UNIVERSITI PUTRA MALAYSIA

EFFECTS OF POLYPHENOLS IN OIL PALM (Elaeis guineensis Jacq.) LEAFLET ON In Vitro RUMEN FERMENTATION, BIOHYDROGENATION AND MICROBIAL POPULATION IN GOATS

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By

ALIF AIMAN ZAKARIA

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

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DEDICATION

TO MY LATE FATHER ZAKARIA HASAN,
MOTHER ROSIDAH JANI
(I LOVE YOU FOREVER),
AND BELOVED FRIENDS

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 POLYPHENOLS IN OIL PALM (*Elaeis guineensis* Jacq.) LEAFLET ON *In Vitro* RUMEN FERMENTATION, BIOHYDROGENATION AND MICROBIAL POPULATION IN GOATS

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October 2016

Chairman: Associate Professor Goh Yong Meng, PhD
Faculty : Veterinary Medicine

The oil palm (*Elaeis guineensis* Jacq.) fronds (OPF) which are made up of petioles and leaves can be used as forage or feed supplement for small ruminants. Their effects on rumen fermentation, biohydrogenation and microbial populations have not been fully investigated. A methanolic extract of the leaves of the OPF was prepared and its polyphenol content and constituents were determined. The polyphenols in the OPLE were mainly tannins with lesser amounts of flavonoids and saponins. The effects of the methanol extract on *in vitro* rumen fermentation, biohydrogenation and microbial populations were assessed using rumen fluid obtained from goats. Two rumen fistulated (Bar-Diamond, Parma, ID, USA) Kacang crossbred male goats weighing 30.39 ± 0.74 Kg that were fed a diet comprising 50% alfalfa and 50% concentrate (DM basis) twice daily at 08:00 and 17:00 h were used in this study. The animals were housed individually in metabolic cages. Water and mineral blocks were available *ad libitum.* Fresh oil palm leaves were obtained from MARDI and immediately a methanolic oil palm leaf extract (OPLE) was prepared. Four treatment groups were designed namely a control group (CON), without OPLE (0%), (T1; low OPLE) containing 2.5 % OPLE, (T2; medium OPLE) containing 5% OPLE and (T3; high OPLE) containing 10% OPLE.

A total of 0.25 g dried treatment feed material was placed in a 100 ml calibrated glass syringe and 30 ml of rumen fluid (from the rumen fistulated goats) were added into each syringe. All the syringes were incubated at 39 ˚C in a water bath incubator for 24 h. The volume of gas produced was measured at 0, 2, 4, 8, 10, 12 and 24 h post-incubation and the pH was recorded at 24 h post-incubation. The *in vitro* gas production of the OPLE methanolic extract after 24 h and the rate of *in vitro* gas production were significantly ($P<0.05$) higher in the CON compared with the treatment groups. In addition, there was a significant ($P<0.05$) decrease in rumen methane gas production in the treatment groups. There was no significant difference in the rate of *in vitro* biohydrogenation between the CON and treatment groups ($P>0.05$) although there was a decreasing trend in the latter. A Real-time Polymerase Chain Reaction technique was performed using the Bio-Rad CFX96 Touch (Bio-Rad Laboratories, Hercules, CA, USA) using optical grade plates to determine microbial populations and species. There was no significant difference ($P>0.05$) in the total bacteria and total protozoa population between the CON and treatment groups. However, there was a significant decrease ($P<0.05$) in the *Butyrivibrio* genus population in the treatment groups compared to the CON group. Similarly, there was a significant decrease ($P<0.05$) in the total methanogenic bacteria population in the treatment groups compared to the CON group. Although the OPLE methanolic extract reduced rumen total gas production and methane production, it did not affect rumen fermentation as reflected by normal pH values and volatile fatty acid (VFA) production. The overall results suggest that the OPLE can be used as a feed supplement for small ruminants as it does not affect rumen fermentation parameters, namely rumen pH and VFA production. In fact, it decreased methane production which would help in reducing pollution and tended to reduce biohydrogenation which should lead to the production of healthier meat. Further *in vivo* investigations are required to confirm these findings.

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

KESAN POLIFENOL DALAM DAUN KELAPA SAWIT (*Elaeis guineensis* Jacq.) TERHADAP FERMENTASI, BIOHIDROGENASI DAN POPULASI MIKROB RUMEN *In Vitro* DALAM KAMBING

Oleh

ALIF AIMAN ZAKARIA

Oktober 2016

Pengerusi : Profesor Madya Goh Yong Meng, PhD
Fakulti : Perubatan Veterinar

Pelepah kelapa sawit (*Elaeis guineensis* Jacq.) yang terdiri dari petiole dan daun boleh digunakan sebagai makanan ternakan atau makanan tambahan bagi ruminan. Daun kelapa sawit segar diperoleh dari MARDI dan ekstrak methanol daun kelapa sawit (OPLE) telah disediakan dan kandungan polifenol serta jenisnya telah ditentukan. Kebanyakan polifenol dalam OPLE adalah jenis tannin dengan sedikit flavonoid dan saponin. Kesan OPLE yang kaya dengan polifenol ke atas fermentasi, biohidrogenasi dan populasi mikrob *in vitro* telah di nilai menggunakan cecair rumen daripada kambing.The effects of the methanol extract on *in vitro* rumen fermentation, biohydrogenation and microbial populations were assessed using rumen fluid obtained from goats. Dua kambing jantan kacukan Kacang dipasang dengan fistula rumen (Bar-Diamond, Parma, ID, Amerika Syarikat) seberat 30.39 ± 0.74 Kg yang diberi makan diet yang terdiri daripada 50% alfalfa dan 50% konsentrat (berasaskan DM) sebanyak dua kali setiap hari pada jam 08:00 dan 17:00 h telah digunakan untuk kajian ini. Haiwan itu telah ditempatkan secara berasingan dalam sangkar metabolik. Air dan mineral blok disediakan secara *ad libitum*. Empat kumpulan rawatan telah direka iaitu kumpulan kawalan tanpa OPLE (0%) (CON), OPLE rendah (2.5%) (T1), OPLE sederhana (5%) (T2) dan OPLE tinggi (10%) kumpulan (T3). Sebanyak 0.25 g rawatan kering bahan makanan diletakkan dalam 100 ml ditentu ukur picagari kaca, dan 30 ml cecair rumen (dari kambing berfistula) telah ditambah ke dalam setiap picagari. Semua picagari dieram pada 39 ˚C dalam inkubator mandi air selama 24 jam. Jumlah hasil gas diukur pada 0, 2, 4, 8, 10, 12 dan 24 h dieram dan pH telah direkodkan selepas 24 h. Dalam pengeluaran gas *in vitro* ekstrak metanol OPLE selepas 24 h dan kadar *in vitro* pengeluaran gas menunjukkan peningkatan yang ketara antara kumpulan CON dan rawatan ($P < 0.05$). Pengeluaran gas metana rumen menunjukkan penurunan yang ketara antara kumpulan CON dan rawatan ($P < 0.05$). Kadar biohidrogenasi menunjukkan tiada perbezaan yang signifikan di antara kumpulan CON dan rawatan ($P < 0.05$) tetapi ia menunjukkan trend menurun. Masa nyata Polymerase Chain Reaction dilakukan dengan Bio-Rad CFX96 Touch (Bio-Rad Laboratories, Hercules, CA, Amerika Syarikat) menggunakan plat gred optik. Jumlah bakteria dan jumlah protozoa menunjukkan tiada perbezaan yang signifikan antara kumpulan CON dan rawatan ($P < 0.05$). hasil yang *Butyrivibrio* genus menunjukkan penurunan yang signifikan di antara kumpulan CON dan rawatan (P

<0.05). Jumlah bacteria metanogen menunjukkan penurunan yang signifikan di antara kumpulan CON dan rawatan (P <0.05). Sungguhpun ekstrak metanol OPLE telah mengurangkan jumlah pengeluaran gas dan pengeluaran metana tetapi tiada kesan ke atas fermentasi rumen yang dibuktikan dengan pH dan pengeluaran asid lemak meruap (VFA) yang normal. Keputusan keseluruhannya mencadangkan bahawa OPLE boleh digunakan sebagai makanan tambahan untuk ruminant kecil sebab ianya tiada kesan ke atas parameter fermentasi rumen terutama sekali pH rumen dan pengeluaran VFA. Ianya mengurangkan pengeluaran metana yang akan membantu mengurankan pencemaran udara dan mengurangkan biohidrogenasi rumen ke arah pengeluaran daging yang lebih, sihat. Penyesiatan *in vivo* selanjutnya diperlukan untuk mempastikan kesasihan keputusan-keputusan ini.

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I certify that an Examination Committee has met on (date) to conduct the final examination of Alif Aiman Zakaria on his Master of Science thesis entitled **"Effects of Polyphenols in Oil Palm (*Elaeis guineensis* Jacq.) Leaf on *in vitro* Rumen Fermentation, Biohydrogenation 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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This thesis 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 Supervisor Committee were as follows:

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
ADF	acid detergent fiber
cal	calorie
CLA	conjugated linoleic acid
cm	centimeter
CP	crude protien
CT	condensed tannins
d	day
DM	dry matter
DNA	deoxyribonucleic acid
EE	ether extract
FA	fatty acid
FAME	fatty acid methyl esters
FID	flame ionization detector
g	gram
GAE	gallic acid equivalent
h	hour
HPLC	high performance liquid chromatography
HT	hydrolysable tannins
kg	kilogram
L	liter
M	molar
m	meter
MARDI	Malaysian Agricultural Research and Development Institute
ME	metabolisable energy
mg	milligram
min	minute
mL	milliliter
mm	millimeter
MUFA	monounsaturated fatty acid
µm	micrometer
NDF	neutral detergent fiber
nm	nanometer
OPF	oil palm frond
OPL	oil palm leaf
OPLE	oil palm leaf extract
PCR	polymerase chain reaction
pdiff	*piecewise differentiable*
PUFA	polyunsaturated fatty acid
RNA	ribonucleic acid
SE	standard error
sec	second
SFA	saturated fatty acid
TF	total flavonoids
TP	total polyphenol
TS	total saponins
TT	total tannins
UFA	unsaturated fatty acid

VFA	volatile fatty acid
wk	week

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of Study

The slow development of the ruminant livestock industry in Malaysia had been partly due to the lack of grazing land and insufficient sustainable feed for ruminant livestock production. There have been many investigations on finding alternative feed sources, especially cheap agricultural byproducts, with mixed success. The oil palm fronds (OPF) are one of the most abundant agricultural byproducts, which are available throughout the year and their effective utilization may provide a sustainable feed for the ruminant livestock industry in this tropical region. The total production of this oil palm frond is estimated at 50 million metric ton hectares per year (MPOB, 2014).

The OPF have been shown to have great potential to be used as a feed supplement for small ruminants including sheep (Goh *et al.,* 2002 and Ebrahimi *et al.,* 2012). Their earlier work showed that the OPF can be used as a supplement but not as a whole feed as it is not nutritionally adequate.

The OPF have also been shown to decrease ruminal biohydrogenation in sheep and goats hence increasing the availability of unsaturated fatty acids, namely linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) to be absorbed and deposited in the animal tissues resulting in an increase in the 'more healthy' unsaturated fatty acids (UFA) and a decrease in the 'less healthy' saturated fatty acids (SFA) (Goh *et al.,* 2002 and Ebrahimi *et al.,* 2012). Both earlier investigations also showed changes in the ruminal environment, such as changes in the rumen populations, particularly the protozoal population, without adversely affecting ruminal fermentation characteristics. Thus, the potential of using the OPF as a feed supplement in small ruminant feeds is not solely due to its availability throughout the year but its potential to produce chevon and mutton with less SFA in their meat, which should appeal to the health conscious consumer.

There were attempts to explain the reduction in ruminal biohydrogenation in small ruminants fed OPF supplemented feeds where the presence of unknown substances and the microbial population changes especially the protozoa were provided as likely causes but these changes were not quantified.

The OPF contained large amounts of polyphenols 24.3 mg gallic acid equivalent (GAE)/g dry weight (Runnie *et al.,* 2003), in fact higher than green tea 22.5 mg (GAE)/g dry weight (Runnie *et al.,*2003) and these plant secondary metabolites have been implied to cause a decrease in biohydrogenation (Jayanegara *et al.,* 2012). The changes in ruminal microbial populations can also be quantified using more recent real time PCR methods (Ebrahimi *et al.,* 2012).

Based on the argument presented above, a thorough *in vitro* investigation was carried out to determine the effects of a methanolic extract of polyphenols contained in OPF on the rumen environment and characteristics of rumen fluid from fistulated goats.

1.2 General objective

To determine the amounts of polyphenol and its fractions in oil palm (*Elaeis guineensis* Jacq.) fronds and assess the effects of its methanolic extract on *in vitro* rumen fermentation, biohydrogenation and microbial populations.

1.3 Specific objectives

1. To determine the polyphenol content and its fractions in the leaves of oil palm (*Elaeis guineensis* Jacq.) fronds (OPF).
2. To assess the effects of a polyphenol rich extract of OPF on rumen fermentation characteristics and biohydrogenation *in vitro* using the rumen fluid of fistulated goats.
3. To measure the effects of a polyphenol rich extract of OPF on the rumen microbial (bacteria, protozoa, fungi) populations using the rumen fluid of fistulated goats.

1.4 Hypothesis

Polyphenol rich fractions of OPF decrease the rate of ruminal biohydrogenation through the changes in the rumen microbial populations, without adversely affecting rumen fermentation characteristics *in vitro*.

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