



UNIVERSITI PUTRA MALAYSIA

***EFFECTS OF ANDROGRAPHIS PANICULATA (BURM.F.) WALL. EX
NEES AND ORTHOSIPHON STAMINEUS BENTH SUPPLEMENTATION
ON RUMEN FERMENTATION CHARACTERISTICS AND MICROBIAL
POPULATION IN GOATS FED UREA-TREATED RICE STRAW***

NURDIYANA AQILAH ROSLAN

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FERMENTATION CHARACTERISTICS AND MICROBIAL POPULATION
IN GOATS FED UREA-TREATED RICE STRAW**

By

NURDIYANA 'AQILAH BT. ROSLAN

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

May 2016

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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 *Andrographis paniculata* (BURM.f.) WALL. EX NEES AND *Orthosiphon stamineus* BENTH SUPPLEMENTATION ON RUMEN FERMENTATION CHARACTERISTICS AND MICROBIAL POPULATION IN GOATS FED UREA-TREATED RICE STRAW

By

NURDIYANA 'AQILAH BT. ROSLAN

May 2016

Chairman : Associate Professor Anjas Asmara Samsudin, PhD
Faculty : Agriculture

A. paniculata and *O. stamineus* have been used for centuries in Asia for treating various ailments in humans, notably common colds, influenza besides improving the blood circulatory system. In animals, some studies have shown that both of these herbs improved growth performance and meat quality through their antioxidant properties. The present study examines on the effect of *A. paniculata* and *O. stamineus* supplement on rumen fermentation characteristics and microbial population in goats fed treated rice straw. The *in-vitro* fermentability of urea-treated rice straw based diet supplemented with *A. paniculata* and *O. stamineus* was examined by incubating the tested diet in ruminal fluid obtained from two rumen-fistulated donor goats which had received fresh grass obtained from Ruminant Unit at the Department of Animal Science, Universiti Putra Malaysia. The fermentation process was carried out for 48 hours at 39 °C; using the method by Menke & Steingass (1988). The gas production of experimental diets was examined and the end products of the fermentation were also quantified. Higher cumulative gas was produced from the fermentation of basal diet + 0.5% of *A. paniculata* and 0.5% *O. stamineus* (AO) treatment diet and the gas production patterns of herb-based diets were found similar in trend. Besides, the rates of gas production, *in-vitro* dry matter digestibility and the rumen fermentation profiles have no significant effects ($P>0.05$) on the addition of both herbs.

In the feeding trial, 4 fistulated Boer cross-bred (± 25 kg of body weight) were used to test the effects of dietary treated rice straw supplemented with *A. paniculata* and *O. stamineus* on *in-vivo* rumen parameters and total viable bacterial population in the rumen goats. The study was conducted in 4 periods (4 x 4 Latin square design), where each period was for a duration of 22 days; 10 days of adaptation period, 5 days of sampling and 7 days of change-over. The animals were fed once daily at 0800 (3% body weight) with 60% of urea-treated rice straw and 40 % of one of four concentrate diets: basal diet + 1% *A. paniculata* (AP), basal diet + 1% *O. stamineus* (OS), AO and a basal diet without supplementation of herbs (BD). Clean water was provided *ad libitum* and the animals

were individually penned. Rumen contents were sampled at 0, 2, 4, 6 and 12 hour after the onset feeding and the pH was recorded. In the *in-vivo* study, the pH, ammonia production total volatile fatty acids content, proportions of acetate, propionate and butyrate production were not significantly different ($P>0.05$) among the treatment diets. However, the production of valerate was found to have significant effect ($P<0.05$) on the treatment diet and hour of sampling where the lowest proportion of valerate was observed in treatment AP besides having the highest level of production at 6 hour.

The rumen microbial populations in the ruminal contents were quantified using real-time PCR analysis. Significant reduction of total protozoa, methanogens, *F. succinogens* and *R. albus* number was observed in the herb-supplemented groups ($P<0.05$).

Conclusively, the results of the present study indicated that dietary urea-treated rice straw supplemented with *A. paniculata* and *O. stamineus* was not able to enhance the rumen fermentation characteristics and microbial population which may be caused by the presence of secondary metabolites in the herbs where it is believed to have an inhibitory effect on the growth of microorganism in the rumen.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

KESAN SUPLEMENTASI *Andrographis paniculata* (BURM.f.) WALL. EX NEES DAN *Orthosiphon stamineus* BENTH TERHADAP CIRI-CIRI FERMENTASI RUMEN DAN POPULASI MIKROB DALAM KAMBING YANG DIBERI JERAMI PADI YANG DIRAWAT UREA

Oleh

NURDIYANA 'AQILAH BT. ROSLAN

Mei 2016

Pengerusi : Profesor Madya Anjas Asmara Samsudin, PhD
Fakulti : Pertanian

A. paniculata dan *O. stamineus* telah digunakan selama berabad-abad di Asia untuk merawat pelbagai penyakit pada manusia, terutamanya selsema, influenza, di samping dapat melancarkan sistem peredaran darah. Beberapa kajian telah menunjukkan bahawa kedua-dua herba ini dapat meningkatkan prestasi pertumbuhan dan kualiti daging pada haiwan melalui sifat antioksidan yang terdapat pada *A. paniculata* dan *O. stamineus*. Kajian ini telah dijalankan untuk mengkaji tentang kesan suplementasi *A. paniculata* dan *O. stamineus* terhadap profil fermentasi rumen dan populasi bakteria pada kambing yang diberi makan jerami padi yang telah dirawat. Fermentasi *in-vitro* diet berasaskan jerami padi yang dirawat urea disuplementasi dengan *A. paniculata* dan *O. stamineus*, dicampur dengan cecair rumen yang diambil dari dua ekor kambing penderma yang diberi makan rumput segar di mana ianya diperolehi daripada Unit Ruminan di Jabatan Sains Haiwan, Universiti Putra Malaysia. Proses fermentasi telah dijalankan selama 48 jam pada 39 °C; menggunakan kaedah yang diperkenalkan oleh Menke dan Steingass (1988). Kadar pengeluaran gas diet diukur dan produk akhir fermentasi juga dikaji. Gas kumulatif yang tertinggi dihasilkan oleh diet asas + 0.5% daripada *A. paniculata* dan 0.5% *O. stamineus* (AO) dan corak pengeluaran gas pada diet berasaskan herba didapati mempunyai trend yang sama. Selain itu, kadar pengeluaran gas, *in-vitro* penguraian bahan kering dan profil fermentasi rumen tidak mempunyai kesan yang ketara ($P>0.05$) pada penambahan kedua-dua herba.

Dalam ujian perbandingan makanan, 4 kambing berfistula kacukan Boer (± 25 kg berat badan) telah digunakan untuk menguji kesan pemakanan jerami padi yang dirawat urea serta disuplementasi dengan *A. paniculata* dan *O. stamineus* pada fermentasi *in-vivo* rumen dan jumlah populasi bakteria dalam rumen kambing. Kajian ini telah dijalankan dalam 4 tempoh (4 x 4 reka bentuk persegi Latin), di mana setiap tempoh adalah dalam tempoh 22 hari; 10 hari tempoh penyesuaian, 5 hari pensampelan dan 7 hari perubahan. Haiwan yang diberi makan sekali sehari pada 0800 (3% berat badan) dengan 60% merupakan jerami padi yang dirawat urea dan 40% merupakan salah satu daripada empat

diet berikut: diet asas + 1% *A. paniculata* (AP), diet asas + 1% *O. stamineus* (OS), AO dan diet asas tanpa suplementasi herba (BD). Air bersih dibekalkan secara *ad libitum* dan haiwan telah dikurung secara berasingan. Kandungan rumen telah disampel pada 0, 2, 4, 6 dan 12 jam selepas kambing menerima makanan dan setelah itu pH telah direkodkan. Dalam kajian *in-vivo*, pH, pengeluaran ammonia, jumlah kandungan asid lemak teruap, bahagian asid lemak teruap yang lain seperti asetat, propionat dan butirat tidak berbeza secara ketara ($P > 0.05$) antara diet kajian. Walau bagaimanapun, pengeluaran valerat dan jam persampelan didapati mempunyai kesan yang ketara ($P < 0.05$) pada diet kajian, di mana kadar valerat yang paling rendah diperolehi dalam diet AP di samping mempunyai tahap tertinggi pengeluaran pada 6 jam.

Populasi mikrob yang dikaji dalam kandungan rumen diukur melalui kaedah real-time PCR. Pengurangan yang ketara terhadap oleh jumlah populasi protozoa, methanogens, *F. succinogens* dan *R. Albus* diperhatikan dalam kumpulan yang disuplementasi dengan herba ($P < 0.05$).

Kesimpulannya, hasil kajian ini menunjukkan bahawa pemakanan jerami padi yang dirawat urea serta disuplementasi dengan *A. paniculata* dan *O. stamineus* tidak dapat meningkatkan ciri-ciri fermentasi rumen dan populasi mikrob yang mungkin disebabkan oleh kehadiran metabolite sekunder di dalam herba di mana ia dipercayai mempunyai kesan yang menghalang kepada pertumbuhan mikroorganisma dalam rumen.

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I certify that a Thesis Examination Committee has met on 20 May 2016 to conduct the final examination of Nurdiyana 'aqilah bt. Roslan on her thesis entitled "Effects of *Andrographis paniculata* (Burm.f.) Wall. ex Nees and *Orthosiphon stamineus* Benth Supplementation on Rumen Fermentation Characteristics and Microbial Population in Goats Fed Urea-Treated Rice Straw" 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:

Loh Teck Chwen, PhD

Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Azhar bin Kassim, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Wan Zahari Mohamed, PhD

Professor
Universiti Malaysia Kelantan
Malaysia
(External Examiner)



ZULKARNAIN ZAINAL, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 September 2016

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

Anjas Asmara Samsudin, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Halimatun Yaakub, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

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Committee:

Associate Professor Dr. Anjas Asmara Samsudin

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Name of Member
of Supervisory
Committee:

Associate Professor Dr. Halimatun Yaakub

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADL	Acid detergent lignin
Atm	Atmospheric pressure
AP	Basal diet + 1% <i>A. paniculata</i>
AO	Basal diet + 0.5% of <i>A. paniculata</i> and 0.5% <i>O. stamineus</i>
BD	Basal diet without supplementation of herbs
CaCO ₃	Calcium carbonate
Ca(OH) ₂	Calcium hydroxide
Cm	Centimeter
CH ₄	Methane
CF	Crude fiber
Cq	Quantification cycle
CP	Crude protein
CTAB	Cetyl trimethylammonium bromide
DNA	Deoxyribonucleic acid
DM	Dry matter
DMRT	Duncan multiple range test
E	Amplification efficiency
GC	Gas chromatography
GE	Gross energy
GLM	General Linear Model
G	Gram
H ₂ SO ₄	Sulfuric acid
Kg	Kilogram

HCl	Hydrochloric acid
Hr	Hour
IVDMD	<i>In-vitro</i> dry matter digestibility
L	Liter
M	Molarity
Mg	Milligram
mM	Millimolar
Min	Minute
mL	Milliliter
M. Ton	Metric ton
N	Nitrogen
NaCl	Sodium chloride
Nm	Nanometer
NH ₃	Ammonia
NaOH	Sodium hydroxide
NDF	Neutral detergent fiber
NH ₃	Ammonia
NTC	No-template control
OM	Organic matter
OS	Basal diet + 1% <i>O. stamineus</i>
PKC	Palm kernel cake
Ppm	Part per million
S	Second
SS	Self sufficient
VFA	Volatile fatty acid

RT-PCR

Real-time PCR

Wt

Weight



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CHAPTER 1

GENERAL INTRODUCTION

Despite the attention that the ruminant industry has accepted from the development plans of Malaysia Government, it is still moving slowly compared to the poultry industry (Wan Zahari *et al.*, 2013). Malaysia is currently only 28% self-sufficient (SS) in beef and lower than 10% in milk production. Among the related issues that limiting the ruminant production are the feed supply, nutrition and low productivity. The supplementation of additives and growth promotants besides the availability of practical and cheap feeds to improve the feed utilization efficiency is the current emphasis in ruminant industry (Thao, 2011).

By-product of rice industry for example, the rice straw has draws attention to the fact that supplementation of rice straw with additives and growth promotants that offer certain amount of nutrients is the most practical alternative of improving the efficiency of rice straw utilization. South East Asia is among the countries which the rice straw is grown by small-scale farmers. In this area, the primary crop residue is used to feed more than 90% of the ruminant livestock (Devendra & Thomas, 2002; Sarnklong *et al.*, 2010). However, due to low digestibility and intake associated with various nutrients contents of the rice straw, it fails frequently to meet the needs of the animals (Rahal *et al.*, 1997). Besides, as the rice straw is poorly fermented rates of disappearance in the rumen are low, thus causing the feed intake to reduce (Conrad, 1966). Fadel Elseed, 2005 and Wanapat *et al.*, 2009 has reported that physical and chemical treatments besides supplementing rice straw with feeds of high nutrient contents, the performance of the animal can be improved. Improvement in these animals can be observed through the optimization of rumen function besides the utilization of the rice straw is maximized thus increasing the feed intake. Chenost & Kayouli (1997) emphasized that supplying the rumen microorganisms with the nutritive supplements is necessary for self-multiplication and for straw degradation of the cell walls, which leads to optimum conditions for good cellulolysis maintenance. Concentrates, molasses, multi-nutrient blocks, green leaves, crop residues and locally available by-products are the among of varieties of supplements that can be used (Wan Zahari *et al.*, 2013).

1.1 Research problem

The use of herbs as supplementation is common in ruminant industry, but most of the research conducted were emphasized on their effects on animal performance and nutrient digestibility, with few papers focusing their effect on rumen fermentation characteristics. Currently, interests in natural antioxidant and antibiotics such as herbal plants have increased dramatically since they were considered to be safer than synthetic antibiotics. Thus, this study was conducted to study the effect of herbs supplementation on rumen fermentation characteristics and bacterial population of goats fed urea-treated rice straw.

1.2 Justification

The effects of herbs on rumen microbial population are not well understood. Furthermore, the mechanisms of how these herbs function as a growth promoter are not known. In the past antibiotics were commonly used as a growth enhancer, but this has been banned worldwide due to risk to human health. Other additives such as probiotics, prebiotics and herbs are still being investigated. In animals, some studies have shown that both of *A. paniculata* and *O. stamineus* improved growth performance on chicken and goat meat quality through their antioxidant properties. Therefore, the present study was conducted to study the effect of herbs supplementation on rumen fermentation characteristics and microbial population in goats fed urea treated rice straw.

1.3 Research hypothesis

Supplementation of *A. paniculata* and *O. stamineus* in urea-treated rice straw diet fed to goats are expected to enhance the level of microbes in rumen, hence will improve the performance of goats.

1.4 Objectives of study

The general objective of this study is to determine the effect of *A. paniculata*, *O. stamineus* and their combination on rumen characteristics, digestibility of nutrients and microbial population in goats fed with urea treated straw.

The specific objectives of the study include:

1. To investigate the effect urea-treated rice straw based diets supplemented with *A. paniculata* and *O. stamineus* on rumen *in-vitro* fermentation profiles and digestibility in goats.
2. To determine the effect of *A. paniculata* and *O. stamineus* supplementation on *in-vivo* rumen fermentation parameters in the rumen goats fed with urea-treated rice straw.
3. To determine the rumen microbial population in response to *A. paniculata* and *O. stamineus* supplementation using quantitative real-time PCR analysis in the rumen of goats.

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BIODATA OF STUDENT

The student, Nurdiana 'Aqilah Roslan was born on 10th August 1990 in Kuala Lumpur. She attained her primary school at SK Bandar Baru Sri Damansara (2) and attended her secondary education at SMK Bandar Sri Damansara (2). She had pursued her foundation in pure science at Kolej Matrikulasi Perak (KMPk). She continued her studies in degree level in BSc. Agriculture (Animal Science) at Universiti Malaysia Kelantan (UMK). During her undergraduate studies, her final year research concentrated on the antibacterial activity in *Tualang* honey under provision of Mr. Zulhisyam Abdul Kari and Dr. Mohd. Hafiz Jamaludin. She have undergoes varies of training involving animal production and health in government farm; Infortenak Farm which is located in Sungai Siput, Perak for 4 months. She graduated in 2013. She had enrolled in MSc. (Animal Nutrition) in 2013 at Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia.

LIST OF PUBLICATIONS

- Roslan, N. A.,** Alimon, A. R., Yaakub, H., Jahromi, M. F., Shokri, J. and Samsudin, A. A. (2016). Effects of *Andrographis paniculata* and *Orthosiphon stamineus* supplementation on *in-vivo* rumen fermentation parameters and microbial population in goats fed urea-treated rice straw. *Mal. J. Anim. Sci.*, 19(2):71-82.
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