EFFECTS OF SUBSTITUTING RICE STRAW WITH DIFFERENT LEVELS OF Leucaena leucocephala (Lam.) DE WIT AND Manihot esculenta CRANTZ LEAVES ON RUMEN FERMENTATION CHARACTERISTICS AND MICROBIAL POPULATION IN GOATS

NUR LIYANA AKMAL BINTI HARUN

FP 2015 50
EFFECTS OF SUBSTITUTING RICE STRAW WITH DIFFERENT LEVELS OF *Leucaena leucocephala* (Lam.) DE WIT AND *Manihot esculenta* CRANTZ LEAVES ON RUMEN FERMENTATION CHARACTERISTICS AND MICROBIAL POPULATION IN GOATS

By

NUR LIYANA AKMAL BINTI HARUN

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

December 2015
All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

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

EFFECTS OF SUBSTITUTING RICE STRAW WITH DIFFERENT LEVELS OF *Leucaena leucocephala* (Lam.) DE WIT AND *Manihot esculenta* CRANTZ LEAVES ON RUMEN FERMENTATION CHARACTERISTICS AND MICROBIAL POPULATION IN GOATS

By NUR LIYANA AKMAL BINTI HARUN

December 2015

Chairman : Anjas Asmara @ Ab. Hadi b. Samsudin, PhD
Faculty : Agriculture

The leaves of *L. leucocephala* and *M. esculenta* are among good source of protein and its use in animal feed would further increase the nutritive value of poor quality feed. Generally, the presence of the anti-nutritive factors contained in both of the forages limit their usefulness as animal feed, but not in ruminant. Ruminant have a dynamic and complex rumen ecology that may have the ability to degrade the anti-nutritive factors contained in the *L. leucocephala* and *M. esculenta* leaves. Most of the previous studies are focused on the effect of *L. leucocephala* and *M. esculenta* leaves on performances and health of the animal. Therefore, current study was conducted to evaluate the effect of substitution rice straw with different level of *L. leucocephala* and *M. esculenta* leaves on rumen fermentation characteristics and rumen microbial population in goats. Seven treatment groups; T1: concentrates (C)/rice straw (RS) (40:60) (Control); T2: C/RS/leucaena leaves (40:45:15); T3:C/RS/leucaena leaves (40:30:30); T4: C/RS/leucaena leaves (40:15:45); T5:C/RS/cassava leaves (40:45:15); T6:C/RS/cassava leaves (40:30:30) and T7: C/RS/cassava leaves (40:15:45) were used in this experiment. An *in-vitro* study was carried out to evaluate the effect of substitution of rice straw with different level of *L. leucocephala* and *M. esculenta* leaves diet on rumen gas production and fermentation characteristics. In the *in-vitro* study, the production of propionate and IVDMD were significantly affected with the treatment diets (P<0.05). Propionate production was significantly increased meanwhile the percentages of IVDMD was decreased significantly with T2 showed the highest value in the propionate production and the lowest value of IVDMD. Similar observation were found among treatment diets in rumen pH, rumen ammonia, acetate, butyrate, total VFA production and total gas production of the *in-vitro* study. In the *in-vivo* study, 21 local Boer goats were randomly divided into seven diets and were placed in metabolic cages individually for a period of 7 days as an adjustment period followed by an administration diet period for 10 days. Urine samples were taken daily for 5 days period for urinary purine derivatives analysis and rumen samples were taken at day 10 for rumen fermentation characteristics and rumen microbial population quantification analysis. Result shown that propionate (P<0.05), butyrate (P<0.01), A to P ratio (P<0.05), total VFA production (P<0.01) were affected significantly among the treatment diets. The highest value of acetate, A to P ratio and total VFA were found in
T2 with the lowest value of propionate. Supplementation of *L. leucocephala* and *M. esculenta* leaves diets demonstrated significant increase (P<0.01) in the production of rumen ammonia. There were no differences shown in the rumen pH and production of acetate. Urinary allantoin (P<0.05), xanthine (P<0.05), total PD (P<0.05), microbial purine absorbed (P<0.01) and microbial N supply (P<0.01) were affected significantly with T2 and T6 showed the highest value among the treatment diets whereas production of uric acid, hypoxanthine showed similar observation. In the microbial population study, the supplementation of *L. leucocephala* or *M. esculenta* leaves in the diet had increased the population of total protozoa and *R. flavefacien* significantly (P<0.05) with T4 and T7 showed the highest value, respectively. Meanwhile, the populations of *R. albus*, and *F. succinogenes* were significantly reduced (P<0.05) with T3 and T7 showed the lowest value, respectively. In conclusion, T2 (25% of *L. leucocephala* leaves) and T6 (50% of *M. esculenta* leaves) supplementation diet are suitable diet for goat consumption due to improvement in the VFA production, urinary purine derivative productions, microbial purine absorbed and microbial N supply with moderate amount rumen microbial population.
Abstrak tesis yang dikeluarkan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN PENGGANTIAN JERAMI PADI DENGAN BERLAINAN ARAS DAUN Leucaena Leucocephala (Lam.) DE WIT DAN Manihot esculenta CRANTZ PADA CIRI-CIRI FERMENTASI RUMEN DAN POPULASI MIKROB RUMEN PADA KAMBING

Oleh

NUR LIYANA AKMAL BINTI HARUN

Disember 2015

Pengerusi : Anjas Asmara @ Ab. Hadi b. Samsudin, PhD
Fakulti : Pertanian

Daun *L. leucocephala* dan *M. esculenta* adalah antara sumber protein yang baik dan penggunaannya dalam makian haiwan akan meningkatkan lagi nilai nutrien makanan yang berkualiti rendah. Umumnya, kehadiran faktor anti-nutrien yang terkandung di dalam kedua-dua foraj menghadkan penggunaan mereka sebagai makanan ternakan, tetapi tidak dalam ruminan. Ruminan mempunyai rumen ekologi yang dinamik serta kompleks dan mungkin mempunyai keupayaan untuk mendegradasi faktor anti-nutrien yang terkandung di dalam daun *L. leucocephala* dan *M. esculenta*. Kebanyakkan kajian sebelum ini memberi tumpuan kepada kesan daun *L. leucocephala* dan *M. esculenta* pada prestasi dan kesihatan haiwan. Oleh itu, kajian semasa telah dijalankan untuk menilai penggantian jerami padi dengan perbezaan aras daun *L. leucocephala* dan *M. esculenta* pada ciri-ciri fermentasi rumen dan rumen populasi mikrob dalam kambing.

10 untuk analisis ciri-ciri rumen fermentasi dan kuantifikasi populasi rumen mikrob. Keputusan menunjukkan bahawa asid propionik (P<0.05), asid butyrik (P<0.01), nisbah A kepada P (P<0.05), total asid lemak meruap (P<0.01) telah terkesan secara signifikan di antara rawatan diet. Nilai tertinggi asid asetik, nisbah A kepada P dan total asid meruap telah dijumpai pada T2 dengan asid propionic pada nilai yang terendah. Penggantian jerami padi dengan daun L. leucocephala and M. esculenta diet telah menunjukkan peningkatan yang signifikan (P<0.01) di dalam produksi rumen ammonia. Terdapat tiada perbezaan yang ditunjukkan di dalam pH rumen dan produksi asid asetik. Alantoin urin (P<0.05), xantina (P<0.05), jumlah derivatif purin (P<0.05), penyerapan purin mikrob (P<0.01) dan bekalan N mikrob (P<0.01) telah terjejas secara signifikan dengan T2 dan T6 menunjukkan nilai tertinggi di antara rawatan diet dimana produksi asid urik, hipoxantina menunjukkan pemerhatian yang sama. Di dalam kajian populasi mikrob, penggantian jerami padi dengan daun L. leucocephala dan M. esculenta di dalam diet telah meningkatkan populasi jumlah protozoa serta R. flavfacien dengan ketara (P<0.05) dengan T4 dan T7 masing-masing menunjukkan nilai tertinggi. Sementara itu, populasi R. Albus dan F. succinogenes telah dikurangkan dengan ketara (P<0.05) dengan T3 dan T7 masing-masing menunjukkan nilai terendah. Kesimpulannya, diet T2 (25% daun L. leucocephala diet) dan T6 (50% daun M. esculenta diet) adalah sesuai untuk pengunaan kambing disebabkan penambahbaikan di dalam produksi asid meruap, produksi derivative purin, penyerapan purin mikrob dan bekalan N mikrob dengan populasi rumen mikrob yang sederhana.
ACKNOWLEDGEMENTS

Here, I would like to express my deepest gratitude to my supervisor, Dr. Anjas Asmara @ Ab. Hadi b. Samsudin, whose selfless time and care were sometimes all that kept me going. Thank you for your guidance, patience, knowledge, encouragement and support in completing my project and thesis. Extended gratitude goes to my co-supervisor, Prof. Razak b. Alimon, who had shared with me his knowledge as well as ideas in executing my experiment.

My sincerest thanks to the lecturer and staff of Animal Science Department for the assistance and providing facilities needed in completing my laboratory analyses. I would also want to express my deepest thanks to Dr. Faseleh Jahromi of Animal Production Laboratory in Tropical Agricultural Institute, who has shared his knowledge and facilitate in the microbial quantification analysis by using real-time PCR. Special thanks on Agro-Biotechnology Institute for providing me the instruments needed in completing my quantification assessment. Not to forget, I am indebted to my colleagues who help me through thick and thin. It is indeed a fun learning environment which I would missed, greatly.

For my beloved parents, Dr. Harun b. Ongah and Ruaidah bt. Yahya, I want to thank both of you from the bottom of my heart for your patience and supports in my decision to further my study. I am grateful enough to have both of you as my parents. My sincerest thanks to my husband, Muhammad Afiq Khalit for your support towards the completion of my thesis. Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.
I certify that a Thesis Examination Committee has met on 28 December 2015 to conduct the final examination of Nur Liyana Akmal bt Harun on her thesis entitled "Effects of Substituting Rice Straw with Different Levels of Leucaena leucocephala (Lam.) de Wit and Manihot esculenta Crantz Leaves on Rumen Fermentation Characteristics and Microbial Population in Goats" 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:

**Dahlan bin Ismail, PhD**
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

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

**Wan Khatijah binti Embong, PhD**
Professor
University of Malaya
Malaysia
(External Examiner)

---

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

Date: 24 March 2016
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:

**Anjas Asmara @ Ab. Hadi Bin Samsudin, PhD**  
Senior Lecturer  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Abdul Razak Bin Alimon, PhD**  
Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

---

**BUJANG 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 No.: Nur Liyana Akmal binti Harun, GS35571
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 Rule 41 in Rules 2003 (Revision 2012-2013) were adhered to.

Signature: ________________________________________________
Name of Chairman of Supervisory Committee: Dr. Anjas Asmara @ Ab. Hadi Bin Samsudin

Signature: ________________________________________________
Name of Member of Supervisory Committee: Professor Dr. Abdul Razak Bin Alimon
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiv</td>
</tr>
</tbody>
</table>

## CHAPTER

### 1 INTRODUCTION

### 2 LITERATURE REVIEW

- 2.1 Goat nutrition in the tropics
- 2.2 Leguminous forages as animal feed
  - 2.2.1 *Leucaena leucocephala*
  - 2.2.2 *Manihot esculenta*
- 2.3 Nutritional quality of *L. leucocephala* and *M. esculenta*
- 2.4 Presence of anti-nutritive factor in *L. leucocephala* and *M. esculenta*
  - 2.4.1 Mimosine
  - 2.4.2 Hydrogen cyanide
  - 2.4.3 Tannin
- 2.5 Rumen fermentation characteristics as affected by *L. leucocephala* and *M. esculenta* supplementations
- 2.6 Urinary purine derivatives and its relation to microbial N supply
- 2.7 Microbial populations in the ruminant
- 2.8 Conclusion

### 3 GENERAL MATERIAL AND METHODS

- 3.1 Experimental diets
- 3.2 Proximate analyses
  - 3.2.1 Dry matter determination
  - 3.2.2 Organic matter determination
  - 3.2.3 Crude protein determination
  - 3.2.4 Crude Fat determination
  - 3.2.5 Neutral detergent fibre determination
  - 3.2.6 Acid detergent fibre determination
  - 3.2.7 Acid detergent lignin determination

### 4 THE EFFECT OF SUBSTITUTING RICE STRAW WITH DIFFERENT LEVELS OF *L. leucocephala* (Lam.) DE WIT AND *M. esculenta* CRANTZ LEAVES DIET ON IN-VITRO RUMEN FERMENTATION CHARACTERISTICS OF GOATS

22
### 4.1 Introduction

22

### 4.2 Materials and method

23

4.2.1 Animal, sample and rumen liquor sampling

23

4.2.2 Buffer and minerals solutions preparations

23

4.2.3 Preparation of media

23

4.2.4 Preparation of the incubation syringes

23

4.2.5 Incubation procedure

24

4.2.6 Gas production determinations

24

4.2.7 *In-vitro* dry matter digestibility (IVDMD)

25

4.2.8 pH determination

25

4.2.9 Rumen ammonia determination

25

4.2.10 Volatile fatty acid determination

26

4.2.11 Statistical analyses

26

### 4.3 Results

26

4.3.1 Rumen pH

26

4.3.2 Rumen ammonia nitrogen

26

4.3.3 Volatile fatty acid

26

4.3.4 Total *In-vitro* gas production

27

4.3.5 *In-vitro* dry matter digestibility

27

### 4.4 Discussions

29

4.4.1 Rumen pH

29

4.4.2 Rumen ammonia nitrogen

29

4.4.3 Volatile fatty acid

30

4.4.4 Total gas production and *in-vitro* dry matter digestibility

30

### 4.5 Conclusion

31

### 5 THE EFFECT OF SUBSTITUTE RICE STRAW WITH DIFFERENT LEVELS OF L. leucocephala (Lam.) DE WIT AND M. esculenta CRANTZ LEAVES DIET ON RUMEN FERMENTATION CHARACTERISTICS AND URINARY PURINE DERIVATIVES OF GOATS

32

5.1 Introduction

32

5.2 Material and methods

32

5.2.1 Animal, management and facilities

32

5.2.2 Experimental design and procedure

32

5.2.3 Sampling technique and procedures

32

5.2.4 Rumen pH determination

33

5.2.5 Ammonia-N determination

33

5.2.6 VFA determination

33

5.2.7 Urinary purine derivatives determination

33

5.2.8 Microbial purine absorption and microbial N supply determination

34

5.3 Statistical analyses

34

5.4 Results

34

5.4.1 Rumen pH

34

5.4.2 Rumen ammonia-N

35

5.4.3 Rumen volatile fatty acid

35

5.4.4 Urinary purine derivatives determinations

37

5.5 Discussions

39

5.5.1 Rumen pH

39
5.5.2 Rumen ammonia-N
5.5.3 Volatile fatty acid
5.5.4 Urinary purine derivatives
5.6 Conclusion

6 EFFECT OF SUBSTITUTING RICE STRAW WITH DIFFERENT LEVEL OF L. leucocephala (Lam.) DE WIT and M. esculenta CRANTZ LEAVES DIETS ON RUMEN MICROBIAL POPULATION OF GOATS

6.1 Introductions
6.2 Materials and method
  6.2.1 Preparation of rumen sample
  6.2.2 Deoxyribonucleic acid (DNA) extractions
  6.2.3 Standard curve preparation
  6.2.4 Real time PCR assay
  6.2.5 Statistical analysis
6.3 Results
  6.3.1 Rumen microbial population
6.4 Discussions
6.5 Conclusion

7 GENERAL DISCUSSION

8 CONCLUSION

REFERENCES
APPENDICES
BIODATA OF STUDENT
LIST OF PUBLICATIONS
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Chemical compositions of <em>L. leucocephala</em> and <em>M. esculenta</em> foliage</td>
</tr>
<tr>
<td>3.1</td>
<td>Treatments diet</td>
</tr>
<tr>
<td>3.2</td>
<td>Nutrient composition (% of dry matter) of the diet components</td>
</tr>
<tr>
<td>3.3</td>
<td>Nutrient composition (% of dry matter) of the treatment diets</td>
</tr>
<tr>
<td>4.1</td>
<td><em>In-vitro</em> gas production, dry matter digestibility and fermentation profile of the treatment diets containing different level of <em>L. leucocephala</em> and <em>M. esculenta</em> leaves</td>
</tr>
<tr>
<td>5.1</td>
<td>The effects of substitution rice straw with different level of <em>L. leucocephala</em> and <em>M. esculenta</em> leaves on rumen fermentation profile of goats</td>
</tr>
<tr>
<td>5.2</td>
<td>The effects of substitution rice straw with different level of <em>L. leucocephala</em> and <em>M. esculenta</em> leaves on urinary purine derivatives, microbial purine absorption and microbial N supply</td>
</tr>
<tr>
<td>6.1</td>
<td>Primers used for real-time PCR assay to target total bacteria, total protozoa, methanogens, <em>R. albus</em>, <em>R. flavefaciens</em> and <em>F. succinogenes</em>.</td>
</tr>
<tr>
<td>6.2</td>
<td>The effects of substitution rice straw with different level of <em>L. leucocephala</em> and <em>M. esculenta</em> leaves on rumen microbial population in goats (log10 copy no. per ml DNA extract)</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>7</td>
<td>Mimosine transformation into dihydroxypyridine by rumen microorganism</td>
</tr>
<tr>
<td>2.2</td>
<td>9</td>
<td>The mechanism of hydrogen cyanide formation</td>
</tr>
<tr>
<td>2.3</td>
<td>10</td>
<td>Tannin classifications</td>
</tr>
<tr>
<td>2.4</td>
<td>13</td>
<td>Purine nucleotides degradation and purine derivatives formation</td>
</tr>
<tr>
<td>4.1</td>
<td>24</td>
<td><em>In-vitro</em> gas production technique</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

In Malaysia, small ruminant subsectors plays inconsiderable role in the livestock industry. Statistics shows that Malaysia self-sufficiency level for beef and mutton were increased in 4.7% over the past five years (DVS, 2014) with average 0.68 kg per capita consumption in Peninsular Malaysia, alone. Currently, market niches for small ruminant product are developing as they did not only cater for religious purposes but also from middle-east restaurant. However, the increasing awareness in Malaysian population regarding healthy lifestyle with high income level makes the never ending demand.

A lot of measures have been taken by the government and non-government agencies to further improve the industry especially the small scale farmers. These include campaign, policy and allocations aiming at increasing the domestic supply in the industry. Nevertheless, the key limiting factors that discourage the small scale farmer to further expand their livestock project was feeding cost. In the times of need, they would rely on concentrates to feed the animals especially during rainy season and thus increasing the cost of feed. Low nutritional value of locally found grass and rice straw were the other options of feeding the animals.

In improving nutritional value of animal feed, locally found legumes forage shows high potential to be used in the animal feed ration. Leguminous forage such as Leucaena leucocephala and Manihot esculenta are widely known and available abundantly in tropical country and traditionally has been used as livestock feed due to its high nutritional values. The usage of these forages in the animal feed shows auspicious result in growth rate, dry matter (DM) digestibility (Adejumo and Ademosun, 1991), DM intake (Srivastava and Sharma, 1997) for leucaena and DM intake, nutrient digestibility and weight gain (Hue et al., 2010; Phengvichith and Ledin, 2007; Chunjula et al., 2004) for M. esculenta regardless of feeding forms and methods.

Most of the plants have a mechanism in which to defense themselves against predators. Aside from physical forms like having thorns, there is secondary compound in aiding a defense mechanism from predators like insects. These secondary compounds like mimosine in L. leucocephala and hydrogen cyanide in M. esculenta affects the nutritive value of forages and animal consumed them (Aganga and Tshwenyane, 2003; Oni et al., 2010). These forages are naturally high in crude protein (CP) where the CP content are 22.5 and 24 g/kg DM, respectively (Speedy and Pugliese, 1992). The consumption of these high quality forages with low quality straw may enhance their diet regimes quality despite the presence of anti-nutritive factors which may limits the usage of the forages as well as decrease their nutritional value as sole feed. Thus, the consumption of high protein forages may increase the microbial protein synthesis as more N is available for the microbes to utilize for their growth.
Foregut fermenter like ruminant was classified by their digestion fermentation site, pre-gastric fermentation chamber. In this chamber, a vast population of microorganism co-existed together with the host animal where they are involved in symbiotic relationship in the feed digestion processes. With the help of microbial population in the rumen, carbon dioxide, hydrogen, methane gas, and short-chain fatty acid such as acetate, propionate and butyrate were produced (Stevens and Hume, 1998). These short-chain fatty acids are the sourced of energy used by the host animal (Godoy-Vitorino et al., 2012). Most of the studies were focused on rumen fermentation product in relation to diets, species, breed, age, sex and physiological status of the animal (Sokerya et al., 2010; Oni et al., 2010; Kang et al., 2012) regardless of rumen microorganism involved.

Rumen microbial population is a stable and dynamic ecosystem where it change considerably through a set of constrain regardless of natural environment or feed-associated constraint such as the presence of anti-nutritive compounds (Kamra, 2005). In the rumen fed with plant contains secondary compound, some microbes may thrive and some may limits their growth due to its anti-microbial activity. Extensive research has been done on the response of anti-nutritive compound regardless of its form towards animal growth performance, nutrient digestibility and fermentation product (Yami et al., 2000; Oduguwa et al., 2013; Tan et al., 2011). However, the respond of feeding plant-containing anti-nutritive compound on the rumen microorganism were still unclear.

Utilisations of rice straw in the tropical region are not uncommon practices among farmers. The availability throughout the year and can be easily incorporated in the animal feed makes this crop residue an easy option compared with green forages. Fermentation rice straw in rumen would result in higher methane production due to an increase in retention time in the gastrointestinal tract as rice straw is highly fibrous and low in digestibility. Hence, the combination use of rice straw as an inexpensive roughage source with L. leucocephala and M. esculenta foliage as N source may activates microbial fermentation in straw which leads to the final result being improve rumen fermentation condition as well as rumen microbial population without increasing N losses to the environment. The combination of rice straw and high quality forage, L. leucocephala and M. esculenta offers on farm practicality with high quality of diets being offered to the animals. However, an optimum level of combination must be explored so that the diet can be fed to the animal without deteriorating the rumen function of the animal and environment.
Therefore, this study were conducted to test the hypothesis that such substitution of rice straw with certain level of *L. leucocephala* and *M. eculenta* leaves diet may improve rumen fermentation condition and microbial population as such that would increase in the microbial N supply. Hence, the objectives of this study were;

1. to investigate the *in-vitro* fermentation characteristics on different level of *L. leucocephala* and *M. esculenta* leaves diet,
2. to investigates the effect of substituting rice straw with different level of *L. leucocephala* and *M. esculenta* on *in-vivo* rumen fermentation characteristics and urinary purine derivatives of the goats,
3. to quantify the rumen microbial community of goats as affected by substitution of rice straw with different level of *L. leucocephala* and *M. esculenta* leaves diets using real-time PCR.
REFERENCES


56


Buitrago, A.(1990). La yuca en la alimentación animal. CIAT.


Jetana, T., Thongruay, S., Uswang, S. and Hengtrakulsin, R. (2012). A comparative study on mimosine, 3, 4-dihydroxy pyridone (3, 4-DHP) and 2, 3-dihydroxy pyridone (2, 3-DHP), purine derivatives (PD) excretion in the urine, thyroid hormone and blood metabolites profiles of Thai swamp buffalo (Bubalus bubalis) and Murrah buffalo (Bubalus bubalis). Tropical Animal Health and Production. 44(4): 887-897.


Karda, I.W. (2007). Effects of leucaena (L. leucocephala cv Tarramba) leucaena with urea or leucaena with sucrose supplementation on intake and digestibility of rhodes grass (Chloris gayana cv callide) hay fed to sheep. E- journal Universitas Udayana. 10(2): 13.


71


