UNIVERSITI PUTRA MALAYSIA

EFFECTS OF TREE LEAF FORAGE SUPPLEMENTATION ON RUMEN FERMENTATION AND MICROBIAL PROFILE OF GOATS

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By

AHMED MUIDEEN ADEWALE

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

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DEDICATION

This thesis is dedicated to Allah (S.W.T), my father, mother and my siblings with love, humility and respect

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

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

Increase in the cost of livestock production with the use of conventional feed is distressful, threatening the food security and livestock sustenance especially ruminant. Alternative to this is to diversify and quest for other source of feed such as forages which are inexpensive, high proximity and high nutritive value for animal optimum utilization. Hence, the purpose of the present study.

In the first experiment, evaluation of nutritive and anti-nutritive factor of three selected tree forages (*Kleinhovia hospita, Leucaena leucocephala* and *Gliricidia sepium*) and their effect on the *in vitro* rumen fermentation were investigated. The proximate analysis was determined by AOAC procedure, *in vitro* cumulative gas production using Ørskov and McDonald model, metabolizable energy determined by Menke and Steingass equation method and rumen fermentation by gas chromatography. The crude protein (CP) of the forages (19%, 23.3% and 20.8% respectively) were more than the 12% CP requirement of ruminant. The NDF and ADF which to some extent dictate the feed intake and the digestibility respectively was found to be lower in KH compared to LL and GS. The lower value is an indication of higher feed intake and digestibility. In the *in vitro* study, the net gas production (NGP), *in vitro* dry matter digestibility and metabolizable energy was also high in KH than LL and GS. Furthermore, the result from the *in vitro* rumen fermentation indicated that the total volatile fatty acid (TVFA) was significantly different ($P<0.05$) among the forages with KH having the highest followed by LL and GS. There was no significant difference among the forages in the acetic acid concentration. The propionic concentration in KH was higher compared to LL and GS while the concentration of butyric acid and AP ratio in KH was the lowest followed by LL and GS. This shows better utilization of dietary component of KH than others.

In the second experiment, KH and LL were selected from the result of the first experiment to formulate diet for the feeding trial of four fistulated Boer male goat in a

4X4 Latin square design. The experiment was made up of four dietary treatments which were: Diet 1 was made up of only concentrate, Diet 2 with inclusion of KH, Diet 3 with inclusion of LL and Diet 4 with mixture of KH and LL (KHLL). The effect of the dietary treatment with sampling time (0, 2, 4, 6 and 12 h) on rumen fermentation and microbial profile were investigated. The rumen fermentation result shows a fluctuation of the dietary treatment with sampling time. The TVFA among the dietary treatments were not significantly different ($P>0.05$) at 12 h postprandial but the propionic acid concentration were significantly different among the dietary treatments with KHLL having the highest concentration followed by KH, LL and control. In the butyric acid concentration at 12 h postprandial, the control, KH and LL were similar and significantly different ($P<0.05$) from KHLL which recorded the lowest butyric acid concentration and this was a confirmation of the efficient utilization of the dietary energy. In the concentration of acetic acid, the level was noted to increase until 4 h postprandial before gradual decline with the KHLL recorded to be higher ($P<0.05$) than the rest. Similarly, the NH_3 concentration of KHLL is significantly different ($P<0.05$) from other diets which were similar. From the *in vivo* microbial population profile result, the total bacteria were noted to be at the peak of their population at 4 h postprandial before gradual decline in all treatment diets. At 12 h postprandial, the total protozoa and methanogens were found decreasing with sampling time and the forage inclusion diets (KHLL, KH and LL) were significantly lower ($P<0.05$) than the control. The number of cellulolytic bacteria *R. albus*, *R. flavefaciens* and *Fibrobacter succinogenes* were more in the forage inclusion diet than the control at 4 h postprandial. Based on the outcome of the present study, it can be concluded that the KHLL inclusion diet could be efficiently used in goat diet without compromising the nutrient potential by reducing the methanogen and protozoa numbers, enhancing propionic acid, acetic acid and nutrient utilization. In addition, acetate is essential in the formation of milk fat and propionate aid the synthesis lactose (milk sugar) as well as increase milk yields in dairy animals.

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

KESAN-KESAN MAKANAN TAMBAHAN FORAJ DAUN POKOK KEPADA FERMENTASI RUMEN DAN PROFIL MIKROB PADA KAMBING

Oleh

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Peningkatan dalam kos pengeluaran ternakan menggunakan makanan konvensional adalah membimbangkan, mengancam keselamatan makanan dan juga pendapatan industri ternakan terutamanya ruminan. Alternatif dalam hal ini adalah dengan mempelbagaikan dan mengusahakan pencarian sumber makanan yang lain seperti foraj yang tidak mahal, mudah didapati dan berkhasiat tinggi untuk pengambilan optimum bagi haiwan. Berikutan itu tujuan kajian ini.

Dalam ujikaji pertama, penilaian faktor pemakanan dan anti-pemakanan untuk tiga pokok foraj dipilih (*Kleinhovia hospita, Leucaena leucocephala* dan *Gliricidia sepium)* dan kesannya terhadap penapaian rumen *in vitro* telah dikaji. Analisis anggaran ditentukan dengan langkah-langkah AOAC, penghasilan gas kumulatif *in vitro* menggunakan model Ørskov dan McDonald, tenaga yang boleh dimetabolismakan yang ditakrifkan oleh cara persamaan Menke dan Steingass dan penapaian rumen oleh gas kromatografi. Protein kasar (CP) foraj (19%, 23.3% dan 20.8% secara individu) adalah lebih daripada 12% keperluan CP oleh ruminan. NDF dan ADF yang sedikit sebanyak menentukan pengambilan makanan dan penghadaman masing-masing didapati lebih rendah dalam KH berbanding LL dan GS. Nilai yang makin rendah adalah indikasi dari pengambilan makanan dan penghadaman yang makin tinggi. Dalam kajian *in vitro*, penghasilan gas bersih (NGP), penghadaman bahan kering *in vitro* dan tenaga yang boleh dimetabolismakan juga tinggi dalam KH berbanding LL dan GS. Tambahan pula, hasil daripada penapaian rumen *in vitro* menunjukkan bahawa jumlah yang tidak menentu asid lemak meruap (TVFA) adalah sangat berbeza ($P < 0.05$) di antara foraj dengan KH mempunyai kandungan yang tertinggi diikuti oleh LL dan GS. Tidak ada perbezaan yang ketara antara foraj dalam kepekatan asid asetik. Kepekatan propionik dalam KH adalah tinggi berbanding dengan LL dan GS manakala kepekatan asid butirik dan nisbah AP dalam KH adalah yang terendah diikuti oleh LL dan GS. Ini menunjukkan penggunaan komponen pemakanan KH adalah lebih baik daripada yang lain.

Dalam ujikaji kedua, KH dan LL telah dipilih berdasarkan hasil daripada ujikaji pertama untuk merangka diet bagi percubaan pemberian makanan kepada empat kambing Boer jantan yang telah difistula dalam 4X4 rekabentuk Latin ganda dua. Ujikaji ini terdiri daripada empat rawatan pemakanan iaitu: Diet 1 yang terdiri daripada konsentrat sahaja, Diet 2 dengan kemasukan KH, Diet 3 dengan kemasukan LL dan Diet 4 dengan campuran KK dan LL (KHLL). Kesan rawatan diet dengan masa persampelan (0, 2, 4, 6 dan 12 jam) pada penapaian dan profil mikrob rumen telah dikaji. Hasil penapaian rumen menunjukkan turun naik rawatan pemakanan bersama-sama dengan masa persampelan. TVFA di antara rawatan pemakanan adalah tidak berbeza secara ketara ($P > 0.05$) pada 12 jam pasca prandial tetapi kepekatan asid propionik berbeza secara ketara antara rawatan diet dengan KHLL mempunyai kepekatan tertinggi diikuti oleh KH, LL dan kawalan. Dalam kepekatan asid butirik pada 12 jam pasca prandial, kawalan, KH dan LL adalah sama dan jauh berbeza ($P < 0.05$) daripada KHLL yang telah mencatatkan kepekatan asid butirik yang terendah dan ini adalah satu pengesahan bagi penggunaan tenaga pemakanan yang cekap. Dalam kepekatan asid asetik, tahap kepekatan didapati telah meningkat sehingga 4 jam pasca prandial sebelum beransur-ansur berkurangan, dengan KHLL direkodkan sebagai yang tertinggi ($P < 0.05$) berbanding dengan yang selebihnya. Begitu juga dengan kepekatan NH_3 bagi KHLL yang dipengaruhi secara bererti ($P < 0.05$) daripada diet lain yang serupa. Daripada hasil keputusan profil populasi mikrob *in vivo*, jumlah bakteria telah didapati berada di puncak populasi mereka pada 4 jam pasca prandial sebelum beransur-ansur berkurangan dalam semua diet rawatan. Pada 12 jam pasca prandial, jumlah protozoa dan methanogens didapati telah berkurangan bersama-sama dengan masa persampelan dan diet makanan ternakan dengan kemasukan foraj (KHLL, KH dan LL) adalah jauh lebih rendah ($P < 0.05$) berbanding kawalan. Bilangan bakteria selulotik *R. albus*, *R. flavefaciens* dan *Fibrobacter succinogenes* didapati tinggi dalam diet makanan ternakan dengan kemasukan foraj berbanding kawalan pada 4 jam pasca prandial.

Berdasarkan hasil kajian ini, dapat disimpulkan bahawa diet makanan ternakan dengan kemasukan KHLL boleh digunakan dalam diet kambing secara cekap tanpa menjejaskan potensi nutrien dengan mengurangkan bilangan methanogen dan protozoa, meningkatkan asid propionik, asid asetik dan penggunaan nutrien. Sebagai tambahan, asetat adalah penting dalam pembentukan lemak susu dan propionat membantu mengsintesis laktosa dan juga meningkatkan penghasilan susu dalam ternakan tenusu.

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APPROVAL

I certify that a Thesis Examination Committee has met on 01/04/ 2016 to conduct the final examination of AHMED MUIDEEEN ADEWALE on his thesis entitled "Effects of tree leaf forage supplementation on rumen fermentation and microbial profile of goats" **i**n accordance with universities and university College 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 degree of Master of Science (MSc).

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LIST OF ABBREVIATIONS

%	percent
µg	micro gram
µl	micro liter
µm	micrometer
µmol	Micromole
ADF	Acid detergent fiber
ADL	Acid detergent lignin
ANOVA	Analysis of variance
Ca	Calcium
$CaCl_2•2H_2O$	calcium chloride dehydrate
CF	Crude fiber
Cfu	colony forming units
CH_4	Methane
Cm	Centimeter
CO_2	Carbon dioxide
CP	Crude protein
CT	Condensed tannin
CTAB	Cetyltrimethylammonium bromide
D	Day
DH_2O	distilled water
DM	Dry matter
DNA	deoxyribonucleic acid
EE	Ether extract
EDTA	ethylene diamine tetraacetic acid
FAO	Food and Agricultural Organization
G	gram
GS	*Gliricidia sepium*
H	hour
H_2SO4	Tetraoxosulphate (vi) acid
HT	Hydrolised tannin
IVDMD	*In vitro* dry matter digestibility
K	Potassium
KCl	potassium chloride
Kg	kilogram
KH	*Kleinhovia hospital*
KH_2PO_4	monopotassium phosphate
L	liter
LL	*Leucaena leucocephala*
LWG	Live weight gain
M	Mole
ME	Metabilisable energy
Mg	Magnesium
$MgCl_2•6H_2O$	magnesium chloride hexahydrate
Min	minute
MJ/Kg	megajoules per kilogram

Ml	milliliter
Mm	Millimolar
Mmol	Millimole
N	Nitrogen
Na_2HPO_4	disodium phosphate
NaCl	sodium chloride
NDF	Neutral detergent fiber
NH_3	Ammonia
Nm	Nanometer
ºC	degree Celsius
P	Phosphorous
PEG	Polyethylene glycol
Ppm	Part per million
PVPP	Polyvinyl polyprrolidone
Rpm	Revolutions per minute
S	second
SEM	Standard error of mean
TVFA	Total volatile fatty acid
UPM	Universiti Putra Malaysia
USDA	United States Department of Agriculture
v/v	volume per volume

CHAPTER 1

GENERAL INTRODUCTION

Increase in human population at a geometric progression concurrently with rapid economy growth with effect of urban transformation, increase income and changes in consumer preference have stimulated the hike in animal protein demand (Devendra, 2006). However, in the United States, where the world largest economic activities take place is not left out of the drastic increase in the demand for animal protein source. This is due to the inflow of diverse ethnic population and increasing cognizance of the nutritional attribute of meat from goat (Knight *et al*., 2006). The global trade capacity for lamb, meat from goat and mutton was approximately US$5.9 billion in 2006 and was anticipated to increase to US$7.2 billion by 2011 (Parker and Lilly, 2005) while Malaysia market potential was approximately US$31.7 million in 2006 and was anticipated to increase to US$42.66 million in 2011.

However, in the last three decade, Malaysia has experienced a tremendous increase in demand for animal based protein source due to increase per capital consumption of the major meat type (Bisant, 2006). Furthermore, the aggregate of Malaysia population has increased to 2.5% annual average growth rate over this period from 13.9 million to 27.7 million in 1980 and 2008 respectively (Department of Statistics Malaysia, 2008). However, the medium earning in nominal terms increased by 7.4% and actual earnings rose by 3.5% over the same time frame. Hence, there is need to improve livestock production through better feeding management.

Feed constitute a larger percentage of the cost of production in most of the livestock industry. Feeding small ruminant well is of fundamental importance for the success of small ruminant industry. Since good nutrition is an identification for good health, effective reproduction, proper maintenance, fast growth rate, high milk yield and a successful goat system (Peacock, 1996).

However, the major limiting factor to increase production of livestock in developing countries like Malaysia is the instability of quality and quantity of conventional feeds. This is due to changes in climate, water scarcity, depletion of land and increase in resources competition for food, feed and fuel production. This will cause an additional challenge for the livestock sector in the long haul. The natural resources and environmental significance of livestock production have been the concern of researchers in recent time as well as deliberation on climatic change.

In order to meet the demand of people on livestock products, by reducing the difference between available product and the huge demand, there is need to improve livestock production through feeding strategy to protect the threatened food security. The use the non-conventional feeds like forages which are not competed for by human for consumption, capable of growing in poor soils and has the ability to withstand drought as well as erosion could be the best alternative.

Some studies have been done on tree forages due to their undisputable potentials in terms of nutrient and availability as protein supplement either as meal mixture or as sole in animal diet but more are yet to be done (Rubanza *et al*., 2007; Jones *et al*, 1994; Rubanza *et al*., 2003). The use of these tree forages has recorded a lot of success (Salgado *et al*., 2013). However, the use of *Kleinhovia hospita* and other tree forages known for their exceptional properties has not been explored in animal feeding (Arung *et al*., 2012). Since small ruminant could not meet their nutritional requirement especially during the dry season, this calls for the supplementation of high protein tree forages (*Kleinhovia hospita, Leucaena leucocephala* and *Gliricidia sepium*) in goat fed with treated rice straw which will be investigated to know the effect on rumen fermentation profile and rumen microbial population in the present study.

1.1 Objectives

I. To evaluate the nutritive value and anti nutritive factor of selected tree forages (*Kleinhovia hospita*, *Leucaena leucocephala* and *Gliricidia sepium)* and their effects on the *in vitro* rumen fermentation and gas production in goats.

II. To determine the effect of partial replacement of concentrate with selected tree forages in goat fed treated rice straw diet on the *in vivo* rumen fermentation profile and microbial population.

1.2 Hypothesis

Dietary supplementation of selected tree forages will improve the rumen fermentation profile and microbial population of goat fed with treated rice straw based diet.

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