics and Ecology*. 27(4): 445-459.

De Groot, J. C., Williams, B. A., Oostdam, A. J., Boer, H., and Tamminga, S. (1998). The use of cumulative gas and volatile fatty acid production to predict *in vitro* fermentation kinetics of Italian ryegrass leaf cell walls and contents at various time intervals. *British Journal of Nutrition*. 79(06): 519-525.

De Oliveira, S. G., Berchielli, T. T., dos Santos, M., Primavesi, O., Frighetto, R., and Lima, M. A. (2007). Effect of tannin levels in sorghum silage and concentrate supplementation on apparent digestibility and methane emission in beef cattle. *Animal Feed Science and Technology*. 135(3): 236-248.

Dehority, B. (1978). Specificity of rumen ciliate protozoa in cattle and sheep. *The Journal of Protozoology*. 25(4): 509-513.

Dehority, B. (2003). Rumen Microbiology. Nottingham University Press Nottingham: UK.

Dehority, B. A., and Odenyo, A. A. (2003). Influence of diet on the rumen protozoal fauna of indigenous African wild ruminants. *Journal of Eukaryotic Microbiology*. 50(3): 220-223.

Deng, W., Xi, D., Mao, H., and Wanapat, M. (2008). The use of molecular techniques based on ribosomal RNA and DNA for rumen microbial ecosystem studies: a review. *Molecular biology reports*. 35(2): 265-274.

Denman, S. E., and McSweeney, C. S. (2006). Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. *FEMS Microbiolology Ecolology*. 58(3): 572-582.

Denman, S. E., Tomkins, N. W., and McSweeney, C. S. (2007). Quantitation and diversity analysis of ruminal methanogenic populations in response to the antimethanogenic compound bromochloromethane. *FEMS Microbiology Ecology*. 62(3): 313-322.

Diaz-Hernandez. (2000). Tannin with anthelmintic properties J.D. Brooker (Ed.), Proceedings of the International Workshop on Tannin in Livestock and Human Nutrition, ACIAR Proceedings No. 92 (2000), pp. 140–154.

Dierenfeld, E. S., du Toit, R., and Braselton, W. E. (1995). Nutrient composition of selected browses consumed by black rhinoceros (*Diceros bicornis*) in the Zambezi Valley, Zimbabwe. *Journal of Zoo and Wildlife Medicine*. 220-230.

Dijkstra, J., Forbes, J. M., and France, J. (2005). Quantitative aspects of ruminant digestion and metabolism: CAB International.

D'Mello, J. F., and Devendra, C. (1995). *Tropical legumes in animal nutrition*: CAB International.

Eadie, J. M. (1962). Inter-relationships between certain rumen ciliate protozoa. *Journal of General Microbiology*. 29(4): 579-588.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., and Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*. 27(16): 2194-2200.

Ellis, J., Dijkstra, J., Bannink, A., Kebreab, E., Hook, S., Archibeque, S., and France, J. (2012). Quantifying the effect of monensin dose on the rumen volatile fatty acid profile in high-grain-fed beef cattle. *Journal of Animal Scienec*. 90(8): 2717-2726.

Eugène, M., Archimede, H., and Sauvant, D. (2004). Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants. *Livestock Production Science*. 85(1): 81-97.

Faint, M., McNeill, D., Stewart, J., Castillo, A., Acasio, R., and Lynch, J. (1998). Palatability of *Leucaena* to ruminants. Paper presented at the *Leucaena*-Adaptation, Quality and Farming Systems.

FAOSTAT. (2006). FAO statistical databases. Food and Agric. Organ. of the United Nations, Rome. FAOSTAT: FAO statistical databases. Food and Agric. Organ. of the United Nations, Rome.

FAOSTAT. (2014). Animal production online database. Food and Agriculture Organization, Rome, Italy.

Field, J., and Lettinga, G. (1992). Toxicity of tannic compounds to microorganisms *Plant polyphenols*. Springer: pp. 673-692.

Field, J., Kortekaas, S., and Lettinga, G. (1989). The tannin theory of methanogenic toxicity. *Biological Wastes*. 29(4): 241-262.

Finlay, B. J., Esteban, G., Clarke, K. J., Williams, A. G., Embley, T. M., and Hirt, R. P. (1994). Some rumen ciliates have endosymbiotic methanogens. *FEMS Microbiology Letter*s. 117(2): 157-161.

Firkins, J., and Yu, Z. (2006). Characterisation and quantification of the microbial populations in the rumen. Ruminant physiology, digestion, metabolism and impact of nutrition on gene expression, immunology and stress, 19-54.

Foo, L. Y., and Porter, L. J. (1980). The phytochemistry of proanthocyanidin polymers. *Phytochemistry*. 19(8): 1747-1754.

Foo, L., Lu, Y., McNabb, W., Waghorn, G., and Ulyatt, M. (1997). Proanthocyanidins from *Lotus pedunculatus*. *Phytochemistry*. 45(8): 1689-1696.

Foo, L., Newman, R., Waghorn, G., McNabb, W., and Ulyatt, M. (1996). Proanthocyanidins from *Lotus corniculatus*. *Phytochemistry*. 41(2): 617-624.

Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D. W., and Myhre, G. (2007). Changes in atmospheric constituents and in radiative forcing. Chapter 2 *Climate Change 2007. The Physical Science Basis*.

Forster, R. J., Gong, J., and Teather, R. M. (1997). Group-specific 16S rRNA hybridization probes for determinative and community structure studies of *Butyrivibrio fibrisolvens* in the rumen. *Applied Environmental Microbiology*. 63(4): 1256-1260.

Frutos, P., Hervas, G., Giráldez, F. J., and Mantecón, A. R. (2004). Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research*. 2(2): 191-202.

Fu, C., Loo, A. E. K., Chia, F. P. P., and Huang, D. (2007). Oligomeric proanthocyanidins from mangosteen pericarps. *Journal of Agricultural and Food Chemistry*. 55(19): 7689-7694.

Galimberti, A., Spada, M., Russo, D., Mucedda, M., Agnelli, P., Crottini, A., and Casiraghi, M. (2012). Integrated operational taxonomic units (IOTUs) in echolocating bats: a bridge between molecular and traditional taxonomy.

Garcia, G., Ferguson, T., Neckles, F., and Archibald, K. (1996). The nutritive value and forage productivity of *Leucaena leucocephala*. *Animal Feed Science and Technology*. 60(1): 29-41.

Getachew, G., Blümmel, M., Makkar, H., and Becker, K. (1998). *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. *Animal Feed Science and Technology*. 72(3): 261-281.

Getachew, G., Said, A., and Sundstøl, F. (1994). The effect of forage legume supplementation on digestibility and body weight gain by sheep fed a basal diet of maize stover. *Animal Feed Science and Technology*. 46(1): 97-108.

Ghali, M., Scott, P., and Al Jassim, R. (2004). Characterization of *Streptococcus bovis* from the rumen of the dromedary camel and Rusa deer. *Letters in applied microbiology*. 39(4): 341-346.

Grainger, C., Clarke, T., Auldist, M., Beauchemin, K., McGinn, S., Waghorn, G., and Eckard, R. J. (2009). Potential use of Acacia mearnsii condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. *Canadian Journal of Animal Science*. 89(2): 241-251.

Griswold, K. E., White, B. A., and Mackie, R. I. (1999). Proteolytic activities of the starch-fermenting ruminal bacterium, *Streptococcus bovis*. *Current microbiology*. 39(4): 180-186.

Gross, G. (1999). Biosynthesis of hydrolysable tannins. In: Pinto, B. (Ed.), comprehensive natural products chemistry: carbohydrates and their derivatives including tannins. cellulose, and related lignins., 3. *Elsevier, Amsterdam.*, pp. 799-826.

Guindon, S., and Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic biology*. 52(5): 696-704.

Hagerman, A. E. (1989). Chemistry of tannin-protein complexation chemistry and significance of condensed tannins (pp. 323-333): Springer.

Hagerman, A. E., and Butler, L. G. (1978). Protein precipitation method for the quantitative determination of tannins. *Journal of agricultural and food chemistry*. 26(4): 809-812.

Hagerman, A. E., and Butler, L. G. (1980). Condensed tannin purification and characterization of tannin-associated proteins. *Journal of agricultural and food chemistry*. 28(5): 947-952.

Hagerman, A. E., Riedl, K. M., Jones, G. A., Sovik, K. N., Ritchard, N. T., Hartzfeld, P. W., and Riechel, T. L. (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of agricultural and food chemistry*. 46(5): 1887-1892.

Hagerman, A., and Butler, L. G. (1991). Tannins and lignins. *Herbivores: their interactions with secondary plant metabolites*. 1:355-388.

Hagerman, A.E. Modified Vanillin Assay (for molecular weight estimation). Avaliable online: http://www.users.miamioh.edu/hagermae/Modified%20Vanillin%20Assay%20for%20MW%20determination.pdf (accessed on 12 November 2013).

Hamady, M., Walker, J. J., Harris, J. K., Gold, N. J., and Knight, R. (2008). Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. *Nature methods*. 5(3): 235-237.

Harborne, J. (1999). Recent advances in chemical ecology. *Natural product reports*. 16(4): 509-523.

Hariadi, B. T., and Santoso, B. (2010). Evaluation of tropical plants containing tannin on *in vitro* methanogenesis and fermentation parameters using rumen fluid. *Journal of the Science of Food and Agriculture*. 90(3): 456-461.

.

Hart, J. H., and Hillis, W. (1972). Inhibition of wood-rotting fungi by ellagjtaiinins in the heartwood of quercus alba. *Phytopathology*. 62:620

Hart, K., Yanez-Ruiz, D., Duval, S., McEwan, N., and Newbold, C. (2008). Plant extracts to manipulate rumen fermentation. *Animal Feed Science and Technology*. 147(1): 8-35.

Haslam, E. (1974). Polyphenol–protein interactions. *Biochemical Journal*. 139(1), 285-288.

Haslam, E. (1989). Plant polyphenols: vegetable tannins revisited: CUP Archive.

Haslam, E. (1996). Natural polyphenols (vegetable tannins) as drugs: possible modes of action. *Journal of natural products*. 59(2): 205-215.

Haslam, E. (1998). *Practical polyphenolics: From structure to molecular recognition and physiological function*; Cambridge University Press: Cambridge, UK.

Hegarty, R. (1999a). Mechanisms for competitively reducing ruminal methanogenesis. *Crop and Pasture Science*. 50(8): 1299-1306.

Hegarty, R. (1999b). Reducing rumen methane emissions through elimination of rumen protozoa. *Crop and Pasture Science*. 50(8): 1321-1328.

Hemingway, R. W. (1989). Structural variations in proanthocyanidins and their derivatives *Chemistry and significance of condensed tannins* (pp. 83-107): Springer.

Hess, H., Tiemann, T., Noto, F., Carulla, J., and Kreuzer, M. (2006). Strategic use of tannins as means to limit methane emission from ruminant livestock. *Paper presented at the International Congress Series*. 1293:164–167.

Hess, H.-D., Kreuzer, M., Dıaz, T., Lascano, C. E., Carulla, J. E., Soliva, C. R., and Machmüller, A. (2003a). Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid. *Animal feed science and technology*. 109(1): 79-94.

Hess, H.-D., Monsalve, L., Lascano, C. E., Carulla, J. E., Diaz, T., and Kreuzer, M. (2003b). Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* fruits: effects on *in vitro* ruminal nitrogen turnover and methanogenesis. *Crop and Pasture Science*. 54(7): 703-713.

Hirata, A., and Murakami, K. S. (2009). Archaeal RNA polymerase. *Current opinion in structural biology*. 19(6): 724-731.

Hobson, P. N., and Stewart, C. S. (1997). The rumen microbial ecosystem: 2nd edition. New York: Blackie Academic and Professional.

Hook, S. E., Northwood, K. S., Wright, A. D., and McBride, B. W. (2009). Long-term monensin supplementation does not significantly affect the quantity or diversity of methanogens in the rumen of the lactating dairy cow. *Appl Environ Microbiol*. 75(2): 374-380. doi: 10.1128/aem.01672-08.

Hook, S. E., Wright, A. D., and McBride, B. W. (2010). Methanogens: methane producers of the rumen and mitigation strategies. *Archaea*. 945785. doi: 10.1155/2010/945785.

Horigome, T., Kumar, R., and Okamoto, K. (1988). Effects of condensed tannins prepared from leaves of fodder plants on digestive enzymes *in vitro* and in the intestine of rats. *British journal of nutrition*. 60(02): 275-285.

Horvarth, P. J. (1981). The nutritional and ecological significance of Acer-tannins and related polyphenols: MSc thesis, *Cornell University*, Ithaca, New York, USA.

Hove, L., Topps, J., Sibanda, S., and Ndlovu, L. (2001). Nutrient intake and utilisation by goats fed dried leaves of the shrub legumes *Acacia angustissima*, *Calliandra calothyrsus* and *Leucaena leucocephala* as supplements to native pasture hay. *Animal Feed Science and Technology*. 91(1): 95-106.

Huang, X. D., Liang, J. B, Tan, H. Y, Yahya, R., and Ho, Y. W. (2011a). Effects of *Leucaena* condensed tannins of differing molecular weights on *in vitro* CH_4 production. *Animal Feed Science and Technology*. 166: 373-376.

Huang, X. D, Liang, J. B., Tan, H. Y., Yahya, R., Khamseekhiew, B., and Ho, Y. W. (2010). Molecular weight and protein binding affinity of *Leucaena* condensed tannins and their effects on *in vitro* fermentation parameters. *Animal feed science and technology*. 159(3): 81-87.

Huang, X. D., Liang, J. B., Tan, H . Y., Yahya, R., Long, R., Ho, Y.W. (2011b). Protein-binding affinity of *Leucaena* condensed tannin of differing molecular weights. *Journal of Agricutudal and Food Chemistry*. 59: 10677–10682.

Huws, S. A., Kim, E. J., Lee, M. R., Scott, M. B., Tweed, J. K., Pinloche, E., Scollan, N. D. (2011). As yet uncultured bacteria phylogenetically classified as *Prevotella*, *Lachnospiraceae incertae sedis* and unclassified *Bacteroidales*, *Clostridiales* and *Ruminococcaceae* may play a predominant role in ruminal biohydrogenation. *Environmental microbiology*. 13(6): 1500-1512.

IAEA. (2010). *World Energy Outlook 2010* (No. 978-92-64-08624-1), Vienna, Austria.

IPCC, (2013). Climate Change 2013: Physical Science Basis, Anthropogenic and Natural Radiative Forcing: Cambridge University Press Cambridge, UK, and New York.

IPCC. (2007). Summary for Policymakers, In: Metz, B., Davidson, O.R., Bosch, P.R., Dave, R., Meyer, L.A (Eds.), Climate Change 2007: Mitigation of climate change, Contribution of Working Group III to the Fourth Assessment Report of the Intergovermental Panel on Climate Change, Cambridge University Press, Cambridge, UK and New York, NY, USA.

Iqbal, M. F., Cheng, Y.-F., Zhu, W.Y., and Zeshan, B. (2008). Mitigation of ruminant methane production: current strategies, constraints and future options. *World Journal of Microbiology and Biotechnology*. 24(12): 2747-2755.

Irbis, C., and Ushida, K. (2004). Detection of methanogens and proteobacteria from a single cell of rumen ciliate protozoa. *The Journal of general and applied microbiology*. 50(4), 203-212.

Jansman, A. (1993). Tannins in feedstuffs for simple-stomached animals. *Nutrition Research Reviews*. 6(01): 209-236.

Janssen, P. H., and Kirs, M. (2008). Structure of the archaeal community of the rumen. *Applied and Environmental Microbiology*. 74(12): 3619-3625.

Jarvis, G. N., Strömpl, C., Burgess, D. M., Skillman, L. C., Moore, E. R., and Joblin, K. N. (2000). Isolation and identification of ruminal methanogens from grazing cattle. *Current microbiology*. 40(5): 327-332.

Joblin, K. (1999). Ruminal acetogens and their potential to lower ruminant methane emissions. *Crop and Pasture Science*. 50(8): 1307-1314.

Joblin, K. (2005). Methanogenic archaea. in methods in gut microbial ecology for ruminants ed. Makkar, H.P.S. and McSweeney, C.S, Netherlands IAEA, pp. 47–53.

Johnson, K. A., and Johnson, D. E. (1995). Methane emissions from cattle. *Journal of animal science*. *73*(8): 2483-2492.

Jones, G., Jakober, K., Bae, H., McAllister, T., Cheng, K., and Muir, A. (1993). *Some interactions between condensed tannins of forage legumes, bovine serum albumin and five strains of proteolytic rumen bacteria.* Paper presented at the Proceedings of the VII World Conference on Animal Production. Edmonton, Alberta, Canada.

Jones, G., McAllister, T., Muir, A., and Cheng, K.J. (1994). Effects of sainfoin (*Onobrychis viciifolia Scop*.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. *Applied and Environmental Microbiology*. 60(4): 1374-1378.

Jones, R. (1979). The value of *Leucaena leucocephala* as a feed for ruminants in the tropics. *World animal review*. 31(1): 3-2.

Jones, R., and Barnes, P. (1996). *In vitro* digestibility assessment of tropical shrub legumes using rumen fluid or faecal fluid as the inoculum source. *Tropical Grasslands*. 30: 374-377.

Jones, W. T., and Mangan, J. L. (1997). Complexes of the condensed tannins of sainfoin with fraction 1 leaf protein and with sub maxillary mucoprotein and the reversal by polyethylene glycol and pH. *Journal of the Science Food and Agriculture*. 28(2): 126-136.

Kabasa, J., Opuda-Asibo, J., and Ter Meulen, U. (2000). The effect of oral administration of polyethylene glycol on faecal helminth egg counts in pregnant goats grazed on browse containing condensed tannins. *Tropical Animal Health and Production*. 32(2): 73-86.

Kamalak Filho, A., JMP, C. O., Gurbuz, Y., Ozay, O., and Ozkan, C. O. (2004). Chemical composition and its relationship to *in vitro* dry matter digestibility

of several tannin-containing trees and shrub leaves. *Livestock Research for Rural Development*. 16: 1-8.

Kamra, D., Patra, A., Chatterjee, P., Kumar, R., Agarwal, N., and Chaudhary, L. (2008). Effect of plant extracts on methanogenesis and microbial profile of the rumen of buffalo: a brief overview. *Animal Production Science*. 48(2): 175-178.

Kamra, D., Sawal, R., Pathak, N., Kewalramani, N., and Agarwal, N. (1991). Diurnal variation in ciliate protozoa in the rumen of black buck (*Antilope cervicapra*) fed green forage. *Letters in applied microbiology*/. 13(3): 165-167.

Karnati, S., Yu, Z., Sylvester, J., Dehority, B., Morrison, M., and Firkins, J. (2003). Technical note: specific PCR amplification of protozoal 18S rDNA sequences from DNA extracted from ruminal samples of cows. *Journal of animal science*. 81(3): 812-815.

Kawamoto, H., Nakatsubo, F., and Murakami, K. (1996). Stoichiometric studies of tannin-protein co-precipitation. *Phytochemistry*. 41(5): 1427-1431.

Khamseekhiew, B. (2006). Characteristics and protein binding affinity of condensed tannins in *Leucaena* species. PhD. Thesis. *Universiti Putra Malaysia, Serdang, Malaysia*.

Kim, M., Morrison, M., and Yu, Z. (2011). Status of the phylogenetic diversity census of ruminal microbiomes. *FEMS Microbiology Ecology*. 76(1): 49-63. doi: 10.1111/j.1574-6941.2010.01029.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular evolution*. 16 (2): 111-120.

Kišidayová, S., Varadyova, Z., Zeleňák, I., and Siroka, P. (2000). Methanogenesis in rumen ciliate cultures of *Entodinium caudatum* and *Epidinium ecaudatum* after long-term cultivation in a chemically defined medium. *Folia microbiologica*. 45(3): 269-274.

Kittelmann, S., and Janssen, P. H. (2011). Characterization of rumen ciliate community composition in domestic sheep, deer, and cattle, feeding on varying diets, by means of PCR-DGGE and clone libraries. *FEMS microbiology ecology*. 75(3): 468-481.

Kittelmann, S., Seedorf, H., Walters, W. A., Clemente, J. C., Knight, R., Gordon, J. I., and Janssen, P. H. (2013). Simultaneous amplicon sequencing to explore co-occurrence patterns of bacterial, archaeal and eukaryotic microorganisms in rumen microbial communities. *PLoS One*. 8(2): e47879.

Klein, D., Janda, P., Steinborn, R., Muller, M., Salmons, B., and Gunzburg, W. H. (1999). Proviral load determination of different feline immunodeficiency virus isolates using real-time polymerase chain reaction: influence of mismatches on quantification. *Electrophoresis*. 20: 291-299.

Kocherginskaya, S. A., Aminov, R. I., and White, B. A. (2001). Analysis of the rumen bacterial diversity under two different diet conditions using denaturing gradient gel electrophoresis, random sequencing, and statistical ecology approaches. *Anaerobe*. 7(3): 119-134.

Koenig, K., Newbold, C., McIntosh, F., and Rode, L. (2000). Effects of protozoa on bacterial nitrogen recycling in the rumen. *Journal of animal science*. 78(9), 2431-2445.

Koike, S., and Kobayashi, Y. (2001). Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*. *FEMS microbiology letters*. 204(2): 361-366.

Kok, C. M., Sieo, C. C., Tan, H. Y., Saad, W. Z., Liang, J. B., and Ho, Y. W. (2013). Anaerobic cellulolytic rumen fungal populations in goats fed with and without *Leucaena leucocephala* hybrid, as determined by real-time PCR. *Journal of Microbiology*. 51(5): 700-703.

Komolong, M. K. (2003). Condensed tannin of fresh *Leucaena* forages and their role in protein digestion and nitrogen retention of weaner sheep. PhD Thesis. The University of Queensland.

Komolong, M., Barber, D., and McNeill, D. (2001). Post-ruminal protein supply and N retention of weaner sheep fed on a basal diet of lucerne hay (*Medicago sativa*) with increasing levels of quebracho tannins. *Animal Feed Science and Technology*, 92(1), 59-72.

Kong, Y., Teather, R., and Forster, R. (2010). Composition, spatial distribution, and diversity of the bacterial communities in the rumen of cows fed different forages. *FEMS Microbiology Ecology*. 74(3): 612-622.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., and Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied Environmental Microbiology*. 79(17): 5112-5120.

Kraus, T., Yu, Z., Preston, C., Dahlgren, R., and Zasoski, R. (2003). Linking chemical reactivity and protein precipitation to structural characteristics of foliar tannins. *Journal of Chemical Ecology*. 29(3): 703-730.

Krause, D., and Russell, J. (1996). How many ruminal bacteria are there? *Journal of Dairy Science*. 79(8): 1467-1475.

Kumar, R., and Horigome, T. (1986). Fractionation, characterization and protein-precipitating capacity of the condensed tannins from *Robinia pseudoacacia* L. leaves. *Journal of Agricultural and Food Chemistry*. 34(3): 487-489.

Kumar, R., and D'mello, J. (1995). Anti-nutritional factors in forage legumes. In: D'Mello, J.P.F., Devendra, C. (Eds.),. *Tropical legumes in animal nutrition. CAB International, Wallingford, Oxon, UK*. 95-133.

Kumar, R., and Singh, M. (1984). Tannins: their adverse role in ruminant nutrition. *Journal of Agricultural and Food Chemistry*. 32(3): 447-453.

Kumar, R., and Vaithiyanathan, S. (1990). Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. *Animal Feed Science and Technology*. 30(1): 21-38.

Kumar, S., Puniya, A. K., Puniya, M., Dagar, S. S., Sirohi, S. K., Singh, K., and Griffith, G. W. (2009). Factors affecting rumen methanogens and methane mitigation strategies. *World Journal of Microbiology and Biotechnology*. 25(9): 1557-1566.

Lane, D. (1991). 16S/23S rRNA sequencing. in Nucleic Acid Techniques in Bacterial Systematics, eds Stackebrandt E, Goodfellow M (Wiley, New York). pp 115–175.

Lascano, C., Avila, P., and Stewart, J. (2003). Intake, digestibility and nitrogen utilization by sheep fed with provenances of *Calliandra calothyrsus* Meissner with different tannin structure. *Archivos Latinoamericanos de Producción Animal*. 11(1): 21-28.

Latham, M., and Wolin, M. (1977). Fermentation of cellulose by *Ruminococcus flavefaciens* in the presence and absence of *Methanobacterium ruminantium*. *Applied and Environmental Microbiology*. 34(3): 297-301.

Lazarus, S. A., Adamson, G. E., Hammerstone, J. F., and Schmitz, H. H. (1999). High-performance liquid chromatography/mass spectrometry analysis of proanthocyanidins in foods and beverages. *Journal of agricultural and food chemistry*. 47(9): 3693-3701.

Le Houerou, H. (1980). The current state of knowledge. In: Proceedings of the International Symposium on Browse in Africa, Addis Ababa, April 8–12, *Browse in Africa*: International Livestock Centre for Africa Addis Ababa, Ethiopia.

Leinmüller, E., Steingass, H., and Menke, K. (1991). Tannins in ruminant feedstuffs. *Biannual Collection of Recent German Contributions Concerning Development through Animal Research*. 33: 9-62.

Leng, J., Zhong, X., Zhu, R., Yang, S., Gou, X., and Mao, H. (2011). Assessment of protozoa in *Yunnan Yellow* cattle rumen based on the 18S rRNA sequences. *Molecular biology reports*. 38(1): 577-585.

Leser, T. D., Amenuvor, J. Z., Jensen, T. K., Lindecrona, R. H., Boye, M., and Møller, K. (2002). Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. *Applied and Environmental Microbiology*. 68(2): 673-690.

Li, Y. G., Tanner, G., and Larkin, P. (1996). The DMACA–HCl protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. *Journal of the Science of Food and Agriculture*. 70(1): 89-101.

Li, Z., Wright, A. D. G., Liu, H., Fan, Z., Yang, F., Zhang, Z., and Li, G. (2015). Response of the rumen microbiota of sika deer (*Cervus nippon*) fed different concentrations of tannin rich plants. *PLoS ONE*. 10(5): e0123481. doi:10.1371

Liang, J., and Khamseekhiew, B. (2006). *Binding affinity of condensed tannins for protein complexes of selected Leucaena species.* Paper presented at the Proceedings of the XII AAAP Animal Science Congress, BEXCO, Busan, Korea.

Lindmark, D. G., and Müller, M. (1973). Hydrogenosome, a cytoplasmic organelle of the anaerobic flagellate Tritrichomonas foetus, and its role in pyruvate metabolism. *Journal of Biological Chemistry*. 248(22): 7724-7728.

Liu, Y., and Whitman, W. B. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annals of the New York Academy of Sciences*. 1125(1): 171-189.

Liu, Z., DeSantis, T. Z., Andersen, G. L., and Knight, R. (2008). Accurate taxonomy assignments from 16S rRNA sequences produced by highly parallel pyrosequencers. *Nucleic acids research*. 36(18): e120. doi: 10.1093/nar/gkn491.

Loman, N. J., Misra, R. V., Dallman, T. J., Constantinidou, C., Gharbia, S. E., Wain, J., and Pallen, M. J. (2012). Performance comparison of benchtop high-throughput sequencing platforms. *Nature biotechnology*. 30(5): 434-439.

Longo, C., Abdalla, A., Liebich, J., Janzik, I., Hummel, J., Correa, P., Burauel, P. (2013). Evaluation of the effects of tropical tanniferous plants on rumen microbiota using qRT PCR and DGGE analysis. *Czech J. Anim. Sci*. 58: 106-116.

Lowry, J. (1989). Green-leaf fractionation by fruit bats: is this feeding behaviour a unique nutritional strategy for herbivores? *Wildlife Research*. 16(2): 203-206.

Lueders, T., and Friedrich, M. W. (2003). Evaluation of PCR amplification bias by terminal restriction fragment length polymorphism analysis of small-subunit rRNA and *mcr*A genes by using defined template mixtures of methanogenic pure cultures and soil DNA extracts. *Applied and Environmental Microbiology*. 69(1): 320-326.

Luo, R., Liu, B., Xie, Y., Li, Z., Huang, W., Yuan, J., and Liu, Y. (2012). SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *Gigascience*. 1(1): 18.

Luton, P. E., Wayne, J. M., Sharp, R. J., and Riley, P. W. (2002). The *mcr*A gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. *Microbiology*. 148(11): 3521-3530.

Machmüller, A., Soliva, C. R., and Kreuzer, M. (2003). Effect of coconut oil and defaunation treatment on methanogenesis in sheep. *Reproduction Nutrition Development*. 43(1): 41-55.

Mackie, R. I., and Bryant, M. P. (1994). Acetogenesis and the rumen: syntrophic relationships. In: Drake, H.L. (Ed.), Acetogenesis. Chapman and Hall, New York, NY, USA, pp. 331–364.

Maie, N., Behrens, A., Knicker, H., and Kögel-Knabner, I. (2003). Changes in the structure and protein binding ability of condensed tannins during decomposition of fresh needles and leaves. *Soil Biology and Biochemistry*. 35(4): 577-589.

Makgobatlou. (2004). Effects of tannins at finisher stage on fat content and broiler chicken carcass characteristics. M Sc thesis, University of Limpopo, South Africa.

Makkar, H. (2003). Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research*. 49(3): 241-256.

Makkar, H. P. S., Dawra, R. K., and Singh, B. (1987). Protein precipitation assay for quantitation of tannins: determination of protein in tannin-protein complex. *Analytical Biochemistry*. 166(2): 435-439.

Makkar, H. P., Blümmel, M., and Becker, K. (1995a). *In vitro* effects of and interactions between tannins and saponins and fate of tannins in the rumen. *Journal of the Science of Food and Agriculture*. 69(4): 481-493.

Makkar, H. P., Blümmel, M., and Becker, K. (1995b). Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in *in vitro* techniques. *British Journal of Nutrition*. 73(06): 897-913.

Mané, C., Sommerer, N., Yalcin, T., Cheynier, V., Cole, R., and Fulcrand, H. (2007a). Assessment of the molecular weight distribution of tannin fractions through MALDI-TOF MS analysis of protein-tannin complexes. *Analytical chemistry*. 79(6): 2239-2248.

Mané, V. P., Patil, S. S., Syed, A. A., and Baig, M. M. V. (2007b). Bioconversion of low quality lignocellulosic agricultural waste into edible protein by *Pleurotus sajor*-caju (Fr.) Singer. *Journal of Zhejiang University Science B*. 8(10): 745-751.

Mangino, J., Peterson, K., and Jacobs, H. (2003). Development of an emissions model to estimate methane from enteric fermentation in cattle. U. S. Environmental Protection Agency. Available: http://www.epa.gov/ttn/chief/conference/ei12/green/mangino.pdf [accessed 13.01.15].

Martin, C., Morgavi, D. P., and Doreau, M. (2010). Methane mitigation in ruminants: from microbe to the farm scale. *Animal*. 4(3): 351-365. doi: 10.1017/s1751731109990620.

Matsui, H., Ogata, K., Tajima, K., Nakamura, M., Nagamine, T., Aminov, R. I., and Benno, Y. (2000). Phenotypic characterization of polysaccharidases produced by four *Prevotella* type strains. *Current microbiology*. 41(1): 45-49.

Matthews, S., Mila, I., Scalbert, A., and Donnelly, D. M. (1997). Extractable and non-extractable proanthocyanidins in barks. *Phytochemistry*. 45(2): 405-410.

McAllister, T., Bae, H., Jones, G., and Cheng, K. (1995). Microbial attachment and feed digestion in the rumen. *Journal of animal science*. 72(11): 3004-3018.

McAllister, T. A., Cheng, K. J., Okine, E. K., and Mathison, G. W. (1996). Dietary, environmental and microbiological aspects of methane production in ruminants. *Canadian Journal of Animal Science*. 76(2): 231-243. doi: 10.4141/cjas96-035.

McAllister, T. A., Martinez, T., Bae, H. D., Muir, A. D., Yanke, L. J., and Jones, G. A. (2005). Characterization of condensed tannins purified from legume forages: chromophore production, protein precipitation, and inhibitory effects on cellulose digestion. *Journal of chemical ecology*. 31(9): 2049-2068.

McAllister, T., and Newbold, C. J. (2008). Redirecting rumen fermentation to reduce methanogenesis. *Animal Production Science*. 48(2): 7-13.

McAllister, T., Bae, H., Yanke, L., Cheng, K.-J., and Muir, A. (1994b). Effect of condensed tannins from birdsfoot trefoil on endoglucanase activity and the digestion of cellulose filter paper by ruminal fungi. *Canadian journal of microbiology*. 40(4): 298-305.

McAllister, T., Cheng, K.-J., Okine, E., and Mathison, G. (1996). Dietary, environmental and microbiological aspects of methane production in ruminants. *Canadian Journal of Animal Science*. 76(2): 231-243.

McCaughey, W., Wittenberg, K., and Corrigan, D. (1999). Impact of pasture type on methane production by lactating beef cows. *Canadian Journal of Animal Science*. 79(2): 221-226.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., and Morgan. (2002). Animal nutrition. 6th edition. *Pearson Education Limited*. *Harlow*. *Essex*, UK. Pg, 266-277.

McMahon, L., McAllister, T., Berg, B., Majak, W., Acharya, S., Popp, J., Coulman, E., Y. Wang, Y., and Cheng, K.-J. (2000). A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. *Canadian Journal of Plant Science*. 80(3): 469-485.

McNabb, W. C., Peters, J. S., Foo, L. Y., Waghorn, G. C., and Jackson, F. S. (1998). Effect of condensed tannins prepared from several forages on the *in vitro*

precipitation of ribulose-1, 5-bisphosphate carboxylase (Rubisco) protein and its digestion by trypsin (EC 2.4. 21.4) and chymotrypsin (EC 2.4. 21.1). *Journal of the Science of Food and Agriculture*. 77(2): 201-212.

McNabbl, W., Waghorn, G., Barry, T., and Shelton, I. (1993). The effect of condensed tannins in *Lotus pedunculatus* on the digestion and metabolism of methionine, cystine and inorganic sulphur in sheep. *British Journal of Nutrition*. 70(02): 647-661.

McNeill, D. M., Osborne, N., Komolong, M., and Nankervis, D. (1998). Condensed tannins in the *Leucaena* genus and their nutritional significance for ruminants. In: Shelton, H.M., Gutteridge, R.C., Mullin, B.F., Bray, R.A. (Eds.), *Leucaena* — Adaptation, Quality and Farming Systems. ACIAR Proceedings No. 86: pp. 205–214.

McSweeney, C. S., Palmer, B., Bunch, R., and Krause, D. O. (1999). Isolation and characterization of proteolytic ruminal bacteria from sheep and goats fed the tannin-containing shrub legume *Calliandra calothyrsus*. *Applied Environmental Microbiology*. 65(7): 3075-3083.

McSweeney, C. S, Palmer, B., McNeill, D., and Krause, D. O. (2001). Microbial interactions with tannins: nutritional consequences for ruminants. *Animal Feed Science and Technology*. 91(1): 83-93.

Menke, K. H., and Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Develapment*. 28(1): 7-55.

Min, B. R., Solaiman, S., Shange, R., and Eun, J.-S. (2014). Gastrointestinal bacterial and methanogenic archaea diversity dynamics associated with condensed tannin-containing pine bark diet in goats using 16S rDNA amplicon pyrosequencing. *International journal of microbiology, 2014*. Article ID 141909, http://dx.doi.org/10.1155/2014/141909.

Min, B., Attwood, G., McNabb, W., Molan, A., and Barry, T. (2005). The effect of condensed tannins from Lotus corniculatus on the proteolytic activities and growth of rumen bacteria. *Animal Feed Science and Technology*. 121(1): 45-58.

.

Min, B., Attwood, G., Reilly, K., Sun, W., Peters, J., Barry, T., and McNabb, W. (2002). *Lotus corniculatus* condensed tannins decrease *in vivo* populations of proteolytic bacteria and affect nitrogen metabolism in the rumen of sheep. *Canadian journal of microbiology*. 48(10): 911-921.

Min, B., Barry, T., Attwood, G., and McNabb, W. (2003). The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Animal feed science and technology*. 106(1): 3-19.

Min, B., McNabb, W., Barry, T., Kemp, P., Waghorn, G., and McDonald, M. (1999). The effect of condensed tannins in *Lotus corniculatus* upon reproductive

efficiency and wool production in sheep during late summer and autumn. *The Journal of Agricultural Science*. 132(03): 323-334.

Molan, A., Attwood, G., Min, B., and McNabb, W. (2001). The effect of condensed tannins from *Lotus pedunculatus* and *Lotus corniculatus* on the growth of proteolytic rumen bacteria *in vitro* and their possible mode of action. *Canadian Journal of Microbiology*. 47(7): 626-633.

Monagas, M., Quintanilla-López, J. E., Gómez-Cordovés, C., Bartolomé, B., and Lebrón-Aguilar, R. (2010a). MALDI-TOF MS analysis of plant proanthocyanidins. *Journal of pharmaceutical and biomedical analysis*. 51(2): 358-372.

Monagas, M., Urpi-Sarda, M., Sánchez-Patán, F., Llorach, R., Garrido, I., Gómez-Cordovés, C., Andres-Lacueva C., and Bartolomé, B. (2010b). Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. *Food and function*. 1(3): 233-253.

Montossi, F., Hodgson, J., and Morris, S. (1997). Herbage intake, ingestive behaviour and diet selection, and effects of condensed tannins upon body and wool growth in lambs grazing *Lolium perenne* and *Holcus lanatus* swards in summer. *New Zealand journal of agricultural research*. 40(4): 449-461.

Moss, A. R., Jouany, J.P., and Newbold, J. (2000). Methane production by ruminants: its contribution to global warming. *Paper presented at the Annales de zootechnie*.

Muchuweti, M., Ndhlala, A. R., and Kasiyamhuru, A. (2005). Estimation of the degree of polymerization of condensed tannins of some wild fruits of Zimbabwe (*Uapaca kirkiana* and *Ziziphus mauritiana*) using the modified vanillin-HCl method. *Journal of the Science of Food and Agriculture*. 85(10): 1647-1650.

Mueller-Harvey, I. (2006). Unravelling the conundrum of tannins in animal nutrition and health. *Journal of the Science of Food and Agriculture*. 86(13): 2010-2037.

Mueller-Harvey, I., and Caygill, J. (1999). Tannins: their nature and biological significance. *Secondary plant products: antinutritional and beneficial actions in animal feeding.* 17-39.

Mueller-Harvey, I., McAllan, A., Theodorou, M., and Beever, D. (1988). Phenolics in fibrous crop residues and plants and their effects on digestion and utilization of carbohydrates and proteins in ruminants. *Plant breeding and the nutritive value of crop residues*. 97-132.

Muinga, R., Topps, J., Rooke, J., and Thorpe, W. (1995). The effect of supplementation with *Leucaena leucocephala* and maize bran on voluntary food intake, digestibility, live weight and milk yield of Bos indicus × Bos taurus dairy cows and rumen fermentation in steers offered *Pennisetum purpureum ad libitum* in the semi-humid tropics. *Animal Science*. 60(1): 13-23.

Murray, R., Bryant, A., and Leng, R. (1976). Rates of production of methane in the rumen and large intestine of sheep. *British journal of nutrition*. 36(01): 1-14.

Muyzer, G., De Waal, E. C., and Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied Environmental Microbiology*. 59(3): 695-700.

Naczk, M., Amarowicz, R., Zadernowski, R., and Shahidi, F. (2001). Protein precipitating capacity of condensed tannins of beach pea, canola hulls, evening primrose and faba bean. *Food chemistry*. 73(4): 467-471.

Naczk, M., Oickle, D., Pink, D., and Shahidi, F. (1996). Protein precipitating capacity of crude canola tannins: effect of pH, tannin, and protein concentrations. *Journal of agricultural and food chemistry*. 44(8): 2144-2148.

NAS. (1977). *Leucaena*: promising forage and tree crops for the tropics. National Research Council: Washington, DC: National Academy Press.

Nelson, K. E., Thonney, M. L., Woolston, T. K., Zinder, S. H., and Pell, A. N. (1998). Phenotypic and phylogenetic characterization of ruminal tannin-tolerant bacteria. *Applied Environmental Microbiology*. 64(10): 3824-3830.

Nelson, K., Pell, A., Schofield, P., and Zinder, S. (1995). Isolation and characterization of an anaerobic ruminal bacterium capable of degrading hydrolyzable tannins. *Applied and Environmental Microbiology*. 61(9): 3293-3298.

Newbold, C., Lassalas, B., and Jouany, J. (1995). The importance of methanogens associated with ciliate protozoa in ruminal methane production *in vitro*. *Letters in applied microbiology*. 21(4): 230-234.

Nicholson, M. J., Evans, P. N., and Joblin, K. N. (2007). Analysis of methanogen diversity in the rumen using temporal temperature gradient gel electrophoresis: identification of uncultured methanogens. *Microbial Ecology*. 54(1): 141-150.

Norton, B., Gutteridge, R., and Shelton, H. (1994). The nutritive value of tree legumes. *Forage tree legumes in tropical agriculture.* 177-191.

Norton, B., Lowry, B., and McSweeney, C. (1995). *The nutritive value of Leucaena species.* Paper presented at the ACIAR proceedings.

NRC, 7th revised edn. (2001). National Research Council National Academy Press, Washington, DC.

Nunez-Hernandez, G., Wallace, J., Holechek, J., Galyean, M., and Cardenas, M. (1991). Condensed tannins and nutrient utilization by lambs and goats fed low-quality diets. *Journal of animal science*. 69(3): 1167-1177.

Odenyo, A. A., and Osuji, P. O. (1998). Tannin-tolerant ruminal bacteria from East African ruminants. *Canadian Journal of Microbiology*. 44(9): 905-909.

Okuda, T., Yoshida, T., and Hatano, T. (2000). Correlation of oxidative transformations of hydrolyzable tannins and plant evolution. *Phytochemistry*. 55(6): 513-529.

Ørskov, E., and McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *The Journal of Agricultural Science*. 92(02): 499-503.

Osborne, N. J. T., and McNeill, D. M. (2001). Characterisation of *Leucaena* condensed tannins by size and protein precipitation capacity. *Journal of the Science of Food and Agriculture*. 81(11): 1113-1119.

Otsyina, R., and Dzowela, B. (1995). Importance of Leucaena in Africa. Pg 25-39 in W.M. Ciesla and L.Nshubemuki (eds). *Leucaena psyllied* a threat to agroforestry in Africa. 237 pp. FAO Rome.

Otsyina, R., Norton, B., and Djimde, M. (1999). *Fodder trees and shrubs in arid and semi-arid livestock production systems.* Paper presented at the XVIII international grassland congress.

Ovreås, L., Forney, L., Daae, F. L., and Torsvik, V. (1997). Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Applied environment Microbioloy*. 63(9): 3367-3373.

Parks, D. H., Tyson, G. W., Hugenholtz, P., and Beiko, R. G. (2014). STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics*. 30(21): 3123-3124.

Patra, A. K., and Saxena, J. (2009). Dietary phytochemicals as rumen modifiers: a review of the effects on microbial populations. *Antonie van Leeuwenhoek, 96*(4), 363-375.

Patra, A. K., and Saxena, J. (2011). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture*. 91(1): 24-37.

Paul, K., Nonoh, J. O., Mikulski, L., and Brune, A. (2012). "*Methanoplasmatales*," *Thermoplasmatales*-related archaea in termite guts and other environments, are the seventh order of methanogens. *Applied Environment Microbiology*. 78(23): 8245-8253. doi: 10.1128/aem.02193-12.

Perez-Maldonado, R. A., Norton, B. W., and Kerven, G. L. (1995). Factors affecting *in vitro* formation of tannin-protein complexes. *Journal of the Science of Food and Agriculture*. 69(3): 291-298.

Perez-Maldonado, R., and Norton, B. (1996). Digestion of C-labelled condensed tannins from *Desmodium intortum* in sheep and goats. *British journal of nutrition*. 76(04): 501-513.

Petty, T. W., and Cecava, M. J. (Eds.). (1995). *Beef cattle feeding and nutrition*. Elsevier.

Pinares-Patiño, C., Ulyatt, M., Lassey, K., Barry, T., and Holmes, C. W. (2003). Persistence of differences between sheep in methane emission under generous grazing conditions. *The Journal of Agricultural Science*. 140(2): 227-233.

Porter, L. J., and Woodruffe, J. (1984). Haemanalysis: the relative astringency of proanthocyanidin polymers. *Phytochemistry*. 23(6): 1255-1256.

Pottinger, A., and Hughes, C. (1995). A review of wood quality in *Leucaena*. In: ACIAR Proceedings no. 57: *Leucaena* - opportunities and limitations. 98-102.

Poulsen, M., Schwab, C., Jensen, B. B., Engberg, R. M., Spang, A., Canibe, N., and Urich, T. (2013). Methylotrophic methanogenic *Thermoplasmata* implicated in reduced methane emissions from bovine rumen. *Nature Communications*. 4: 1428. doi: 10.1038/ncomms2432.

Pritchard, D., Martin, P., and O'rourke, P. (1992). The role of condensed tannins in the nutritional value of mulga (*Acacia aneura*) for sheep. *Crop and Pasture Science*. 43(8): 1739-1746.

Provenza, F. D. (1995). Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *Journal of Range Management Archives*. 48(1): 2-17.

Puchala, R., Min, B., Goetsch, A., and Sahlu, T. (2005). The effect of a condensed tannin-containing forage on methane emission by goats. *Journal of animal science*. 83(1): 182-186.

Quail, M. A., Smith, M., Coupland, P., Otto, T. D., Harris, S. R., Connor, T. R., **Bertoni,** A., **Harold, P., Swerdlow, H.P.,** and Gu, Y. (2012). A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC genomics*. 13(1): 341.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., and Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, gks1219.

Rakhmani, S., Brooker, J., Jones, G., and Palmer, B. (2005). Composition of condensed tannins from *Calliandra calothyrsus* and correlation with *in sacco* digestibility. *Animal Feed Science and Technology*. 121(1): 109-124.

Ranilla, M., Jouany, J. P., and Morgavi, D. (2007). Methane production and substrate degradation by rumen microbial communities containing single protozoal species *in vitro*. *Letters in applied microbiology*. 45(6): 675-680.

Reddy, K. M., Feris, K., Bell, J., Wingett, D. G., Hanley, C., and Punnoose, A. (2007). Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. *Applied physics letters*. 90(213902): 213902-1–213902-3.

Reed, J. D. (1995). Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of animal science*. 73(5): 1516-1528.

Regensbogenova, M., Pristas, P., Javorsky, P., Moon-van der Staay, S., Van Der Staay, G., Hackstein, J., and McEwan, N. R. (2004). Assessment of ciliates in the sheep rumen by DGGE. *Letters in applied microbiology*. 39(2): 144-147.

Rusdi, R. (2004). *In vitro* studies of the interaction between selected protein and condensed tannins *Jurnal Agroland*. 11(1): 90-96.

Russell, J. (1998). The importance of pH in the regulation of ruminal acetate to propionate ratio and methane production *in vitro*. *Journal of Dairy Science*. 81(12): 3222-3230.

Russell., J. B . (2002). Rumen microbiology and its role in ruminant nutrition. J. B. Russell Publ., Ithaca, New York.

Sallam, S. M., Bueno, I. C., Nasser, M. E., and Abdalla, A. L. (2010). Effect of eucalyptus (*Eucalyptus citriodora*) fresh or residue leaves on methane emission *in vitro*. *Italian Journal of Animal Science*. 9(3): 58.

Saucier, C., Mirabel, M., Daviaud, F., Longieras, A., and Glories, Y. (2001). Rapid fractionation of grape seed proanthocyanidins. *Journal of agricultural and food chemistry*. 49(12): 5732-5735.

Scalbert, A. (1991). Antimicrobial properties of tannins. *Phytochemistry*. 30(12): 3875-3883.

Schink, B. (1997). Energetics of syntrophic cooperation in methanogenic degradation. *Microbiology and Molecular Biology Reviews*. 61(2): 262-280.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., and Robinson, C. J. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied Environmental Microbiology*. 75(23): 7537-7541.

Schofield, P., Mbugua, D., and Pell, A. (2001). Analysis of condensed tannins: a review. *Animal Feed Science and Technology*. 91(1): 21-40.

Sharp, R., Ziemer, C. J., Stern, M. D., and Stahl, D. A. (1998). Taxon-specific associations between protozoal and methanogen populations in the rumen and a model rumen system. *FEMS microbiology ecology*. 26(1): 71-78.

Shelton, H., and Brewbaker, J. (1998). *Leucaena leucocephala*-the Most Widely Used Forage Tree Legume. Forage Tree Legumes in Tropical Agriculture. *The Tropical Grassland Society of Australia*.

Shin, E., Cho, K., Lim, W., Hong, S., An, C., Kim, E., and Kang, J. (2004). Phylogenetic analysis of protozoa in the rumen contents of cow based on the 18S rDNA sequences. *Journal of applied microbiology*. 97(2): 378-383.

Shinkai, T., and Kobayashi, Y. (2007). Localization of ruminal cellulolytic bacteria on plant fibrous materials as determined by fluorescence in situ hybridization and real-time PCR. *Applied Environmental Microbiology*. 73(5): 1646-1652.

Silanikove, N., Gilboa, N., Perevolotsky, A., and Nitsan, Z. (1996). Goats fed tannin-containing leaves do not exhibit toxic syndromes. *Small Ruminant Research*. 21(3): 195-201.

Silanikove, N., Nitsan, Z., and Perevolotsky, A. (1994). Effect of a daily supplementation of poly (ethylene glycol) on intake and digestion of tannin-containing leaves (*Ceratonia siliqua*) by sheep. *Journal of agricultural and food chemistry*. 42(12): 2844-2847.

Silanikove, N., Perevolotsky, A., and Provenza, F. D. (2001). Use of tannin-binding chemicals to assay for tannins and their negative postingestive effects in ruminants. *Animal Feed Science and Technology*. 91(1): 69-81.

Singleton, V. L. (1981). Naturally occurring food toxicants: phenolic substances of plant origin common in foods. *Advance Food Research*. 27: 149-242.

Sirohi, S., Chaudhary, P., and Goel, N. (2012). Effect of inclusion of *Myristica fragrans* on methane production, rumen fermentation parameters and methanogens population. *Veterinary World*. 5(6): 335-340.

Skerman, P. J., Cameron, D., and Riveros, F. (1988). Tropical forage legumes (2nd Edition, rivised and expanded). FAO.

Skillman, L. C., Toovey, A. F., Williams, A. J., and Wright, A.-D. G. (2006a). Development and validation of a real-time PCR method to quantify rumen protozoa and examination of variability between *Entodinium* populations in sheep offered a hay-based diet. *Applied and environmental microbiology*. 72(1): 200-206.

Skillman, L., Evans, P., Strömpl, C., and Joblin, K. (2006b). 16S rDNA directed PCR primers and detection of methanogens in the bovine rumen. *Letters in applied microbiology*. 42(3): 222-228.

Smith, A. H., Zoetendal, E., and Mackie, R. I. (2005). Bacterial mechanisms to overcome inhibitory effects of dietary tannins. *Microbial Ecology*. 50(2): 197-205.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., and Sirotenko, O. 2007. Agriculture. In: Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Eds B. Metz, O.R. Davidson PR, Bosch R, Dave and Meyer LA. Cambridge University Press, Cambridge: United Kingdom and New York, NY, USA.

Soltan, Y., Morsy, A., Sallam, S., Louvandini, H., and Abdalla, A. (2012). Comparative in vitro evaluation of forage legumes (prosopis, acacia, atriplex, and leucaena) on ruminal fermentation and methanogenesis. *Journal of Animal and Feed Sciences*. 575: 54.

SPSS. (1999). SPSS forWindows (Release 16.0), Standard Version. SPSS, Chicago, IL.

Stanton, T., and Canale-Parola, E. (1980). *Treponema bryantii* sp. nov., a rumen spirochete that interacts with cellulolytic bacteria. *Archives of Microbiology*. 127(2): 145-156.

Steelman, S. M., Chowdhary, B. P., Dowd, S., Suchodolski, J., and Janečka, J. E. (2012). Pyrosequencing of 16S rRNA genes in fecal samples reveals high diversity of hindgut microflora in horses and potential links to chronic laminitis. *BMC veterinary research*. 8(1): 231.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., and Haan, C. D. (2006). *Livestock's long shadow: environmental issues and options*: Food and Agriculture Organization of the United Nations (FAO).

Stewart, C.S., Flint, H.J., F., and Bryant, M.P. (1997). The rumen bacteria. In The Rumen Microbial Ecosystem ed. Hobson PN, Stewart CS, London: Blackie Academic and Professional, pp. 10–72.

Stienezen, M., Waghorn, G., and Douglas, G. (1996). Digestibility and effects of condensed tannins on digestion of sulla (*Hedysarum coronarium*) when fed to sheep. *New Zealand Journal of Agricultural Research*. 39(2): 215-221.

Svedström, U., Vuorela, H., Kostiainen, R., Huovinen, K., Laakso, I., and Hiltunen, R. (2002). High-performance liquid chromatographic determination of oligomeric procyanidins from dimers up to the hexamer in hawthorn. *Journal of Chromatography A*. 968(1): 53-60.

Sylvester, J. T., Karnati, S. K., Yu, Z., Morrison, M., and Firkins, J. L. (2004). Development of an assay to quantify rumen ciliate protozoal biomass in cows using real-time PCR. *The Journal of nutrition*. 134(12): 3378-3384.

Tajima, K., Aminov, R. I., Nagamine, T., Ogata, K., Nakamura, M., Matsui, H., and Benno, Y. (1999). Rumen bacterial diversity as determined by sequence analysis of 16S rDNA libraries. *FEMS Microbiology Ecology*. 29(2): 159-169.

Tajima, K., Arai, S., Ogata, K., Nagamine, T., Matsui, H., Nakamura, M., Aminov. R . I., and Benno, Y. (2000). Rumen bacterial community transition during adaptation to high-grain diet. *Anaerobe*. 6(5): 273-284.

Tajima, K., Nagamine, T., Matsui, H., Nakamura, M., and Aminov, R. I. (2001). Phylogenetic analysis of archaeal 16S rRNA libraries from the rumen suggests the existence of a novel group of archaea not associated with known methanogens. *FEMS microbiology letters*. 200(1): 67-72.

Tan, H. Y., Sieo, C. C., Abdullah, N., Liang, J. B., Huang, X. D., and Ho, Y. W. (2013). Effect of condensed tannins on bovine rumen protist diversity based on 18S rRNA gene sequences. *Journal of Eukaryotic Microbiology*. 60(1): 98-100.

Tan, H., Sieo, C., Abdullah, N., Liang, J., Huang, X., and Ho, Y. (2011a). Effects of condensed tannins from *Leucaena* on methane production, rumen fermentation and populations of methanogens and protozoa *in vitro*. *Animal feed science and technology*. 169(3): 185-193.

Tan, H., Sieo, C., Abdullah, N., Liang, J., Huang, X., and Ho, Y. (2011b). Effects of condensed tannins from *Leucaena* on methane production, rumen fermentation and populations of methanogens and protozoa *in vitro*. *Animal Feed Science and Technology*. 169(3): 185-193.

Tan, H.Y. (2012). Effects of condensed tannins from *Leucaena leucocephala* hybrid on methane mitigation, rumen fermentation and populations of methanogens and protozoa *in vitro*. PhD. Thesis. Universiti Putra Malaysia, Serdang, Malaysia.

Tavendale, M. H., Meagher, L. P., Pacheco, D., Walker, N., Attwood, G. T., and Sivakumaran, S. (2005). Methane production from *in vitro* rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. *Animal Feed Science and Technology*. 123: 403-419.

Taylor, A. W., Barofsky, E., Kennedy, J. A., and Deinzer, M. L. (2003). Hop (*Humulus lupulus* L.) proanthocyanidins characterized by mass spectrometry, acid catalysis, and gel permeation chromatography. *Journal of agricultural and food chemistry*. 51(14): 4101-4110.

Terrill, T., Rowan, A., Douglas, G., and Barry, T. (1992). Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of the Science of Food and Agriculture*. 58(3): 321-329.

Terrill, T., Windham, W., Evans, J., and Hoveland, C. (1990). Condensed tannin concentraton in *Sericea lespedeza* as influenced by preservation method. *Crop ScienecI*. 30: 219-224.

Thoetkiattikul, H., Mhuantong, W., Laothanachareon, T., Tangphatsornruang, S., Pattarajinda, V., Eurwilaichitr, L., and Champreda, V. (2013). Comparative analysis of microbial profiles in cow rumen fed with different dietary fiber by tagged 16S rRNA gene pyrosequencing. *Current microbiology*. 67(2): 130-137.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research*. 22(22): 4673-4680.

Tiemann, T. T., Lascano, C. E., Wettstein, H.-R., Mayer, A. C., Kreuzer, M., and Hess, H. D. (2008). Effect of the tropical tannin-rich shrub legumes *Calliandra calothyrsus* and *Flemingia macrophylla* on methane emission and nitrogen and energy balance in growing lambs. *Animal*. 2(5): 790-799.

Tilley, J., and Terry, R. (1963). A two-stage technique for the *in vitro* digestion of forage crops. *Grass and forage science*. 18(2): 104-111.

Ungerfeld, E., Rust, S., Boone, D., and Liu, Y. (2004). Effects of several inhibitors on pure cultures of ruminal methanogens. *Journal of applied microbiology*. 97(3): 520-526.

Ushida, K., Newbold, C. J., and Jouany, J.-P. (1997). Interspecies hydrogen transfer between the rumen ciliate *Polyplastron multivesiculatum* and *Methanosarcina barkeri*. *The Journal of general and applied microbiology*. 43(2): 129-131.

Van Horn, H., Wilkie, A., Powers, W., and Nordstedt, R. (1994). Components of dairy manure management systems. *Journal of Dairy Science*. 77(7): 2008-2030.

Van Sumere, C., Albrecht, J., Dedonder, A., De Pooter, H., and Pe, I. (1975). Plant proteins and phenolics. *The chemistry and biochemistry of plant proteins*. 11: 211-264.

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., Cheynier, V., and Waters, E. J. (2003). The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *Journal of the Science of Food and Agriculture*. 83(6): 564-573.

Vogels, G. D., Hoppe, W. F., and Stumm, C. K. (1980). Association of methanogenic bacteria with rumen ciliates. *Applied and Environmental Microbiology*. 40(3): 608-612.

Waghorn, G. C. (1990). Beneficial effects of low concentrations of condensed tannins in forages fed to ruminants. In *Microbial and plant opportunities to improve lignocelluloses utilization by ruminants*; Akin, D.E., Ljungdahl, L.G., Wilson, J.R., Harris, P.J., Eds.; Elsevier Science Publishing Co.: New York, NY, USA, pp. 137–147.

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

Waghorn, G. C., and McNabb, W. C. (2003). Consequences of plant phenolic compounds for productivity and health of ruminants. *Proceedings of the Nutrition Society*, *62*(02), 383-392.

Waghorn, G. C., and Shelton, I. (1997). Effect of condensed tannins in *Lotus corniculatus* on the nutritive value of pasture for sheep. *The Journal of Agricultural Science*. 128(03): 365-372.

Waghorn, G. C., and Woodward, S. (2006). Ruminant contributions to methane and global warming—A New Zealand perspective. *Climate Change and Managed Ecosystems*, 233.

Waghorn, G. C., Jones, W., Shelton, I., and McNabb, W. (1990). *Condensed tannins and the nutritive value of herbage.* Paper presented at the Proceedings of the New Zealand Grassland Association.

Waghorn, G. C., Shelton, I., McNabb, W., and McCutcheon, S. (1994). Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. *The Journal of Agricultural Science*. 123(01): 109-119.

Waghorn, G. C., Tavendale, M., and Woodfield, D. (2002). *Methanogenesis from forages fed to sheep.* Paper presented at the Proceedings of the New Zealand Grassland Association. 64:167–171.

Waghorn, G. C., Ulyatt, M., John, A., and Fisher, M. (1987). The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. *British journal of nutrition*. 57(01): 115-126.

Waghorn, G., Clark, H., Taufa, V., and Cavanagh, A. (2008). Monensin controlled-release capsules for methane mitigation in pasture-fed dairy cows. *Animal Production Science*. 48(2): 65-68.

Wang, C., Wang, S., and Zhou, H. (2009). Influences of flavomycin, ropadiar, and saponin on nutrient digestibility, rumen fermentation, and methane emission from sheep. *Animal Feed Science and Technology*. 148(2): 157-166.

Wang, H., Nair, M. G., Iezzoni, A. F., Strasburg, G. M., Booren, A. M., and Gray, J. I. (1997). Quantification and characterization of anthocyanins in Balaton tart cherries. *Journal of agricultural and food chemistry*. 45(7): 2556-2560.

Wang, Y., Douglas, G., Waghorn, G., Barry, T., and Foote, A. (1996). Effect of condensed tannins in *Lotus corniculatus* upon lactation performance in ewes. *The Journal of Agricultural Science*. 126(3): 353-362.

Wang, Y., Waghorn, G., Barry, T., and Shelton, I. (1994). The effect of condensed tannins in *Lotus corniculatus* on plasma metabolism of methionine, cystine and inorganic sulphate by sheep. *British Journal of Nutrition*. 72(06): 923-935.

Waterman, P. G., and Mole, S. (1994). *Analysis of phenolic plant metabolites*: Blackwell Scientific. Publications, Oxford, pp. 238.

Wei, S.-D., Zhou, H.-C., and Lin, Y.-M. (2011). Antioxidant activities of fractions of polymeric procyanidins from stem bark of *Acacia* confusa. *International journal of molecular sciences*. 12(2): 1146-1160.

Wheeler, R., Norton, B., and Shelton, H. (1995). *Condensed tannins in Leucaena species and hybrids and implications for nutritive value.* Paper presented at the ACIAR PROCEEDINGS.

Whitford, M. F., Teather, R. M., and Forster, R. J. (2001). Phylogenetic analysis of methanogens from the bovine rumen. *BMC microbiology*. 1(1): 5.

Williams, A., and Coleman, G. (1992). The rumen protozoa, Brock: Springer Series in Contemporary Bioscience, Springer-Verlag, New York.

Windham,W.R., Petersen, J.C., and Terrill, T.H. (1990). Tannins as anti-quality factors in forage. in D. E. Akin, L. G. Ljungdahl, J.R. Wilson and P.J. Harris, ed. Microbial and plant opportunities to improve lignocellulose utilization by ruminants. Elsevier Science Publishing Co., New York, Pages 127-135.

Wolin, M. J. (1979). The rumen fermentation: a model for microbial interactions in anaerobic ecosystems *Advances in microbial ecology* (pp. 49-77): Springer
.

Wolin, M., Miller, T., and Stewart, C. (1997). Microbe-microbe interactions *The rumen microbial ecosystem*. pp. 467-491: Springer.

Wong, A., Kim, Y., Singh, A., and Ling, W. (2005). Natural durability of tropical species with emphasis on Malaysian hardwoods—variations and prospects. Paper presented at the The international research group on wood preservation, the 36th annual meeting, Bangalore, India.

Wong, C., Chen, C., and Hutton, E. (1998). *Development of acid/psyllid tolerant Leucaena hybrids for ruminant production.* Paper presented at the ACIAR PROCEEDINGS.

Woodward, S. L., Waghorn, G. C., and Laboyrie, P. G. (2004). Condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) reduce methane emissions from dairy cows. Paper presented at the proceedings-new zealand society of animal production. 64:160–164.

Woodward, S., Auldist, M., Laboyrie, P., and Jansen, E. (1999). *Effect of Lotus corniculatus and condensed tannins on milk yield and milk composition of dairy cows*. Paper presented at the Proceedings of the New Zealand Society of Animal Production.

Woodward, S., Laboyrie, P., Jansen, E., and Stone, G. (2000). *Lotus corniculatus* and condensed tannins-effects on milk production by dairy cows. *Asian-Australasian Journal of Animal Sciences*. 13(Suppl.): 521-525.

Woodward, S., Waghorn, G., Watkins, K., and Bryant, M. (2009). Feeding birdsfoot trefoil (*Lotus corniculatus*) reduces the environmental impacts of dairy farming. Paper presented at the Proceedings of the New Zealand Society of Animal Production.

Woodward, S.L., Waghorn, G.C., Ulyatt, M. J., and Lassey, K .R. (2001). Early indications that feeding lotus will reduce methane emission from ruminants. *Proceeding of the New Zealand Society of Animal Production*. 61: 23–26.

Wright, A.-D., Ma, X., and Obispo, N. E. (2008). Methanobrevibacter phylotypes are the dominant methanogens in sheep from Venezuela. *Microb Ecol, 56*(2), 390-394. doi: 10.1007/s00248-007-9351.

Wright, A.-D. G., Auckland, C. H., and Lynn, D. H. (2007). Molecular diversity of methanogens in feedlot cattle from Ontario and Prince Edward Island, Canada. *Applied and Environmental Microbiology*. 73(13): 4206-4210.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., and Diemer, M. (2004). The worldwide leaf economics spectrum. *Nature, 428*(6985), 821-827.

Yanagita, K., Kamagata, Y., Kawaharasaki, M., Suzuki, T., Nakamura, Y., and Minato, H. (2000). Phylogenetic analysis of methanogens in sheep rumen ecosystem and detection of *Methanomicrobium mobile* by fluorescence in situ hybridization. *Bioscience, biotechnology, and biochemistry*. 64(8): 1737-1742.

Yokotsuka, K., and Singleton, V. L. (1987). Interactive precipitation between graded peptides from gelatin and specific grape tannin fractions in wine-like model solutions. *American journal of enology and viticulture*. 38(3): 199-206.

Yu, Y., Lee, C., Kim, J., and Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnology and bioengineering*. 89(6): 670-679.

Zened, A., Sylvie, C., Laurent, C., Jérôme, M., Christophe, K., Olivier, B., and Francis, E. (2013). Microbial ecology of the rumen evaluated by 454 GS FLX pyrosequencing is affected by starch and oil supplementation of diets. *FEMS microbiology ecology*. 83(2): 504-514

.

Zhang, L.-L., Lin, Y.-M., Zhou, H.-C., Wei, S.-D., and Chen, J.-H. (2010). Condensed tannins from mangrove species *Kandelia candel* and *Rhizophora mangle* and their antioxidant activity. *Molecules*. 15(1): 420-431.

Zhang, L.-M., Wang, M., Prosser, J. I., Zheng, Y.-M., and He, J.-Z. (2009). Altitude ammonia-oxidizing bacteria and archaea in soils of Mount Everest. *FEMS Microbiology Ecology*, 70(2): 208-217.

Zhou, H., Qiao, K., Gao, Z., Meehan, M. J., Li, J. W.-H., Zhao, X., Dorrestein, P. C., Vederas, J. C., and Tang, Y. (2010). Enzymatic synthesis of resorcylic acid lactones by cooperation of fungal iterative polyketide synthases involved in hypothemycin biosynthesis. *Journal of the American Chemical Society*. 132(13): 4530-4531.

Zhou, M., Hernandez-Sanabria, E., and Guan, L. L. (2009). Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. *Applied Environmenatl Microbiololgy*. 75(20): 6524-6533. doi: 10.1128/aem.02815-08.

Zhou, Y. W., McSweeney, C. S., Wang, J. K., and Liu, J. X. (2012). Effects of disodium fumarate on ruminal fermentation and microbial communities in sheep fed on high-forage diets. *Animal*. 6(05): 815-823.