UNIVERSITI PUTRA MALAYSIA

EFFECTS OF FUNGAL TREATMENT ON RICE STRAW DIGESTIBILITY AND RUMEN METHANE MITIGATION

MOHD AZLAN BIN PAUZI

ITA 2015 8

EFFECTS OF FUNGAL TREATMENT ON RICE STRAW DIGESTIBILITY AND RUMEN METHANE MITIGATION

By

MOHD AZLAN BIN PAUZI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master Of Science

August 2015

COPYRIGHT

DEDICATION

To My Family

My Parents
Pauzi Bin Che Hassan
Ani Binti Abdullah

My Loyal Wife
Nurul Azlina Binti Takiyudin

My Cute Sons
Muhammad Arif Ikmal
Muhammad Alif Ikhwan

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

EFFECTS OF FUNGAL TREATMENT ON RICE STRAW DIGESTIBILITY AND RUMEN METHANE MITIGATION

By

MOHD AZLAN BIN PAUZI

August 2015

Chairman: **Liang Juan Boo, PhD**
Institute: **Tropical Agriculture**

Rice straw is a major global agro-byproduct with about 90% are produced in Asia. Although some of this agro-byproduct is used as roughage source for ruminant livestock, its low nutritive value and high non-digestible fibre contents limit its use. In addition, fermentation of high fibre diet in the rumen is accompanied by high rate of enteric methane production, which in return causes 7-10 % of the dietary energy loss. Enteric methane production from ruminants contributes about 33-39 % of total methane, a greenhouse gas, from agricultural sector which has been implicated as a major cause of global warming and climate change. Improving the nutritive value of agro-byproduct such as rice straw will allow it to break down quickly, thus reduces its retention time in the rumen and lowers its contribution on methane production. Nutritive values of lignocellulosic materials could be improved by biological treatment using fungi. A recent laboratory study in Universiti Putra Malaysia reported that *Aspergillus terreus* (ATCC 74135) can effectively breakdown lignocellulose component of rice straw and also produce lovastatin which has the potential to mitigate methane production *in vitro*. Thus, the primary objective of this follow-up study was to evaluate the effects of fungal treatment on rice straw digestibility and methane mitigation *in vitro* and *in vivo* in goats.

The objective of the first experiment (Chapter 3) was to develop an up-scale laboratory protocol to treat rice straw with *Aspergillus terreus* (ATCC 74135) to achieve an optimized balance in reduction of lignocellulose content and production of lovastatin in the treated rice straw using solid state fermentation (SSF). The treatments consisted of 12 combinations of three levels (amount) of rice straw (300, 600 and 900 g) and four incubation durations (8, 10, 12 and 14 days) incubated at 25°C, 50% moisture and pH at 6. Untreated rice straw was used as control to compare with the treatment effects. The result showed that all treatments reduced the lignocellulose contents compared to control with hemicellulose content by 7.6 to 26.4% and cellulose content by 4.6 to 21.6%, depending on the treatment. T1 (300 g x 8 days), T2 (300 g x 10 days), T7 (600 g x 12 days) and T8 (600 g x 14 days) produced significantly higher amount of lovastatin ($P<0.05$) than the other treatments, thus the above four treatments were selected for further testing in the second experiment.

The second experiment (Chapter 4) was conducted to evaluate the effect of treated rice straw using the four treatments (T1, T2, T7 and T8) selected from the first experiment and untreated rice straw (as control) on methane production and related fermentation parameters using *in vitro* gas production procedure. Results showed that all treatments significantly reduced total gas (102.33 vs. 51.17 to 67.67 mL), CH_4 (30.21 vs. 13.46 to 18.10 mL) and CH_4: VFA (0.71 vs. 0.38 to 0.46) compared to the control ($P<0.05$). Among the treatments, T8 showed the highest (55.45%) methane reduction compared to the control. All treatments had no effect on *in vitro* dry matter digestibility (IVDMD). Based on its high methane mitigation efficacy, T8 was selected to evaluate the effect of *A. terreus* (ATCC 74135) treated rice straw on *in vivo* methane production in goats.

Eight male cross-bred Boer goats, fitted with rumen fistula, were used in the third experiment (Chapter 5). Rice straw treated using the protocol of T8 (as described in Chapters 3 and 4) was chosen as treatment and untreated rice straw as control in this feed trial. Methane production was estimated hourly for 12 hours (0800-2200h) per day using four open-circuit respiration chambers (~3,000 L). The measurement was repeated three times (triplicates) for each goat. Dry matter digestibility was determined using total fecal collection procedure and rumen liquor and blood samples were collected for VFA, microbial population and blood biochemical profile analysis. Result showed that daily methane production from goats fed on treated rice straw was reduced by 34.08% as compared to the control diet (13.27 vs. 20.13 L/day) ($P<0.01$). The above value increased to 42.36% if methane production was adjusted to per unit digestible DM intake (34.86 vs. 60.48 L). Dry matter digestibility for goats fed with treated rice straw diet improved ($P<0.05$) by 12.60% (80.0 vs. 69.9%). All treatments increased concentration of propionate ($P<0.01$) which led to a lower A/P ratio ($P<0.01$). This shift of VFA pathways to favor propionate production (a source of H_2 sink) could partly explain for the lower methane production in the treatment group. The populations of total methanogens and *Methanobacterials* in goats fed treated rice straw diet were significantly lower than that of the control ($P<0.05$). In contrast, the populations of total bacteria and *Ruminococcus albus* (cellulolytic bacteria) increased significantly in goats fed treated rice straw diet ($P<0.05$). Blood biochemical profile data showed that cholesterol ($P<0.05$) and low density lipoprotein (LDL) ($P<0.01$) in goats in the treatment group were lower than those of the control.

Results from this thesis showed that biological treatment using *A. terreus* (ATCC 74135) reduced the lignocellulose content thus improved the nutritive value of rice straw. The above fungal treatment produced lovastatin which effectively reduced methane production *in vitro* and *in vivo* (in goats) without negative effect to *in vivo* DM digestibility in goats. The reduction in populations of total methanogens and *Methanobacteriales* without negatively affecting other bacteria showed that the effect of lovastatin on methane production is specific on methanogens by inhibiting the activity of HMG-CoA reductase enzyme in the methanogens cell membrane synthesis pathway as reported in the literature. The increase in population of total bacteria and *Ruminococcus albus* (cellulolytic bacteria) further reaffirms the above. This thesis demonstrates that solid state fermentation using the appropriate fungus (e.g. *A. terreus* ATCC 74135) can improve nutritive value of agro-byproduct such as rice straw as ruminant feed and also reduce enteric methane production in ruminant livestock.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan ijazah Sarjana Sains

KESAN RAWATAN KULAT TERHADAP PENGHADAMAN JERAMI PADI DAN PENGURANGAN METANA DARI RUMEN

Oleh

MOHD AZLAN BIN PAUZI

Ogos 2015

Pengerusi: **Liang Juan Boo, PhD**
Institut: **Pertanian Tropika**

Jerami padi merupakan sejenis produk sampingan pertanian global di mana 90% penghasilannya adalah dari Asia. Walaupun sebahagian daripada bahan sampingan pertanian ini digunakan sebagai sumber rufaj untuk ternakan ruminan, nilai pemakanan yang rendah dan kandungan serat tidak hadam yang tinggi menghadkan penggunaannya. Kadar fermentasi diet berserat tinggi di dalam rumen disertai dengan penghasilan metana enterik pada kadar yang tinggi menyebabkan kehilangan tenaga dari diet antara 7-10 %. Pengeluaran metana enterik oleh ruminan menyumbang antara 33-39 % dari jumlah keseluruhan metana, sejenis gas rumah hijau, dari sektor pertanian di mana implikasinya menjadi penyebab utama kepada pemanasan global dan perubahan iklim. Meningkatkan nilai pemakanan produk sampingan pertanian seperti jerami padi membolehkan ia dimetabolismakan dengan cepat, sekaligus mengurangkan masa pengekalan di dalam rumen dan mengurangkan kadar penghasilan metana. Nilai pemakanan bagi bahan ligno-selulosa boleh dipertingkatkan melalui rawatan biologi menggunakan kulat. Kajian terbaru di makmal Universiti Putra Malaysia melaporkan *Aspergillus terreus* (ATCC 74135) berkesan untuk memecahkan komponen ligno-selulosa dalam jerami padi dan menghasilkan lovastatin di mana ia mempunyai potensi untuk mengurangkan pengeluaran metana secara *in vitro*. Tujuan utama kajian lanjutan ini ialah untuk menilai kesan rawatan kulat pada penghadaman jerami padi dan pengurangan metana secara *in vitro* dan *in vivo* pada kambing.

Objektif eksperimen pertama (Bab 3) adalah membangunkan protokol makmal bagi skala yang lebih besar bagi merawat jerami padi menggunakan *Aspergillus terreus* (ATCC 74135) untuk mencapai keseimbangan optimum dalam pengurangan kandungan ligno-selulosa dan penghasilan lovastatin pada jerami padi terawat menggunakan fermentasi fasa pepejal (SSF). Rawatan yang mengandungi 12 kombinasi melibatkan tiga kandungan jerami padi (300, 600 and 900 g) dan empat tempoh pengeraman (8, 10, 12 dan 14 hari) pada suhu pengeraman 25°C, kelembapan 50% dan nilai pH 6. Jerami padi tidak terawat digunakan sebagai kawalan untuk tujuan perbandingan bagi kesan rawatan. Keputusan menunjukkan kesemua rawatan menurunkan kandungan ligno-selulosa berbanding kawalan di mana kandungan hemiselulosa mengalami penurunan antara 7.6 hingga 26.4% selulosa dan kandungan

selulosa antara 4.6 hingga 21.6%, bergantung pada rawatan. Rawatan T1 (300 g x 8 hari), T2 (300 g x 10 hari), T7 (600 g x 12 hari) dan T8 (600 g x 14 hari) menghasilkan lovastatin yang lebih tinggi ($P<0.05$) berbanding dengan rawatan yang lain, oleh sebab itu keempat-empat rawatan di atas dipilih untuk Eksperimen kedua.

Eksperimen kedua (Bab 4) dijalankan untuk menilai kesan jerami padi terawat menggunakan empat rawatan (T1, T2, T7 and T8) yang dipilih daripada Eksperimen 1 dan jerami padi tidak terawat (sebagai kawalan) pada pengeluaran metana dan parameter fermentasi yang berkaitan menggunakan prosedur penghasilan gas *in vitro*. Keputusan menunjukkan kesemua rawatan ketara menurunkan jumlah gas (102.33 vs. 51.17 hingga 67.67 mL), CH_4 (30.21 vs. 13.46 hingga 18.10 mL) dan CH_4:VFA (0.71 vs. 0.38 hingga 0.46) berbanding kawalan ($P<0.05$). Dari semua rawatan, T8 menunjukkan kadar pengurangan (55.45%) metana tertinggi berbanding kawalan. Rawatan didapati tidak memberikan kesan pada penghadaman bahan kering *in vitro* (IVDMD). Berdasarkan keberkesanan pengurangan metana yang tinggi, T8 dipilih untuk dinilai kesan jerami padi dirawat dengan *A. terreus* (ATCC 74135) pada kambing ke atas pengeluaran metana *in vivo*.

Lapan ekor kambing jantan kacukan Boer yang dipasang dengan fistula rumen digunakan pada Eksperimen ketiga (Bab 5). Jerami padi terawat menggunakan protokol T8 (seperti diterangkan pada Bab 3 dan 4) dipilih sebagai rawatan dan jerami padi tidak terawat sebagai kawalan dalam kajian pemakanan. Pengeluaran metana diambil setiap jam selama 12 jam (0800-2200) setiap hari menggunakan empat gerobok pernafasan terbuka (~3,000 L). Pengukuran diulang sebanyak tiga kali (triplikat) untuk setiap ekor kambing. Penghadaman bahan kering ditentukan menggunakan prosedur pengumpulan jumlah tahi dan cecair rumen serta sampel darah dikumpul untuk analisa VFA, populasi mikrob dan profil biokimia darah. Keputusan menunjukkan pengeluaran metana harian daripada kambing yang diberi makanan jerami padi terawat menurun sehingga 34.08% dibandingkan dengan kambing yang diberi makanan kawalan (13.27 vs. 20.13 L/hari) ($P<0.01$). Nilai di atas meningkat sehingga 42.36% sekiranya pengeluaran metana diselaraskan kepada unit pengambilan bahan kering hadam (34.86 vs. 60.48 L). Penghadaman bahan kering pada kambing yang diberi makanan jerami padi terawat meningkat ($P<0.05$) sehingga 12.60% (80.0 vs. 69.9%). Rawatan kulat meningkatkan kandungan propionat ($P<0.01$) yang membawa kepada penurunan nisbah A/P ($P<0.01$). Pertukaran laluan VFA untuk lebih menjurus kepada penghasilan propionat (sumber penenggelaman H_2) merupakan salah satu sebab pengeluaran metana yang lebih rendah bagi kumpulan terawat. Populasi jumlah metanogen dan Metanobaktril dalam kambing yang diberi makanan jerami padi terawat ketara lebih rendah daripada kawalan ($P<0.05$). Berbeza dengan populasi jumlah bakteria dan *Ruminococcus albus* (bakteria selulolitik) di mana jumlahnya meningkat dengan ketara pada kambing yang diberi makanan jerami padi terawat ($P<0.05$). Data profil biokimia darah menunjukkan kolestrol ($P<0.05$) dan LDL ($P<0.01$) dalam kumpulan terawat adalah lebih rendah daripada kumpulan kawalan.

Keputusan dalam tesis ini menunjukkan rawatan biologi menggunakan *A. terreus* (ATCC 74135) menurunkan kandungan ligno-selulosa seterusnya meningkatkan nilai pemakanan jerami padi. Rawatan kulat di atas menghasilkan lovastatin di mana ia berkesan menurunkan pengeluaran metana *in vitro* dan *in vivo* (pada kambing) tanpa

kesan negatif pada penghadaman bahan kering *in vivo* pada kambing. Penurunan populasi jumlah metanogen dan Metanobaktril tanpa mempengaruhi bakteria lain menunjukkan kesan lovastatin kepada pengeluaran metana tertumpu kepada metanogen dengan merencatkan aktiviti enzim HMG-CoA reductase pada laluan sintesis membran sel metanogen seperti yang telah dilaporkan dalam literatur. Peningkatan populasi jumlah bakteria dan *Ruminococcus albus* (bakteria selulolitik) menyokong situasi di atas. Tesis ini menunjukkan fermentasi fasa pepejal menggunakan kulat yang sesuai (e.g. *A. terreus* ATCC 74135) boleh meningkatkan nilai pemakanan bahan sampingan pertanian seperti jerami padi sebagai makanan ruminan dan juga menurunkan pengeluaran metana enterik dalam ternakan ruminan.

ACKNOWLEDGEMENT

I would like to express my gratitude to my supervisor, Dr. Liang Juan Boo. Thank you for your patience, undivided support and unlimited encouragement throughout the period of my study.

I would like to thank my co-supervisor, Professor Dr. Mohamed Ariff Omar for his guidance and advice which help me to overcome the challenges to finish my study.

Thank you to all my fellow students in the Laboratory of Industrial Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, especially to Dr. Mohamad Faseleh Jahromi and Ms Chen Wei Li for your advise and assistance in my study.

Thank you to MARDI for supporting my study financially and helping me by granted permission to do my experiment in Animal Science laboratory at MARDI Serdang .

Special thanks to my wife, Nurul Azlina Takiyudin and my two sweet sons, Muhammad Arif Ikmal and Muhammad Alif Ikhwan. Without your support, it would become much harder for me to accomplish my study. Thanks for patience in enduring long hours I have spent away from you all. I really love you all.

I certify that a Thesis Examination Committee has met on 21 August 2015 to conduct the final examination of Mohd Azlan bin Pauzi on his thesis entitled "Effects of Fungal Treatment on Rice Straw Digestibility and Rumen Methane Mitigation" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Norhani binti Abdullah, PhD
Professor
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Chairman)

Anjas Asmara @ Ab. Hadi bin Samsudin, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Vincenzo Tufarelli, PhD
Senior Lecturer
Universiti of Bari Aldo Moro
Italy
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 5 November 2015

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

Liang Juan Boo, PhD,
Associate Professor
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Chairman)

Mohamed Ariff Omar, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

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

Date: 12 November 2015

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 No.: Mohd Azlan bin Pauzi (GS 27421)

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: Assoc. Prof. Dr. Liang Juan Boo

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Mohamed Ariff Omar

TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENTS		vi
APPROVAL		vii
DECLARATION		ix
LIST OF TABLES		xiii
LIST OF FIGURES		xiv
LIST OF ABBREVIATIONS		xv
CHAPTER		
1	**GENERAL INTRODUCTION**	1
2	**LITERATURE REVIEW**	3
	2.1 Rice straw	3
	2.2 Greenhouse gases (GHG)	4
	2.3 Methane (CH_4)	5
	2.4 Rumen ecosystem	10
	2.5 Methanogens	12
	2.6 Statins	12
	2.7 Lovastatin	14
	2.8 Biological treatment	17
	2.9 Summary	18
3	**EFFECT OF *ASPERGILLUS TERREUS* ON LIGNOCELLULOSE CONTENT AND LOVASTATIN PRODUCTION USING RICE STRAW IN SOLID STATE FERMENTATION**	20
	3.1 Introduction	20
	3.2 Materials and Methods	21
	3.2.1 Substrate	21
	3.2.2 Fungi and preparation of spore suspension	21
	3.2.3 Solid state fermentation	22
	3.2.4 Extraction and determination of lovastatin	22
	3.2.5 Chemical analysis	23
	3.2.6 Statistical analysis	23
	3.3 Results and Discussion	23
	3.3.1 Lignocellulose content	23
	3.3.2 Lovastatin production	26
	3.4 Conclusion	27
4	**EFFECT OF FUNGAL TREATED RICE STRAW ON *IN VITRO* METHANE PRODUCTION AND FEED DIGESTIBILITY**	28
	4.1 Introduction	28
	4.2 Materials and Methods	29

4.2.1 Fermented rice straw 29
4.2.2 *In vitro* gas production 29
4.2.3 Methane production 30
4.2.4 Volatile fatty acids (VFA) analysis 30
4.2.5 *In vitro* dry matter digestibility (IVDMD) 30
4.2.6 Statistical analysis 31
4.3 Results and Discussion 31
4.3.1 Effect on fermented rice straw on gas production 31
4.3.2 Effect of fermented rice straw on VFA production and IVDMD 33
4.4 Conclusion 35

5 EFFECT OF FUNGAL TREATED RICE STRAW ON *IN VIVO* METHANE PRODUCTION 36
5.1 Introduction 36
5.2 Materials and Methods 37
5.2.1 Fermented rice straw 37
5.2.2 Animal and experimental design 37
5.2.3 Digestibility trial 38
5.2.4 Determination of methane production 38
5.2.4.1 Respiration chambers 38
5.2.4.2 Estimation of methane productions 38
5.2.4.3 CH_4 estimation 39
5.2.5 Rumen liquor collection 39
5.2.5.1 VFA analysis 39
5.2.5.2 DNA extraction 40
5.2.5.3 Quantitative real-time PCR 40
5.2.6 Blood analysis for biochemical profile 42
5.2.7 Statistical analysis 42
5.3 Results and Discussion 42
5.3.1 Methane production 42
5.3.2 VFA production 45
5.3.3 Effect on microbial population 45
5.3.4 Effect of fermented rice straw on blood biochemical profile 46
5.4 Conclusion 47

6 SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH 48
6.1 Summary 48
6.2 Conclusion 50
6.3 Recommendations for future research 50

REFERENCES 51
APPENDICES 60
BIODATA OF STUDENT 66
LIST OF PUBLICATIONS 67

LIST OF TABLES

Table	Page
2-1. Rice production and crop residues of the top 10 rice producing countries in the world in 2003 (million tonnes)	3
2-2. Global natural and anthropogenic methane sources (million tonnes)	5
2-3. Global livestock population and production in different production system	7
2-4. Global methane emission from enteric fermentation in 2004 (million tonnes per year)	8
2-5. Global methane emission from manure management in 2004 (million tonnes per year)	9
2-6. Predominant ruminal bacteria, substrate and fermented products	11
3-1. Effect of *A. terreus* ATCC 74135 on lignocellulose composition of rice straw (% of DM) at different amounts and fermentation durations	25
4-1. Effect of fermented rice straw on *in vitro* gas and CH_4 production of 48-h *in vitro* incubation	32
4-2. Effect of untreated & fermented rice straw on VFA production(mM) and IVDMD(%)	34
5-1. Names,sequences,application and references of the primers used in this study	41
5-2. Effect of fermented rice straw fed to animals on CH_4 production and dry matter digestibility	44
5-3. Effect of fermented rice straw on acetate, propionate and total VFA production (mM)	45
5-4. Effect of rice straw and fermented rice straw on microbial population in the rumen fluid (cell/mL)	46
5-5. Effect of rice straw and fermented rice straw on blood biochemical profile	47

LIST OF FIGURES

Figure	Page
2-1. Molecular structure of lovastatin (Hydroxyl form) and HMG-CoA	13
2-2. Cholesterol biosynthetic pathway	13
2-3. Chemical structures of different statins	14
2-4. Lovastatin production pathway	15
2-5. Biosynthetic pathway showing cholesterol production in humans (solid-line markers) and phospholipids production in Archaea (dotted-line markers)	16
3-1. Diagram showing the 12 treatment combination	21
3-2. Lovastatin production by different treatments (T1-T12)	26
4-1. Reduction (CH_4 ml/g DM) in % of *in vitro* methane production from treated rice straw compared to untreated rice straw	33
5-1. Diagram showing the experimental schedule which consisted of 14 days adaptation period,7 days of digestibility trial and 6 days of methane measurement.	38

LIST OF ABBREVIATIONS

%	Percentage
ºC	degree Celcius
A:P	Acetate to propionate ratio
A. Terreus	*Aspergillus terreus*
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
AOAC	Association of Official Analytical Chemists
ATCC	American Type Culture Collection
bp	Base pair
BW	Body weight
$C_{24}H_{36}O_5$	Lovastatin
CH_4	Methane
cm	Centimeter
CP	Crude protein
CO_2	Carbon dioxide
CRD	Completely randomized design
CT	Threshold cycle
CU	Cellulose
d	Day
DM	Dry matter
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
FID	Flame Ionization Detector
g	Gram
GC	Gas Chromatograph
GHG	Greenhouse gases
GLM	General Linear Model
h	Hour
H_2	Hydrogen
H_2O	Water
HC	Hemicellulose
HCl	Hydrochloric acid
HMG-CoA	3-hydroxy- 3-methylglutaryl coenzyme A
HPLC	High Performance Liquid Chromatography
IPCC	Intergovernmental Panel on Climate Change
IVDMD	*In vitro* dry matter digestibility
Kg	Kilogram
L	Liter
LDL	Low-density lipoprotein
M	Molar
M. bryanti	*Methanobacterium bryanti*
M. ruminantium	*Methanobacterium ruminantium*
mg	milligram
mL	Mililiter
mm	Milimeter

mM	Mili molar
M.W.	Molecular weight
µL	micro liter
µm	micro meter
N_2O	Nitrous oxide
NaOH	Sodium hydroxide
NDF	Neutral Detergent Fibre
Nm	Nanometer
PDA	Potato Dextrose Agar
ppb	Part per billion
ppm	Part per million
rpm	Revolutions per minute
SAS	Statistical Analysis System
SD	Standard deviation
SF_6	Sulfur hexafluoride
USFDA	United States Food & Drug Administration
V	Volt
VFA	Volatile fatty acid
W/V	Weight per Volume

CHAPTER 1

GENERAL INTRODUCTION

Rice straw, with global annual production exceeding 570 million tonnes, is one of the most excessive agricultural by-products (Abdel-Mohdy *et al.*, 2009). The traditional method to dispose the rice straw after harvest is by burning leads to health and environmental concerns (Abdel-Mohdy *et al.*, 2009). Small amount of rice straw is used as roughage feed for ruminant animals. However, due to the high lignocellulose but low nitrogen contents, rice straw is poorly fermented in the rumen resulting in low animal productivity. In addition, fermentation of poor quality feed such as rice straw in rumen is associated with high rate of methane production. Biological treatment has been suggested to improve the quality of agricultural biomass such as rice straw as ruminant feed (Alborés *et al*., 2006).

Although greenhouse gases are essential to maintain atmospheric temperature on earth (Moss *et al*., 2000), high concentration of these gases will trap more heat which will increase the atmospheric temperature (IPCC, 2013). Methane is known to be the second most important greenhouse gas after carbon dioxide (Bunglavan, 2014; IPCC, 2007). Although the concentration of methane was lower than carbon dioxide, the global warming potential of methane is 34 times higher than that of carbon dioxide (IPCC, 2013). Enteric methane production from ruminants contributes about 33-39 % of total methane production from agricultural activity (Moss *et al*., 2000). Methane production from rumen fermentation also results in 7 to 10% of dietary energy loss for the host animals (Moss and Givens, 1993). Bio-hydrogen produced from carbohydrate fermentation in the rumen is used by methanogenic archaea in the process of methanogenesis to produce methane (Janssen, 2010). Due to the negative impact of enteric methane on the environment and dietary energy utilization by the host animals, many studies including feeding of methane mitigation agents have been intensively conducted to counter these impacts. One of the approaches is the use of lovastatin to suppress methanogenesis (Miller and Wolin, 2001).

Lovastatin ($C_{24}H_{36}O_5$, M.W. 404.55) is a secondary metabolite produced during the secondary phase of fungi growth (Gupta *et al*., 2007). It is an inhibitor of 3-hydroxy- 3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key enzyme in cholesterol production pathway in humans (Alberts, 1988). Part of the cell membrane synthesis pathway in archaea is similar to that of cholesterol synthesis in humans (Miller and Wolin, 2001) thus making it possible for lovastatin to be used to inhibit the growth of methanogens (Lam and Doolittle, 1992; Wolin and Miller, 1999). Although pure statin can be used to significantly reduce growth and activity of methanogenic archaea without any negative effect on cellulolytic bacteria (Wolin and Miller, 2006), it is too expensive making it irrelevant to be used as feed additive by farmers for methane mitigation.

Aspergillus terreus is one of the fungi capable of producing lovastatin (Lai *et al*., 2003; 2005). This fungus is also able to produce cellulolytic enzymes for degradation of lignocellulolytic components in rice straw (Jahromi *et al.*, 2011). By using submerged fermentation, *A. terreus* was reported as the best lovastatin producer (55mg/l) among 110 fungi examined (Siamak *et al*., 2003). Many studies have been conducted using different substrates such as rice grain, rice husk, wheat bran, soybean and corn for lovastatin production (Wei *et al*., 2007; Jaivel and Marimuthu, 2010; Pansuriya and Singhal, 2010). However, these substrates are normally expensive as they are used as human food and feed for livestock production.

Since rice straw is one of the most excessive agricultural by-products in the world, it is important to find an alternative way to utilize it. Earlier studies conducted in Universiti Putra Malaysia reported that treating rice straw with *A. terreus* reduced lignocellulose content of rice straw and produced lovastatin which inhibited methane production *in vitro* (Jahromi *et al*., 2012; 2013a). Although the use of lovastatin to mitigate methane *in vitro* (Miller and Wolin, 2001) and *in vivo* (Klevenhusen *et al.*, 2011) has been documented, these earlier studies used pure commercial lovastatin (RM 905.02 / 25 mg - http://www.sigmaaldrich.com/catalog/product/sigma/m2147?lang=en&region=MY), which is too expensive for methane mitigation in ruminant livestock under practical farm condition. In addition, fungal treatment also reduces lignocelluloses content of rice straw. Thus the novelty of this thesis is to produce lovastatin from fungal treatment using rice straw to mitigate enteric methane production and to improve its digestibility.

General objective

To evaluate the effects of fungal treatment on rice straw digestibility and methane mitigation *in vitro* and *in vivo* in goats.

Specific objectives:

i) To determine the optimal incubation conditions of rice straw using *A. terreus* (ATCC 74135) in solid state fermentation on larger laboratory bench scales (300-900 g) for the production of lovastatin and to reduce the lignocellulose content of the rice straw.
ii) To investigate the effect of treated rice straw on *in vitro* methane production and *in vitro* dry matter digestibility.
iii) To determine the ability of fermented rice straw, containing lovastatin, to reduce methane production and to alter blood biochemical profile in goats.

REFERENCES

Abdel-Mohdy, F.A., Abdel-Halim, E.S., Abu-Ayana, Y.M. and El-Sawy, S.M., (2009). Rice straw as a new resource for some beneficial uses. *Carbohydrate Polymers.* 75: 44-51.

Akin, D.E., Rigsby, L.L., Sethuraman, A., Morrison III, W.W., Gamble, G.R. and Eriksson, K.E., (1995). Alterations in structure, chemistry, and biodegradability of grass lignocellulose treated with the white rot fungi *Ceriporiopsis subvermispora* and *Cyathus stercoreus*. *Applied and Environmental Microbiology.* 61 (4): 1591-1598.

Alani, F., Grove, J.A., Anderson, W.A. and Moo-Young, M., (2009). Mycophenolic acid production in solid-state fermentation using a packed-bed bioreactor. *Biochemical Engineering Journal.* 44: 106-110.

Alberts, A.W., (1988). Discovery, biochemistry and biology of lovastatin. *The American Journal of Cardiology*. 62 (15): 10-15.

Alberts, A.W., Chen, J., Kuron, G., Hunt, V., Huff, J., Hoffman, C., Rothrock, J., Lopez, M., Joshua, H., Harris, E., Patchett, A., Monaghan, R., Currie, S., Stapley, E., Albers-Schonberg, G., Hensens, O., Hirshfield, J., Hoogsteen, K., Liesch, J. and Springer, J., (1980). Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proceedings of the National Academy of Sciences*. 77 (7): 3957-3961.

Alborés, S., Pianzzola, M.J., Soubes, M. and Cerdeiras, M.P., (2006). Biodegradation of agroindustrial wastes by *Pleurotus spp* for its use as ruminant feed. *Electronic Journal of Biotechnology.* 9 (3): 215–220.

AOAC, (1990). Official Methods of Analysis, Association of Official Analytical Chemists, Washington, DC.

Assis, L.F., Kagohara, E., Omori, A., Comasseto, J.V., Andrade, L.H. and Porto, A.L.M., (2007). Deracemization of (RS)-1-[(4-methylselanyl) phenyl] ethanol and (RS)-1-[(4-ethylselanyl) phenyl] ethanol by strains of *Aspergillus terreus*. *Food Technology and Biotechnology.* 45 (4): 415-419.

Badhan, A.K., Chadha, B.S., Kaur, J., Saini, H.S. and Bhat, M.K., (2007). Production of multiple xylanolytic and cellulolytic enzymes by thermophilic fungus *Myceliophthora sp*. IMI 387099. *Bioresource Technology.* 98: 504-510.

Barrios-Gonzalez, J., Castillo, T.E. and Mejia, A., (1993). Development of high penicillin producing strains for solid state fermentation. *Biotechnology Advances.* 11 (3): 525-537.

Beauchemin, K.A. and McGinn, S.M., (2005). Methane emissions from feedlot cattle fed barley or corn diets. *Journal Animal Science*. 83: 653-661.

Bisaria, R., Madan, M. and Vasudevan, P., (1997). Utilisation of agro-residues as animal feed through bioconversion. *Bioresource Technology.* 59: 5-8.

Brooker, J.D., Lum, D.K., Miller, S., Skene, I. and O'Donovan, L., (1995). Rumen microorganisms as providers of high quality protein. *Livestock Research for Rural Development.* 6 (3): *Article #23*. (http://www.lrrd.org/lrrd6/3/1.htm).

Bunglavan, S.J., (2014). Methanogenesis and recent techniques for mitigation of methanogenesis in ruminants. *Journal of Livestock Science*. 5: 35-48.

Calzada, J., Franco, L., De Arriola, M., Rolz, C. and Ortiz, M., (1987). Acceptability, body weight changes and digestibility of spent wheat straw after harvesting of *Pleurotus sajor-caju*. *Biological Wastes.* 22: 303-309.

Casas López, J.L., Sanchez Perez, J.A., Fernandez Sevilla, J.M., Acien Fernandez, F.G., Molina Grima, E. and Chisti, Y., (2003). Production of lovastatin by *Aspergillus terreus*: effects of the C: N ratio and the principal nutrients on growth and metabolite production. *Enzyme and Microbial Technology.* 33: 270-277.

Conde, K., Roy, S., Freake, H.C., Newton, R.S. and Fernandez, M.L., (1999). Atorvastatin and simvastatin have distinct effects on hydroxy methylglutaryl-CoA reductase activity and mRNA abundance in the guinea pig. *Lipids.* 34 (12): 1327-1332.

De Rosa, M., Gambacorta, A. and Gliozzi, A., (1986). Structure, biosynthesis, and physicochemical properties of archaebacterial lipids. *Microbiological Reviews.* 50 (1): 70-80.

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 Microbiology Ecology.* 58: 572-582.

Domingues, F.C., Queiroz, J.A., Cabral, J.M.S. and Fonseca, L.P., (2000). The influence of culture conditions on mycelial structure and cellulase production by *Trichoderma reesei* Rut C-30. *Enzyme and Microbial Technology*. 26: 394-401.

Drake, D.J., Nader, G. and Forero, L., (2002). Feeding rice straw to cattle. Publication 8079 - University of California, Division of Agriculture and Natural Resources.

Endo, A., Hasumi, K., Yamada, A., Shimoda, R. and Takeshima, H., (1986). The synthesis of compactin (ML-236B) and monacolin K in fungi. *The Journal of Antibiotics*. 39: 1609-1616.

Endo, A., Kuroda, M. and Tsujita, Y., (1976). ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterogenesis produced by *Penicillium citrinium*. *The Journal of Antibiotics*. 29: 1346-1348.

Eriksson, K.E.L., Blanchette, R.A. and Ander, P., (1990). Microbial and Enzymatic Degradation of Wood and Wood Components. *Springer-Verlag Berlin Heidelberg, New York.* 407 pp.

Erwin, E.S., Marco, G.J. and Emery, E.M., (1961). Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *Journal of Dairy Science.* 44: 1768–1771.

Fadel Elseed, A.M.A., (2005). Effect of supplemental protein feeding frequency on ruminal characteristics and microbial N production in sheep fed treated rice straw. *Small Ruminant Research*. 57: 11–17.

FAO, (2006). Livestock long shadow – environmental issues and options. 1-390.

Field, C.B., (2004). The global carbon cycle: integrating humans, climate, and the natural world. Island Prss.

Friedrich, J., Zuzek, M., Bencina, M., Cimerman, A., Strancar, A. and Radez, I., (1995). High-performance liquid chromatographic analysis of mevinolin as mevinolinic acid in fermentation broths. *Journal of Chromatography A.* 704: 363-367.

Frumholtz, P.P., Newbold, C.J. and Wallace, R.J., (1989). Influence of *Aspergillus oryzae* fermentation extract on the fermentation of a basal ration in the rumen

simulation technique (Rusitec). *Journal of Agricultural Science, Cambridge.* 113 (2): 169-172.

Gao, J., Weng, H., Zhu, D., Yuan, M., Guan, F. and Xi, Y., (2008). Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal *Aspergillus terreus* M11 under solid-state cultivation of corn stover. *Bioresource Technology*. 99: 7623-7629.

Gawande, P.V. and Kamat, M.Y., (1999). Production of *Aspergillus* xylanase by lignocellulosic waste fermentation and its application. *Journal of Applied Microbiology*. 87: 511-519.

Giraldo, L.A., Tejido, M.L., Ranilla, M.J., Ramos, S. and Carro, M.D., (2008). Influence of direct-fed fibrolytic enzymes on diet digestibility and ruminal activity in sheep fed a grass hay-based diet. *Journal Animal Science*. 86: 1617–1623.

Goldstein, J.L. and Brown, M.S., (2009). The LDL receptor. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 29: 431-438.

Gupta, K., Mishra, P.K. and Srivastava, P., (2007). A correlative evaluation of morphology and rheology of *Aspergillus terreus* during lovastatin fermentation. *Biotechnology and Bioprocess Engineering*. 12: 140-146.

Hindrichsen, I.K., Wettstein, H.R., Machmüller, A., Soliva, C.R., Bach Knudsen, K.E., Madsen, J. and Kreuzer, M., (2004). Effects of feed carbohydrates with contrasting properties on rumen fermentation and methane release *in vitro*. *Canadian Journal of Animal Science*. 84 (2): 265-276.

Hölker, U., Höfer, M. and Lenz, J., (2004). Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. *Applied Microbiology and Biotechnology*. 64: 175-186.

Howard, R.L., Abotsi, E., Jansen van Rensburg, E.L. and Howard, S., (2003). Lignocellulose biotechnology: issues of bioconversion and enzyme production. *African Journal of Biotechnology*. 2 (12): 602-619.

Huang, X.D., Liang, J.B., Tan, H.Y., Yahya, R. and Ho, Y.W., (2011). Effects of *Leucaena* condensed tannins of differing molecular weights on *in vitro* CH_4 production. *Animal Feed Science and Technology*. 166-167: 373-376.

Hungate, R.E., Smith, W., Bauchop, T., Yu, I. and Rabinowitz, J.C., (1970). Formate as an intermediate in the bovine rumen fermentation. *Journal of Bacteriology*. 102 (2): 389-397.

IPCC, 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V. and Midgley, P.M. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA: 1535 pp.

IPCC, 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R.K and Reisinger, A. (eds.)]. IPCC, Geneva, Switzerland: 104 pp.

IPCC, 2001. Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change [Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K. and Johnson, C.A. (eds.)]. Cambridge

University Press, Cambridge, United Kingdom and New York, NY, USA: 881 pp.

Jackson, M.G., (1978). Rice straw as livestock feed. *Ruminant nutrition : selected articles from the World animal review*: 13 pp. (http://www.fao.org/docrep/004/x6512e/X6512E07.htm#ch7)

Jahromi, M.F., Liang, J.B., Rosfarizan, M., Goh, Y.M., Sokryazdan, P. and Ho, Y.W., (2013a). Lovastatin-enriched rice straw enhances biomass quality and suppresses ruminal methanogenesis. *BioMed Research International.* Article ID 397934 : 13 pp.

Jahromi, M.F., Liang, J.B., Ho, Y.W., Rosfarizan, M., Goh, Y.M., Sokryazdan, P. and Chin, J., (2013b). Lovastatin in *Aspergillus terreus*: Fermented Rice Straw Extracts Interferes with Methane Production and Gene Expression in *Methanobrevibacter smithii*. *BioMed Research International.* Article ID 604721 : 10 pp.

Jahromi, M.F., Liang, J.B., Ho, Y.W., Rosfarizan, M., Goh, Y.M., and Sokryazdan, P., (2012). Lovastatin production by *Aspergillus terreus* using agro-biomass as substrate in solid state fermentation. *Journal of Biomedicine and Biotechnology*. 2012, Article ID 196264 : 11 pp.

Jahromi, M.F., Liang, J.B., Rosfarizan, M., Goh, Y.M., Sokryazdan, P. and Ho, Y.W., (2011). Efficiency of rice straw lignocelluloses degradability by *Aspergillus terreus* ATCC 74135 in solid state fermentation. *African Journal of Biotechnology.* 10 (21): 4428–4435.

Jaivel, N. and Marimuthu, P., (2010). Optimization of lovastatin production in solid state fermentation by *Aspergillus terreus*. *International Journal of Engineering Science and Technology.* 2 (7): 2730-2733.

Jalc, D. and Laukovã, A., (2002). Effect of nisin and monensin on rumen fermentation in the artificial rumen. *Berliner und Manchener tierarztliche Wochenschrift.* 115 (1-2): 6-10.

Jalc, D., Nerud, F. and Siroka, P., (1998). The effectiveness of biological treatment of wheat straw by white-rot fungi. *Folia Microbiologica.* 43 (6): 687-689.

Jalc, D., (1994). Straw enrichment for fodder production by fungi. *Agricultural Applications*. 11: 19-38.

Janssen, P.H., (2010). Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. *Animal Feed Science and Technology*. 160: 1-22.

Janzen, H.H., (2004). Review : Carbon cycling in earth systems – a soil science perspective. *Agriculture, Ecosystems & Environment*. 104: 399-417.

Jia, Z., Zhang, X., Zhao, Y. and Cao, X., (2010). Enhancement of Lovastatin Production by Supplementing Polyketide Antibiotics to the Submerged Culture of *Aspergillus terreus*. *Applied Biochemistry and Biotechnology.* 160: 2014-2025.

Johnson, D.E. and Ward, G.M., (1996). Estimates of animal methane emissions. *Environmental Monitoring Assessment.* 42: 133–141.

Johnson, K. and Johnson, D., (1995). Methane emissions from cattle. *Journal of Animal Science*. 73: 2483-2492.

Kamra, D. N., (2005). Rumen microbial ecosystem. *Current Science.* 89 (1): 124-135.

Kashyap, P., Sabu, A., Pandey, A., Szakacs, G. and Soccol, C.R., (2002). Extra-cellular L-glutaminase production by *Zygosaccharomyces rouxii* under solid-state fermentation. *Process Biochemistry.* 38: 307-312.

Kirk, T.K. and Moore, W.E., (1972). Removing lignin from wood with white-rot fungi and digestibility of resulting wood. *Wood and Fiber Science.* 4: 72-79.

Klevenhusen, F., Duval, S., Zeitz, J.O., Kreuzer, M. and Soliva C.R., (2011). Diallyl disulphide and lovastatin: effects on energy and protein utilisation in, as well as methane emission from, sheep. *Archives of Animal Nutrition*. 65 (4): 255-266.

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: 361-366.

Konrad, Z. and Eichler, J., (2002). Lipid modification of proteins in Archaea: attachment of a mevalonic acid-based lipid moiety to the surface-layer glycoprotein of *Haloferax volcanii* follows protein translocation. *Biochemical Journal.* 366: 959-964.

Kumar, D., Jain, V.K., Shanker, G. and Srivastava, A., (2003). Citric acid production by solid state fermentation using sugarcane bagasse. *Process Biochemistry.* 38: 1731-1738.

Lai, L.S.T., Tsai, T.H., Wang, T.C. and Cheng, T.Y., (2005). The influence of culturing environment on lovastatin production by *Aspergillus terreus* in submerged cultures. *Enzyme and Microbial Technology.* 36: 737-748.

Lai, L.S.T., Pan, C.C. and Tzeng, B.K., (2003). The influence of medium design on lovastatin production and pellet formation with a high-producing mutant of *Aspergillus terreus* in submerged cultures. *Process Biochemistry*. 38: 1317-1326.

Lam, W.L. and Doolittle, W.F., (1992). Mevinolin-resistant mutations identify a promoter and the gene for a eukaryote-like 3- hydroxy-3-methylglutaryl-coenzyme A reductase in the archaebacterium *Haloferax volcanii*. *The Journal Of Biological Chemistry*. 267 (9): 5829-5834.

Lane, D.J., (1991). 16S/23S rRNA sequencing. Nucleic Acid Techniques in Bacterial Systematics (StackebrandtE & GoodfellowM, eds). John Wiley & Sons, New York.

Liu, J.X. and Ørskov., E.R., (2000). Cellulase treatment of untreated and steam pre-treated rice straw-effect on *in vitro* fermentation characteristics. *Animal Feed Science and Technology*. 88: 189-200.

Mackie, R.I., McSweeney, C.S. and Klieve, A.V., (2002). Microbial ecology of the ovine rumen. *Sheep nutrition*. M. Freer and H. Dove, ed. CABI, New York : 71-94.

Mao, H.L., Wang, J.K., Zhou, Y.Y. and Liu, J.X., (2010). Effects of addition of tea saponins and soybean oil on methane production, fermentation and microbial population in the rumen of growing lambs. *Livestock Science.* 129: 56-62.

Martin, C., Ferlay, A., Chilliard, Y. and Doreau, M., (2007). Rumen methanogenesis of dairy cows in response to increasing levels of dietary extruded linseeds. *2nd Int. Symp. Energy Protein Metab. Nutr.EAAP publication, Vichy, France*. 124: 609–610.

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

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 Development*. 28: 7-55.

Miller, T.L. and Wolin, M.J., (2001). Inhibition of Growth of Methane-Producing Bacteria of the Ruminant Forestomach by Hydroxymethylglutaryl~SCoA Reductase Inhibitors. *Journal Dairy Science*. 84: 1445–1448.

Mohammed, N., Ajisaka, N., Lila, Z.A., Hara, K., Mikuni, K., Hara, K., Kanda, S. and Itabashi, H., (2004). Effect of Japanese horseradish oil on methane production and ruminal fermentation *in vitro* and in steers. *Journal of Animal Science.* 82: 1839-1846.

Moe, P.W. and Tyrrell, H.F., (1979). Methane production in dairy cows. *Journal Dairy Science*. 62: 1583-1586.

Morgavi, D.P., Martin, C. and Boudra, H., (2013). Fungal secondary metabolite from *Monascus* spp. reduce rumen methane production *in vitro* and *in vivo*. *Journal Animal Science*. 91: 848-860.

Mosier, A.R., Duxbury, J.M., Freney, J.R., Heinemeyer, O., Minami, K. and Johnson, D.E, (1998). Mitigating agricultural emissions of methane. *Climatic Change*. 40: 39-80.

Moss, A.R., Jouany, J.P. and Newbold, J., (2000). Methane production by ruminants: its contribution to global warming. *Annales de zootechnie*. 49 (3): 231-253.

Moss, A.R. and Givens, D.I., (1993). Effect of supplement type and grass silage: concentrate ratio on methane production by sheep. *Proc. Brit. Soc. Anim. Prod.* Paper no. 52.

Muetzel, S., Hunt, C.L. and Tavendale, M.H., (2011). Brief Communication: Evaluating rumen fluid from sheep and cattle as inoculum in a newly developed automated *in vitro* rumen batch culture system. *Proceedings of the New Zealand Society of Animal Production*. 71: 240-242.

Navarro, C.L., Cau, P. and Lvy, N., (2006). Molecular bases of progeroid syndromes. *Human Molecular Genetics.* 15: 151-161.

Oenema, O., Wrage, N., Velthof, G.L., Groenigen, J.W., Dolfing, J. and Kuikman, P.J., (2005). Trends in global nitrous oxide emissions from animal production systems. *Nutrient Cycling in Agroecosystems*. 72: 51-65.

Oeztuerk, H., Emre, B., Sagmanligil, V., Piskin, I., Fidanci, U.R. and Pekcan, M., (2010). Effects of nisin and propolis on ruminal fermentation *in vitro. Journal of Animal and Veterinary* Advances. 9 (21): 2752-2758.

Osman, M.E., Khattab, O.H., Zaghlol, G.M. and El-Hameed, R.M.A., (2011). Optimization of Some Physical and Chemical Factors for Lovastatin Productivity by Local Strain of *Aspergillus terreus*. *Australian Journal of Basic and Applied Sciences.* 5 (6): 718-732.

Pansuriya, R.C. and Singhal, R.S., (2010). Response surface methodology for optimization of production of lovastatin by solid state fermentation. *Brazilian Journal of Microbiology*. 41: 164-172.

Pei-Jun, L., De-bing, J., Qi-xing, Z. and Chun-gui, Z., (2004). Optimization of solid fermentation of cellulase from *Trichoderma koningii*. *Journal of Environmental Sciences.* 16 (5): 816-820.

Penazola, W., Molina, M.R., Brenes, R.G. and Bressani, R., (1985). Solid-state fermentation: an alternative to improve the nutritive value of coffee pulp. *Applied and Environmental Microbiology*. 49 (2): 388-393.

Raghavarao, K.S.M.S., Ranganathan, T.V. and Karanth, N.G., (2003). Some engineering aspects of solid-state fermentation. *Biochemical Engineering Journal*. 13: 127-135.

Reddy, D. V., (1996). Evaluation of rice straw-poultry droppings based rations supplemented with graded levels of rice bran in fistulated buffaloes. *Animal Feed Science Technology*. 58: 227-237.

Rosmiza, M.Z., Davies, W.P., Rosniza Aznie, C.R., Mazdi, M. and Jabil, M.J., (2014). Farmers' knowledge on potential uses of rice straw: An assessment in MADA and Sekinchan, Malaysia. *GEOGRAFIA Online ™ Malaysian Journal of Society and Space*. 10 (5): 30-43.

Rouviere, P.E and Wolfe, R.S., (1988). Novel Biochemistry of Methanogenesis. *The Journal Of Biological Chemistry*. 263 (17): 7913-7916.

Rozman, D. and Monostory, K., (2010). Perspectives of the non-statin hypolipidemic agents. *Pharmacology and Therapeutics.* 127: 19-40.

Russell, J.B. and Rychlik, J.L., (2001). Factors that alter rumen microbial ecology. *Science*. 292 (5519): 1119-1122.

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

Samuel, B.S., Hansen, E.E., Manchester, J.K., Coutinho, P.M., Henrissat, B., Fulton, R., Latreille, P., Kim, K., Wilson, R.K. and Gordon, J.I., (2007). Genomic and metabolic adaptations of *Methanobrevibacter smithii* to the human gut. *Proceedings of the National Academy of Sciences.* 104: 10643-10648.

Sarnklong, C., Cone, J.W., Pellikaan, W. and Hemdriks, W.H., (2010). Utilization of Rice Straw and Different Treatments to Improve Its Feed Value for Ruminants: A Review. *Asian-Australia Journal Animal Sciences*. 23 (5): 680 – 692.

SAS, (2008). SAS Institute Inc., 2008. SAS OnlineDoc 9.2. SAS Institute Inc., Cary, NC.

Sekar, C., Rajasekar, V.W. and Balaraman, K., (1997). Production of cyclosporin A by solid state fermentation. *Bioprocess and Biosystems Engineering.* 17: 257-259.

Selim, A.S.M., Pan, J., Takano, T., Suzuki, T., Koike, S., Kobayashi, Y. and Tanaka, K., (2004). Effect of ammonia treatment on physical strength of rice straw, distribution of straw particles and particle-associated bacteria in sheep rumen. *Animal Feed Science and Technology*. 115: 117-128.

Shaligram, N.S., Singh, S.K., Singhal, R.S., Szakacs, G. and Pandey, A., (2008). Compactin production in solid-state fermentation using orthogonal array method by *P. brevicompactum*. *Biochemical Engineering Journal.* 41: 295-300.

Sharma, R.K. and Arora, D.S., (2013). Fungal degradation of lignocellulosic residues: An aspect of improved nutritive quality. *Crit Rev Microbiol, Early Online*: 1–9.

Shen, H. S., Sundstøl, F., Eng, E. R. and Eik, L. O., (1999). Studies on untreated and urea-treated rice straw from three cultivation seasons: 3. Histological investigations by light and scanning electron microscopy. *Animal Feed Science and Technology*. 80: 151-159.

Shen, H. S., Ni, D. B. and Sundstøl, F., (1998a). Studies on untreated and urea-treated rice straw from three cultivation seasons: 1. Physical and chemical

measurements in straw and straw fractions. *Animal Feed Science and Technology*. 73: 243-261.

Shen, H. S., Sundstøl, F. and Ni, D. B., (1998b). Studies on untreated and urea-treated rice straw from three cultivation seasons 2. Evaluation of straw quality through *in vitro* gas production and *in sacco* degradation measurements. *Animal Feed Science and Technology*. 74: 193-212.

Shrikumar, S., (2003). Current industrial practice in solid state fermentations for secondary metabolite production: the Biocon India experience. *Biochemical Engineering Journal.* 13: 189-195.

Siamak, M.S., Nasrin, M., Saeid, H., Farzaneh, A.M., Saeid, M. and Mohammad, R.B., (2003). Screening of Lovastatin Production by Filamentous Fungi. *Iran Biomed Journal.* 7 (1): 29-33.

Sigma Malaysia., (2015). Price of M2147 Sigma (Mevinolin from Aspergillus sp). http://www.sigmaaldrich.com/catalog/product/sigma/m2147?lang=en&region=MY

Singhania, R.R., Patel, A.K., Soccol, C.R. and Pandey, A., (2009). Recent advances in solid-state fermentation. *Biochemical Engineering Journal.* 44: 13-18.

Siti Humairah S., (2010). Characterization of films from rice straw *hemicellulose*. UITM final project report: 1-14.

Smith, K.A and Conen, F., (2004). Impacts of land management on fluxes of trace greenhouse gases. *Soil Use and Management*. 20: 255-263.

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

Subramaniyam, R. and Vimala, R., (2012). Solid state and submerged fermentation for the production of bioactive substances: a comparative study. *International Journal of Science and Nature*. 3 (3): 480-486.

Sylvester, J.T., Karnati, S.K.R., 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: 3378-3384.

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

Tobert, J.A., (2003). Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. *Nature Reviews Drug Discovery*. 2: 517-526.

Tomasini, A., Fajardo, C. and Barrios-GonzaÂ´ lez, J., (1997). Gibberellic acid production using different solid-state fermentation systems. *World Journal of Microbiology and Biotechnology.* 13: 203-206.

Vadiveloo, J., (2003). The effect of agronomic improvement and urea treatment on the nutritional value of Malaysian rice straw varieties. *Animal Feed Science and Technology*. 108: 133-146.

Vadiveloo, J., (2000). Nutritional properties of the leaf and stem of rice straw *Animal Feed Science and Technology*. 83: 57-65.

Valera, H.R., Gomes, J., Lakshmi, S., Gururaja, R., Suryanarayan, S. and Kumar, D., (2005). Lovastatin production by solid state fermentation using *Aspergillus flavipes*. *Enzyme and Microbial Technology.* 37: 521-526.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A., (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*. 74: 3583-3597.

Wanapat, M., Polyorach, S., Boonnop, K., Mapato, C. and Cherdthong, A., (2009). Effect of treating rice straw with urea or urea and calcium hydroxide upon intake, digestibility, rumen fermentation and milk yield of dairy cows. *Livestock Science*. 125: 238-243.

Wei, P., Xu, Z. and Cen, P., (2007). Lovastatin production by *Aspergillus terreus* in solid-state fermentation. *Journal of Zhejiang University-Science A*. 8 (9): 1521-1526.

Wolin, M.J. and Miller, T.L., (2006). Control of rumen methanogenesis by inhibiting the growth and activity of methanogens with hydroxymethylglutaryl-SCoA inhibitors. The 2nd International Conference on Greenhouse Gases and Animal Agriculture. *Elsevier*. 1293: 131-137.

Wolin, M.J. and Miller, T.L., (1999). Method for inhibiting growth of methanogens. U.S. Patent No. 5,985,907.

Wright, A.D.G. and Klieve, A.V., (2011). Does the complexity of the rumen microbial ecology preclude methane mitigation? *Animal Feed Science and Technology*. 166-167: 248-253.

Yamakawa, M., Abe, H. and Okamoto, M., (1992). Effect of incubation with edible mushroom, *Pleurotus ostreatus*, on voluntary intake and digestibility of rice straw by sheep. *Animal Science Technology (Jpn)*. 63 (2): 129-133.

Yang, S., Yan, Q., Jiang, Z., Li, L., Tian, H. and Wang, Y., (2006). High-level of xylanase production by the *thermophilic Paecilomyces themophila J18* on wheat straw in solid-state fermentation. *Bioresource Technology*. 97: 1794-1800.

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: 670-679.