UNIVERSITI PUTRA MALAYSIA

EFFECTS OF SUPPLEMENTING OIL PALM (Elaeis guineensis Jacq.) LEAF METHANOLIC EXTRACTS ON RUMEN FERMENTATION AND MEAT QUALITY OF BOER GOATS

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By

WISAM SALIM RASHEED AL-JUMAILI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

October 2018

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DEDICATION

To the soul of my dear lost brother “Wea’am”

And

To my teachers who providing me with best education

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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

Red meat and its meat products have been associated with significant risks of chronic metabolic disorders in human, as well as about one-fifth of annual global green-house gas emissions. Therefore, production of meats with healthier fatty acid profiles that are also environmentally sustainable, is of major interest to the meat industry. Oil palm fronds (OPF) have been used in ruminant feeds as a source of roughage. The incorporation of OPF in the diet has been shown to improve meat quality, and reduced saturated fatty acids (SFA) content in the meat. Being a readily available and abundant agriculture byproduct, OPF has the potential of being utilized as a feed source. The potency of OPF in improving rumen fermentation and nutrient digestion is related to the phytochemical compounds found in OPF. Therefore, it is logical to investigate the feasibility of using OPF extracts to improve ruminal nutrient digestion and meat quality, while restricting methane emissions from the ruminant. In view of the significance and potential applications of oil palm leaves extract (OPLE), the current study investigated the effects of OPLE on growth performance, carcass characteristics, meat quality and tissue fatty acid profiles in Boer goats, fed diets supplemented with different concentrations of OPLE. Nutritional and physiological changes in the rumen as a result of OPLE supplementation such as changes in rumen fermentation, gas production and as well as the composition of microbial populations were evaluated.

The present study was conducted in three phases at Universiti Putra Malaysia (UPM). In the first phase, oil palm leaves were obtained and subjected to extraction and characterization procedures. Oil palm leaf extract (OPLE) was extracted using methanol and characterized. Anti-oxidant activity of OPLE and the associated compounds were identified using gas chromatography-mass spectrometry (GC-MS).

In the second phase of the study, the effects of OPLE on *in vitro* methane production, *in vitro* and *in vivo* rumen fermentation, bio-hydrogenation and microbial population were determined. In the third phase, the effects of OPLE supplementation was performed on 21 Boer goats. The study investigated the growth performance, nutrient digestibility, carcass characteristics, meat quality, tissue fatty acid profile and rumen characteristics following OPLE supplementation at different concentrations in goats. Rumen bio-hydrogenation and fermentation, fatty acid composition of the rumen digests, lipid content of the liver, muscles, subcutaneous fatty acid and adipose tissue were also determined. Other parameters that were investigated, included the PPARα, PPARγ and Stearoyl CoA desaturase (SCD) gene expression in LD muscle and liver.

Results showed that oil palm leaves produced significant amount of methanolic OPLE extract. Methanolic extraction of oil palm leaves produced higher (8.28%) phenolic compounds compared to hexane (3.10%) and chloroform (3.08%). The results also confirmed that OPLE possessed potent anti-oxidant activity as well as strong scavenging activity of 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The GC-MS results revealed the presence of 56 compounds in OPLE.

In vitro gas and methane production in goats was also reduced significantly following dietary OPLE supplementation in goats. There were also changes in the rumen bacterial flora following OPLE supplementation, an indication that the OPLEcompounds had selective antimicrobial effects. Additionally, acetic and butyric acid concentrations were increased, while propionic acid contents declined. The volatile fatty acids (VFA) content were no different between OPLE supplemented and non-supplemented groups in both *in vitro* and *in vivo* studies. Overall, the totalVFA concentration was not affected by the addition of 7.5 mg/kg dry matter (DM) of extract compared to control, suggesting that the OPLE did not affect ruminal digestion adversely. Significant reduction in the concentration of saturated fatty acids (SFA) in the OPLE treated groups was also recorded.

The present study also established that OPLE caused induction in color stability, reduction in lipid oxidation, cholesterol levels, *in vitro* bio-hydrogenation as well as reduction in SFA in meat samples and stabilization in meat pH. Other findings included the fact that OPLE supplement did not affect rumen fermentation and nutrients digestibility despite the positive effect of OPLE supplement on rumen bio-hydrogenation (BH).

Based on the findings of the study, it is concluded that OPLE had the potential to be employed as a supplement in ruminant diet which could enhance the production of higher quality ruminant meat. Using OPLE as supplement in goat fattening diets alsomitigated gas and methane production in rumen without affecting rumen fermentation. The 56 compounds identified in the study could be further refined to serve as feed supplements in industries associated with animal nutrition and production.

Keywords:
Oil palm, plant secondary metabolites, methane, meat quality, goat.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

SUPLEMENTASI EKSTRAK METANOLIK DEDAUN KELAPA SAWIT (*Elaeis guineensis* Jacq.) DAN KESANNYA KE ATAS FERMENTASI RUMEN DAN KUALITI DAGING PADA KAMBING BOER

Oleh

WISAM SALIM RASHEED AL-JUMAILI

Oktober 2018

Pengerusi : Profesor Madya Goh Yong Meng, PhD
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Daging merah dan hasilan dagingnya berkait rapat dengan risiko penyakit metabolik pada manusia. Penghasilan daging merah juga menyumbang kepada seperlima daripada jumlah penghasilan tahunan gas rumah hijau sedunia. Justeru itu, pengeluaran daging dan produk daging yang mempunyai profil lemak yang sihat yang lestari merupakan fokus utama industri daging. Dedaun pelepah kelapa sawit (OPF) banyak digunakan sebagai foraj makanan ruminan. Penambahan OPF dalam diet kambing ternyata boleh meningkatkan kualiti daging, dan mengurangkan kandungan asid lemak tepu (SFA) dalam daging. OPF mempunyai potensi untuk digunakan sebagai sumber makanan haiwan kerana ia merupakan produk sisa pertanian yang mudah diperolehi. Keupayaan OPF untuk memperbaiki profil penapaian dan penghadaman nutrien di dalam rumen berkait rapat dengan komposisi fitokimia pada OPF. Sehubungan itu, adalah logik untuk mengkaji potensi penggunaan estrak dedaun pelepah kelapa sawit (OPLE) dalam meningkatkan kecekapan penghadaman nutrien dan kualiti daging, di samping menghadkan penghasilan gas metana daripada ruminan. Berdasarkan kepada signifikan dan potensi aplikasi OPLE dalam industry ternakan, kajian ini betujuan untuk menentukan prestasi tumbesaran, ciri karkas, kualiti daging dan profil asid lemak tisu pada kambing Boer yang diberi diet berbilang kepekatan OPLE. Perubahan nutrisi dan fisiologi pada rumen akibat suplementasi OPLE seperti perubahan dalam penapaian rumen, produksi gas serta populasi mikrob turut dicerap.

Kajian ini telah dijalankan dalam tiga fasa di Universiti Putra Malaysia (UPM). Di fasa pertama, dedaun pelepah kelapa sawit telah diperolehi untuk pengekstrakan dan pencirian bahan. Ekstrak daun kelapa sawit (OPLE) (metanolik) telah ditentukan dan dicirikan. Aktiviti anti-oksidan OPLE dan sebatian berkaitan telah dikenalpasti dengan menggunakan gas kromotografi-mass spectrometri (GC-MS).

Dalam fasa kedua kajian, kesan OPLE ke atas produksi metana secara *in vitro*, penapaian rumen secara *in vitro* dan *in vivo*, biohidrogenasi dan populasi mikrob telah dijalankan. Di fasa ketiga, kesan penambahan OPLE telah dilakukan pada 21 ekor kambing Boer. Kajian ini mengkaji kesan OPLE keatas prestasi pertumbuhan, keupayaan penghadaman nutrien, ciri karkas, kualiti daging, profil tisu asid lemak tepu dan ciri penapaian rumen telah ditentukan. Biohidrogenasi rumen dan penapaian, komposisi asid lemak rumen, kandungan lipid hati, otot, asid lemak subkutanus dan tisu adipos telah ditentukan. Lain-lain parameter yang telah ditentukan termasuklah PPARα, PPARγ dan expresi gen Stearoyl CoA desaturase (SCD) dalam otot LD dan hati.

Keputusan menunjukkan bahawa dedaun pelepah kelapa sawit mempunyai jumlah ekstrak methanolik yang signifikan. Pengekstrakan methanolik dari daun kelapa sawit memberi sebatian fenolik lebih tinggi (8.28%) berbanding heksana (3.10%) dan kloroform (3.08%). Keputusan juga menunjukkan OPLE mempunyai aktiviti antioksidan termasuklah aktiviti penipisan 2,2-Diphenyl-1-picrylhydrazyl (DPPH). Keputusan GC-MS menunjukkan kewujudan 56 kompoun dalam ekstrak OPLE.

Pengurangan pengeluaran gas *in vitro* dan metana yang ketara telah dicerap pada kambing yang dirawat dengan OPLE berbanding kumpulan kawalan. Perubahan dalam flora bakteria rumen turut dicerap pada kambing yang diberi suplemen OPLE. Ini menunjukkan bahawa kompaun OPLE mempunyai kesan antibakteria yang memilih. Di samping itu, kandungan asid asetik dan butyrik meningkat sementara asid propionik menunjukkan pengurangan. Walaubagaimanapun, kandungan asid lemak yang meruap (VFA) tidak berbeza di antara semua kumpulan rawatan dalam eksperimen *in vitro* dan *in vivo*. Kepekatan VFA tidak terjejas oleh penambahan 7.5 mg/kg berat kering (DM) ekstrak berbanding kumpulan kawalan. Ini membawa erti bahawa penambahan OPLE tidak menjejaskan proses penghadaman dalam rumen. Pengurangan kepekatan asid lemak tepu (SFA) yang ketara dalam kumpulan yang dirawat dengan OPLE turut dicerap.

Kajian ini juga mendapati bahawa OPLE membawa kepada kestabilan warna, penurunan pengoksidaan lipid, paras kolestrol, penghidrogenanbio *in vitro* dan pengurangan SFA dalam sampel daging, di samping menstabilkan pH daging. Lain-lain penemuan termasuklah ketiadaan kesan ke atas penapaian rumen dan kebolehhadaman nutrient, walaupun penambahan OPLE telah memberi kesan kepada penghidrogenanbio (BH) dalam rumen.

Kesimpulannya, OPLE mempunyai potensi untuk digunakan sebagai suplemen diet ruminan untuk meningkatkan pengeluaran daging ruminan berkualiti tinggi. Penggunaan OPLE sebagai suplemen dalam diet penggemukan kambing juga boleh mengurangkan penghasilan gas dan metana dalam rumen, tanpa memberi kesan kepada aktiviti penapaian rumen. 56 sebatian yang dikenalpasti dalam kajian ini boleh

diperinci dan dikaji untuk dijadikan sebagai suplemen makanan untuk industri makanan dan produksi haiwan.

Kata kunci:
Kelapa sawit, metabolit sekunder tumbuhan, metana, kualiti daging, kambing.

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

ADF	acid detergent fiber
ANOVA	analysis of variance
^{o}C	degrees centigrade
^{o}C/min	degrees centigrade per minute
cal	calorie
cm	centimeter
cm^2	square centimeter
d	day
DM	dry matter
FAR	fatty acid ratio
FE	feed efficiency
g	gram
GLM	general linear model
h	hour
kcal	kilo calories
Kg	kilogram
L	liter
LD	*longissimus dorsi*
m	meter
mm	millimeter
MDA	malondialdehyde
ME	metabolizable energy
min	minute
mmol/L	millimoles per liter

μmol/L	micromoles per liter
mg	milligram
mg/L	milligrams per liter
mL	milliliter
mL/min	milliliters per minute
MUFA	monounsaturated fatty acids / monoenoic fatty acids
n-6: n-3 ratio	total n-6 PUFA to total n-3 PUFA ratio
NDF	neutral detergent fiber
OPF	oil palm fronds
OPL	oil palm leaves
OPLE	oil palm leaves extract
PPA	peroxisome proliferators-activated receptors
PSM	plants secondary metabolites
PUFA	polyunsaturated fatty acids
PUFA: SFA ratio	total PUFA to total SFA ratio
SCD	stearoyl-CoA desaturase
SEM	standard error of mean
SFA	saturated fatty acids
TBARS	thiobarbituric acid reactive substances
UFA	unsaturated fatty acids
UFS: SFA ratio	total UFA to total SFA ratio
VFA	volatile fatty acid
WBSF	Warner-Bratzler shear force

CHAPTER 1

GENERAL INTRODUCTION

Meat protein is an important source of nutrients for human populations. Evolving demographics and expanding economies throughout the world meant that more and more populations would have access to this high-quality food source, and with it ailments associated with over consumption of meats like high saturated fatty acids and cholesterol. Apart from that, intensive animal production also resulted in potentially damaging environmental consequences, such as pollution and excessive greenhouse gas emission. In fact, the animal production industry is responsible for about 15-21 % annual greenhouse gas emission globally (Moss *et al*., 2000). Red meat and its products are rich in energy, macronutrient (protein and fat), salt content and nutritional characteristics which contribute to tastefulness and satiety. The presence of a wide range of micronutrients such as minerals (heme iron, zinc) and vitamins (vitamin A, B12), logically make it an indispensable source of essential nutrients for humans (Mcafee et al., 2010).

The goat is an important source of animal protein for humans in many countries (Webb *et al*., 2005). The global acceptance of goat meat has been on the rise primarily because it is leaner compared to beef and mutton (Mahgoub et al., 2002). In fact the importance of goats in global production of meat, milk and hire is becoming ever important by the years (Skapetas and Bampidis, 2016).

In human nutrition, dietary recommendations that to reduce the risk of cardio vascular disease (CVD) are to reduce the daily energy intakes of total fat, saturated fatty acids (SFA) as well as trans fatty acids, in order to mitigate their cholesterol raising effects (Gidding *et al*., 2005). On the other hand, current human diets in the developed world are usually and unfortunately characterized by high levels of SFA, with low levels of UFA (Simopoulos *et al*., 1999). Several studies have associated the high fat content of red meat with CVD and colon cancer (Mcafee *et al*., 2010).

Ruminant meats are generally associated with higher saturated and trans fats. This is because of the biohydrogenation (BH) of UFA as they enter the rumen environment (Jenkins *et al*., 2008). Rumen BH constitutes the fundamental reason for the saturation of UFA and increase in SFA in the rumen and consequently in animal products (meat and milk) (Kim *et al*., 2009). Rumen BH also occurs in tandem with methanogenesis as feed materials are being subjected to microbial digestion in the foregut. The process of methanogenesis in the rumen is a significant avenue through huge quantities of dietary energy are lost (Patra *et al*., 2017). The emission of methane has been reported to contribute in global warming as greenhouse gas (GHG) (Patra *et al*., 2017). Methane emissions (CH4) are generated by microbial fermentation of feed components in anaerobic conditions. Larger percentage of methane is produced in the rumen (87%) while the rest comes from large and small intestines. The methane generated in the

rumen is released mainly through the process of eructation (Boadi *et al*., 2004). The reason leading to the formation of methane in the rumen is the urgent need to remove hydrogen that are typically generated when feedstuffs are degraded by rumen microbes. The methanogens are responsible in the production of methane (Boadi *et al*., 2004), where hydrogen is bonded to carbon creating methane gas that is energy dense and much more stable than hydrogen under the oxygen rich environment which methane is released to. These would have the net effect of reducing the overall hydrogen content in the rumen.

Therefore, mitigating methane emission involved balancing the efficiency of nutrient digestion in the rumen that involved BH, to that of the need to constantly remove ruminal hydrogen through methanogenesis. Several approaches have been attempted to modify rumen BH, this is to reduce the quantity of SFA in the meat, increase the quantity of UFA and to reduce rumen methanogenesis. Many chemical feed additives such as antibiotics, ionophores, methane inhibitors and defaunation agents have been introduced into ruminant nutrition to promote growth, improve feed utilization and decrease methane production (Guan *et al*., 2006; Hristov *et al*., 2015).

Instead of using of chemicals, one of these proposals is through the use of plant secondary metabolites. Compounds such as polyphenols specially plants phenolic extracts have been incorporated in ruminants feed (Vasta and Luciano, 2011; Henke *et al*., 2017). Polyphenols have been reported to possess the ability to modify rumen biohydrogenation, thereby mitigating the saturation of UFA in the rumen (Dschaak *et al*., 2011). Plant extracts have also been shown to be able to mitigate methane production in ruminants (Saeid Jafari, Goh, et al., 2016). These are further supported by similar work using other plants leave extracts with rich concentrations of bioactive PSM such as saponins, tannins, flavonoids and several other metabolites (Patra and Saxena, 2010), to modify rumen BH (Vasta *et al*., 2009), thereby mitigating the production of methane (Goel and Makkar, 2012). Therefore, it is the aim of the current work to further explore this possibility utilizing polyphenols derived from oil palm fronds. The oil palm frond is an abundant resource in Malaysia. The produced amounts of oil palm fronds were 51 and 48 million tons in 2008 and 2011, respectively (MPOB, 2009; Zawawi *et al*., 2012) .

The oil palm (*Elaeis guinensis*) fronds (OPF) has been employed in ruminants feeding as a source of roughage (Ebrahimi *et al*., 2013; Wan Zahari *et al*., 2003). The incorporation of OPF in goat fattening diet has been reported to improved meat quality and reduced SFA in the meat (Ebrahimi *et al*., 2012). However, feeding the OPF to animals remained a major logistical and transportation challenge. Therefore, it is believed that by extracting the polyphenols from OPF, and utilizing this extract would solve the logistical issues in the use of OPF in ruminant diets. The extraction of Oil Palm Leaves Extract (OPLE) from OPF using methanol was carried out by Jaffri (2009) and it was found to be rich in polyphenols with substantial antioxidant properties. The phenolic component of the OPLE has been investigated and has been shown not to cause any apparent *in vivo* toxicity in animals (Anyanji *et al*., 2013; Syahmi *et al*., 2010). Despite the enormous significance of the OPLE as reported by

several authors, the current research is the sole study so far that extracted OPLE in larger quantities with consistent quality control in order to measure OPLE's impact on the growth performance, carcass characteristics, meat quality and tissue fatty acid profiles in goats. The current research also attempted to determine changes in rumen fermentation and rumen microbial populations in the goat as a result of feeding diets supplemented with different concentration of OPLE. Malaysia produces huge quantities of OPF as byproduct from the palm-oil industry that remained largely unutilized. Thus, there is a need to explore use the OPLE as a feed supplement and potential phytochemical additive in animals. This is would be a welcoming solution to improve animal production, while at the same time addressing the environmental impacts due to methane emission, and the accumulation of underutilized waste from the oil palm industry.

Therefore, the current study was designed with the following hypothesis and objectives:

Hypothesis

Inclusion of OPLE as a feed supplement in goat diets will mitigate *in vitro* methane emission, modify rumen digestion and fermentation characteristics and reduce meat cholesterol and SFA's resulting in healthier meat.

Objectives

The present study was carried out with the following objectives:

1. To determine the phytochemical composition of oil palm leaves methanolic extract.
2. To assess the effect of OPLE on *in vitro* methane production, and associated rumen fermentation characteristics.
3. To determine the growth performance, carcass characteristics, meat quality, feed digestibility, rumen characteristics, as well as the relative gene expressions of PPAR's and SCD in goats fed diets supplemented with different concentrations of OPLE.

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LIST OF PUBLICATIONS

Al-Jumaili, W. S., Goh, Y. M., Jafari, S., Rajion, M. A., Jahromi, M. F., & Ebrahimi, M. (2017). An in vitro study on the ability of tannic acid to inhibit methanogenesis and biohydrogenation of C18 PUFA in the rumen of goats. *Annals of Animal Science*, *17*(2), 491–502. https://doi.org/10.1515/aoas-2016-0059.

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