UNIVERSITI PUTRA MALAYSIA

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

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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

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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

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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.

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Abstrak tesis yang dikemukakan 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 makanan 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*. Kebanyakan 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. Tujuh kumpulan rawatan; T1:konsentrat (K)/jerami padi (JP) (40:60) (Kontrol); T2:K/JP/daun leucaena (40:45:15); T3:K/JP/daun leucaena (40:30:30); T4:K/JP/daun leucaena (40:15:45); T5:K/JP/daun leucaena (40:45:15); T6:K/JP/daun leucaena (40:30:30) dan T7:K/JP/daun leucaena (40:15:45) telah digunakan di dalam ekperimen ini. Satu kajian *in-vitro* telah dijalankan untuk menilai kesan penggantian jerami padi dengan perbezaan aras daun *L. leucocephala* dan *M. esculenta* di dalam diet pada pengeluaran gas rumen dan ciri-ciri fermentasi. Total pengeluaran gas yang sama diperhatikan apabila perbandingan dibuat diantara diet dengan diet kontrol. Dalam kajian *in-vitro*, produksi asid propionic dan ketercernaan bahan kering *in-vitro* telah terjejas secara signifikan dengan diet rawatan ($P<0.05$). Produksi propionik telah meningkat secara signifikan sementara peratus ketercernaan bahan kering *in-vitro* telah berkurangan secara signifikan dengan T2 menunjukkan nilai tertinggi di dalam produksi asid propionic dan nilai terendah di dalam ketercernaan bahan kering *in-vitro*. Pemerhatian yang serupa telah didapati di antara diet rawatan pada pH rumen, rumen ammonia, asid asetik, asid butyric, total asid lemak meruap dan total produksi gas meruap dalam kajian *in-vitro.* Dalam kajian *in-vitro*, 21 kambing Boer tempatan telah dibahagi secara rawak kepada 7 diet dan telah diletakkan di dalam kandang metabolic secara individual selama 7 hari sebagai tempoh adaptasi diikuti dengan tempoh pentadbiran rawatan selama 10 hari. Sampel air kencing diambil setiap hari selama 5 hari untuk analisis derivatif purin dalam urin dan sampel rumen telah diambil pada hari

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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. flavefacien* 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.

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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 Ruhaidah 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.

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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.

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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
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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.

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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.

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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.

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