UNIVERSITI PUTRA MALAYSIA

RUMEN METABOLISM, MEAT QUALITY AND GENE EXPRESSION CHANGES ASSOCIATED WITH Nigella sativa L. SEEDS AND Rosmarinus officinalis L. LEAF SUPPLEMENTATION IN DORPER SHEEP

KIFAH ODHAIB JUMAAH

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By

KIFAH ODHAIB JUMAAH

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

November 2017

DEDICATION

This thesis is dedicated to

My father and mother

My husband and son

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

RUMEN METABOLISM, MEAT QUALITY AND GENE EXPRESSION CHANGES ASSOCIATED WITH *Nigella sativa* L. SEEDS AND *Rosmarinus officinalis* L. LEAF SUPPLEMENTATION IN DORPER SHEEP

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Chairman : Associate Professor Awis Qurni Sazili, PhD
Faculty : Agriculture

The use of herbs in animal nutrition represents a potent strategy for achieving desired production targets with minimal or no negative impact on animal health and environment. Nonetheless, the impact of herbs on rumen metabolism, growth performance, and product quality is generally less consistent in the published literature. Thus, there is need for additional studies in different production systems to permit tailored decision and informed choices in the utilization of medicinal plants in ruminant nutrition.

The current study examined the effects of *Nigella sativa* (NS) seeds, *Rosmarinus officinalis* (RO) leaves on *in vitro* and *in vivo* rumen metabolism, growth performance, immune response, meat quality and gene expression in Dorper lambs. The study was partitioned into three experiments.

The results in present study are illustrated that the effects of different levels of NS seeds and RO leaves on *in vitro* gas production, rumen fermentation, fatty acids composition and the apparent biohydrogenation of oleic, linoleic and linolenic acids using rumen liquor from Dorper lambs. The NS seeds and RO leaves were supplemented at the rate of 0, 0.5, 1, 1.5 and 2% (w/w) DM of basal substrate [60% forage (urea treated rice straw) and 40% concentrate] and incubated for 24 h at 39°C. Substrates containing RO and NS had greater ($P<0.05$) gas production than the control substrates. The volume of gas produced increased as the levels of RO and NS increased up to 1.5% and decreased afterwards. Supplementation of RO and NS did not affect ($P>0.05$) *in vitro* dry matter digestibility, *in vitro* organic matter digestibility, rumen pH, CH_4 and NH_3-N, total volatile fatty acids (VFA) and the molar proportion of acetate, propionate and butyrate. The RO supplements reduced the ruminal concentration of C18:0 and increased the ruminal concentration of

C18:1n-9 in a dose dependent manner. The supplementation of RO leaves reduced (P<0.05) the apparent biohydrogenation of C18:1n-9 but had no effect (P>0.05) on the apparent biohydrogenation of C18:2n-6 and C18:3n-3.

The results indicated after treatment with *Nigella sativa L.* seeds, *Rosmarinus officinalis L*. leaves and their combination on rumen metabolism, nutrient intake and digestibility, growth performance, immune response and blood metabolites in Dorper lambs. Twenty-four entire male Dorper lambs (18.68±0.6 kg, 4-5 months old) were randomly assigned to a concentrate mixture containing on a dry matter basis either, no supplement (control, T1), 1% *Rosmarinus officinalis* leaves (T2), 1% *Nigella sativa*seeds (T3), or 1% *Rosmarinus officinalis* leaves + 1% *Nigella sativa* seeds (T4). The lambs had *ad libitum* access to urea-treated rice straw (UTRS) and were raised for 90 days. Supplemented lambs had greater (P<0.05) intake of DM and UTRS than the control lambs. Total and daily weight gain were greater (P< 0.05) in T2 lambs than those fed other diets. The T3 and T4 lambs had greater (P< 0.05) ruminal pH than the T1 and T2 lambs. Supplemented lambs improved ruminal total volatile fatty acids, acetate, propionate and reduced NH_3-N, methane gas and C18:0 than the control lambs. Supplemented greater (P< 0.05) serum IgA and IgG compared with the control lambs.

On the other hand, there are detectable evident about ameliorative changes of NS seeds, RO leaves and their combination utilized on carcass attributes, gene expression, lipid oxidation and physicochemical properties of LD, ST and SS muscles in Dorper lambs. The results show that the T2 lambs had greater (P< 0.05) slaughter and cold carcass weights than the control lambs. Meat from supplemented lambs had lower (P<0.05) cooking and drip losses, shear force, lightness, and lipid oxidation and greater (P< 0.05) redness compared with the control meat. *Postmortem* ageing influenced meat quality in Dorper lambs. The impact of dietary supplements on muscle FA varied with muscle type. Furthermore, NS and RO decreased serum total cholesterol, triglycerides, LDL-C and had no effect (P> 0.05) on the expression of SCD and LPL genes in LD and ST muscles in Dorper lambs. The T2 diet up regulated the expression of PRKAA2 gene in LD and ST muscles and up regulated the expression of SREBP1 in LD and ST muscles in Dorper lambs.

Overall, our results concluded that *Nigella sativa* seeds and *Rosmarinus officinalis* leaves variation in the efficacy of in ruminant nutrition. Dietary supplementation of *Nigella sativa* seeds and *Rosmarinus officinalis* leaves had beneficial effects on rumen metabolism, immune response and meat quality in Dorper lambs.

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

PERUBAHAN METABOLISME RUMEN, KUALITI DAGING DAN EKSPRESI GEN BERKAIT DENGAN DENGAN SUPLEMENTASI BIJI *Nigella sativa* L. DAN DAUN *Rosmarinus officinalis* L.PADA BEBIR DORPER

Oleh

KIFAH ODHAIB JUMAAH

November 2017

Pengerusi : Profesor Madya Awis Qurni Sazili, PhD
Fakulti : Pertanian

Penggunaan ramuan herba dalam makanan haiwan meupakan strategi yang berkesan untuk mencapai sasaran pengeluaran, tanpa menjejaskan kesihatan haiwan dan persekitaran. Walau bajaimanapuu, dapatan kajian kesan herba terhadap metabdisme rumen, prestasi tumbesaran kualiti produk adalah tidak konsisten. Sehubunyan itu, kajian lanjutan adalah diperlukan dalam sistem penghasilan yang berbeza bagi memastikan keputusan yang tepat dapat dibuat tentang penggunaan ramuan herba dalam pemakanan ruminan. Kajian ini adalah uatak menilai kesan biji benih *Nigella sativa* (NS), daun *Rosmarinus officinalis* (RO) ke atas metabolisme rumen *in-vitro* dan *in-vivo*, tumbesaran, gerkbalas imun, kualiti daging dan ekspresi gen pada bebiri Dorper. Penyelidikan ini terbahagi kepada tiga eksperimen. Keputusan dalam kajian *in-vitro* ini menunjukkan kesan terhadap tahap yang berbeza dalam biji benih NS dan daun RO dalam penghasilan gas, fermentasi rumen, komposisi asid lemak dan biohidrogenasi asid oleik, linoleik dan linolenik menggunakan fermentasi rumen daripada kambing biri-biri jenis Dorper. Biji benih NS dan daun RO telah ditambah dengan substrat basal pada kadar 0, 0.5, 1, 1.5 and 2% (w/w) bahan kering [60% makananan yang mengandungi (urea dirawat dengan jerami padi) dan kepekatannya pada 40%] dan dibiarkan selama 24 jam pada suhu 39°C. Substrat yang mengandungi RO dan NS mempunyai penghasilan gas lebih besar berbanding substrat terkawal. Isipadu gas yang terhasil meningkat seiring dengan paras RO dan NS yang meningkat pada 1.5% dan menurun kemudiannya. Suplementasi dengan RO dan NS tidak memberikan apa-apa kesan dalam *in-vitro* pencernaan bahan kering, *in-vitro* pencernaan bahan organik, pH rumen, CH_4 dan NH_3-N, jumlah asid lemak meruap (VFA) dan kadaran molar asetat, propionat dan butirat. Suplementasi dengan RO menurunkan kepekatan rumina pada C18:0 dan meningkatkan kepekatan rumina pada C18:1n-9 terhadap dos dependen. Manakala, suplementasi dengan daun RO menurunkan ($P<0.05$) biohidrogenasi dengan ketara pada C18:1n-9 tetapi tiada kesan ($P>0.05$) pada C18:2n-6 and C18:3n-3. Keputusan ini menunjukkan selepas

suplementasi dengan biji benih *Nigella sativa L.*, daun *Rosmarinus officinalis L* dan kombinasi kedua-duanya terhadap metabolisma rumen, pengambilan nutrisi dan pencernaan, prestasi tumbesaran, tindak balas imun dan metabolit darah untuk bebiri jenis Dorper. Dua puluh empat ekor bebiri jantan jenis Dorper (18.68±0.6 kg, berumur 4-5 bulan) telah dipilih secara rawak dan disuplementasikan dengan campuran bahan kering mengandungi sama ada dengan tiada makanan tambahan (kawalan, T1), 1% daun *Rosmarinus officinalis (T2), 1%* biji benih *Nigella sativa (T3),* atau kombinasi 1% daun *Rosmarinus offininalis* + 1% biji benih *Nigella sativa* (T4). bebiri telah diberi makan jerami padi-urea (UTRS) secara *ad libitum* dan dijaga selama 90 hari. Bebiri yang sudah dirawat menunjukkan kesan pengambilan DM dan UTRS yang besar ($P<0.05$) berbanding bebiri terkawal. Jumlah berat harian bebiri T2 menunjukkan perbezaan ketara ($P<0.05$) daripada bebiri yang dirawat dengan makanan tambahan lain. Bebiri T3 dan T4 menunjukkan pH rumina yang ketara berbanding bebiri T1 dan T2. Bebiri yang sudah disuplementasikan dengan makanan tambahan menunjukkan prestasi yang baik terhadap jumlah asid lemak meruap, asetat, propionate dan penurunan NH_3-N, gas metana dan C18:0 berbanding bebiri terkawal. Bebiri yang disuplementasikan juga menunjukkan perbezaan ketara dengan IgA dan IgG serum berbanding bebiri. Selain itu, bukti menunjukkan perubahan yang baik terhadap sifat bangkai, ekspresi genetik, oksidasi lipid dan keadaan fisio-kimia pada otot LD, ST dan SS pada bebiri jenis Dorper dengan suplementasi biji benih NS, RO dan kombinasi kedua-duanya. Keputusan penyembelihan dan berat sejuk bebiri T2 menunjukkan kesan ketara ($P<0.05$) berbanding bebiri. Daging bebiri yang dirawat menunjukkan penurunan pada keadaan dimasak dan keupayaan untuk kehilangan jus daging (*drip losses*), daya ricih (*shear force*), keringanan dan oksida lipid serta perubahan ketara ($P<0.05$) pada warna kemerahan daging berbanding dengan daging bebiri terkawal. Penuaan *postmortem* mempengaruhi kualiti daging bebiri jenis Dorper.

Pengambilan suplemen memberikan kesan terhadap otot lemak asid (FA) dengan jenis otot-otot yang lain. Tambahan pula, NS dan RO menurunkan jumlah kolesterol, trigliserida, LDL-C dalam serum dan tiada kesan ketara ($P> 0.05$) pada ekspresi gen SCD dan LPL dalam otot LD dan ST pada bebiri jenis Dorper. T2 menunjukkan ekspresi gen PRKAA2 dan SREBP1 menaik pada otot LD dan ST kepada bebiri jenis Dorper. Keseluruhannya, keputusan ini telah menunjukkan biji *Nigella sativa* dan daun *Rosmarinus officinalis* memberikan kesan variasi pada nutrisi ruminan. Suplementasi diet biji *Nigella sativa* dan daun *Rosmarinus officinalis* memberikan kesan yang baik pada metabolisma rumen, tindak balas imun dan kualiti daging bebiri.

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

Awis Qurni Sazili, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Anjas Asmara @ Ab. Hadi Samsudin, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Shokri Jusoh, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Signature: _____
Name of Chairman of Supervisory Committee: Associate Professor Dr. Awis Qurni Sazili

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Anjas Asmara @ Ab. Hadi Samsudin

Signature: _____
Name of Member of Supervisory Committee: Dr. Shokri Jusoh

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ADF	acid detergent fiber
ANOVA	analysis of variance
BH	Biohydrogenation
^{o}C	degrees centigrade
^{o}C/min	degrees centigrade per minute
cal	Calorie
CLA	conjugated linoleic acid
cm	Centimetre
cm^2	square centimetre
d	Day
DM	dry matter
FA	fatty acids
FE	feed efficiency
g	Gram
GLM	generalized linear model
h	Hour
Kcal	Kilocalories
L	Liter
LD	*longissimus dorsi*
m	Meter
MDA	Malondialdehyde
min	Minute
mm	Milimeter
mmol/L	milimole per liter
MRA	metmyoglobin reducing activity
µL	Microliter
µ ol/L	micromole per liter
mg	Milligram
mg/L	milligram per lit r
mL	Millilitre

mL/min	millilitre p r minute
MUFA	monounsat rated fatty acids
NS	*Nigella sativa*
n-6/n-3	total n-6 PUFA to total n-3 PUFA ratio
NDF	neutral detergent fib e
PUFA	polyunsaturated fatty acids
RO	*Rosmarinus officinalis*
SEM	standard error of means
SFA	saturated fatty acids
SS	*Supraspinatus*
ST	*semitendinosus*
TBARS	thiobarbituric acid reactive substances
UFA	unsaturated fatty acids
VFA	volatile fatty acids
WHC	water holding capacity

CHAPTER 1

GENERAL INTRODUCTION

Recently, the increase in human population, rapid urbanization and rising incomes have stimulated an increased demand for animal products (Makkar and Beever, 2013). This scenario presents animal scientists with a significant challenge of developing strategies to enhance sustainable animal production to meet the consumers' demand (Adeyemi *et al*., 2015b).

Ruminant production plays a significant role in sustainable livestock production because they can convert plant materials not suitable for human consumption into high quality protein (Wanapat *et al*., 2008). Small ruminant are critical components of production systems throughout the world and they are essential in agricultural systems. Therefore, lambs and goats give rise to large percentage of consumption by global human populace in terms of the dietary protein consumed. About 4% of the meat eaten in the European Union is from ruminant source (EU 2004). Also, in Spain, about 239,500 t of lamb meat is produced yearly occupying the second largest producer of lamb meat (MAPA, 2002). Nonetheless, the pursuit for large-scale animal production to meet the prevailing consumers' demand could compromise animal health and product quality and have negative impact on the environment (Wanapat *et al*., 2008; Vasta and Luciano 2011). Thus, enhancing livestock production without jeopardizing environmental sustainability, animal health and product quality is a continued research endeavor.

The need to improve the productivity of livestock enterprise and animal health lend credence to the use of antibiotics in animal nutrition (Guler *et al*., 2006; Kim *et al*., 2013). Nonetheless, antibiotics could leave residues in animal tissues, which could induce subsequent emergence of resistant strains of microorganisms (Russell and Houlihan 2003; Kim *et al*., 2013) capable of endangering the health of livestock and human. This scenario has given the impetus to explore alternatives to antibiotics in animal nutrition (Guler *et al*., 2006; Kim *et al*., 2013). Consequently, as replacement for the synthetic growth promoters (antibiotics), natural products (herbs and spice) as natural feed additives come to the attention to enhance physiological or pharmacological functions (Ando *et al*., 2003).

Herbs represent an effective substitute for antibiotics in animal nutrition and a potent modifier of rumen metabolism capable of suppressing methanogenesis and improving animal's health, performance and product quality (Yang *et al*., 2007; Vasta and Luciano 2011; Wanapat *et al*., 2013). The manipulation of rumen microbial ecosystem represents an effective strategy for promoting animal health, product quality and the environmental sustainability of ruminant production (Wanapat *et al*., 2013; Adeyemi, 2015). The effects of herbs on ruminants are premised on the chemical nature of the plant secondary metabolites (PSM), the

quantity in the feed, abundance and variety of rumen microflora and ruminant species (McSweeney *et al*., 2001). The scientific literature is replete with studies that have investigated the influence of dietary supplementation of medicinal herbs on rumen metabolism, production performance and product quality in ruminants (Karami *et al*., 2010; Vasta and Luciano 2011; Wanapat *et al*., 2013). Nonetheless, the findings are generally less consistent. Thus, there is need for further research in diverse production systems to allow informed choices and tailored decision in the use of herbs in ruminant nutrition as natural feed additives.

Using *Nigella sativa* (NS) and *Rosmarinus officinalis* (RO) as natural feed additives are examples of medicinal herbs in animal nutrition. *Nigella sativa* and *Rosmarinus officinalis* have been reported as aromatic herbs and they are very rich polyphenols and flavonoids with high antioxidant properties (Michel *et al*., 2011; Sasaki *et al*., 2013; Vatansev *et al*., 2013). In addition, Studies with NS and RO as an option growth booster in livestock production has been established to enhanced carcass trails growth rate and digestibility (Hassan and Hassan 2009; Jordán *et al*. 2010; Nieto *et al*. 2010, 2011; Hassan *et al*. 2011).

Oxidation which is one of the main reason of meat quality impairment as a result of presence of prominent amount of unsaturated lipids, metal catalysts, heme pigments and a range of oxidizing agents in the muscle tissue. Oxidative impairment that occur in any meat sample manifests in form of poor shelf life, off flavor, drip losses, toxic compounds formation, discoloration and nutrient (Contini *et al*., 2014; Palmieri and Sblendorio, 2007). Therefore, enhancing the antioxidant properties of meat could assist to improving meat quality. *Rosmarinus officinalis* and *Nigella sativa* are a medicinal herbs widely used around the world. Of the natural antioxidants, rosemary and *Nigella sativa* have been widely accepted as one of the herbs with the high antioxidant activity (Klančnik *et al*., 2009). The use of these herbs and their by-products as natural antioxidants in feed for ewes could be a simple and interesting opportunity to replace synthetic antioxidants and to improve the quality of lamb meat.

However, feed and animal feeding form one of the basic rock of every livestock. Its enormous role in animal productivity, producer incomes, land use, product quality and safety, health and welfare, water pollution, household security and greenhouse gas emission has been observed (FAO, 2012). In addition, feed accounted for about 70% of the total cost of production regardless of the livestock system either ruminant or non-ruminant and this has placed feed at the fore front in the livestock production sector in order to achieve sustainability (Makkar and Beever, 2013; Buza *et al*., 2014). A question arose as to whether the addition of small amounts of *Nigella sativa* seeds or *Rosmarinus officinalis* leaves to forage or concentrate based diets would result in further improvement of the growth rate of sheep. Therefore, the present study was carried out assess the effects of *Nigella sativa* seeds or *Rosmarinus officinalis* leaves on rumen metabolism, growth performance, meat quality, immune response and gene expression in Dorper lambs. It was hypothesized

that *Nigella sativa* seeds, *Rosmarinus officinalis* leaves and both as natural feed additives would improve rumen metabolism, growth performance, carcass traits, meat quality, immune response and gene expression in Dorper lambs.

Thus, the objectives of the study were:

1. To ascertsin the suitable level of *Nigella sativa* seeds and *Rosmarinus officinalis* leaves on *in vitro* rumen fermentation and apparent biohydrogenation of fatty acids using rumen liquor from Dorper lambs.
2. To determine the effects of *Nigella sativa* seeds, *Rosmarinus officinalis* leaves and their blend on nutrient intake and digestibility, growth performance, and rumen metabolism, some blood parameters and immune response in Dorper lambs.
3. To examine the effects of *Nigella sativa* seeds, *Rosmarinus officinalis* leaves and their combination on carcass traits, fatty acid composition, oxidative stability, quality attributes and gene expression in different muscles in Dorper lambs.

Presentation of the thesis

This thesis consisted of eight chapters. The first two chapters discussed the framework of the experimental research. Chapter 1 provides the justification, hypotheses and the objectives of the study. Chapter 2 presents the review of literature covering the distribution, economic and nutritional importance of sheep, fats and fatty acids in ruminants, rumen ecosystem, uses of medicinal plants to manipulate rumen metabolism, fatty acid composition and meat quality attributes in ruminants. The morphology and medicinal properties of *Nigella sativa* and *Rosmarinus officinalis* and the role of nutrition on gene expression, immune response and meat quality in ruminants were also reviewed. Chapters 3, 4 and 5 present the experimental works for this study. Chapter 6 describes the major findings and highlights the practical importance. Chapter 7 presents the summary, conclusions and recommendations for future studies.

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LIST OF PUBLICATIONS

Odhaib, K.J., Sazili, A.Q., and A.L. Alimon (2015). The effect of *Nigella sativa* and *Rosmarinus officinalis* on in vitro rumen fermentation. Green technology farming for sustainable livestock production. Proceeding of the 2nd ASEAN Regional Conference on Animal production and 36th Annual Conference of the Malaysian Society of Animal Production 2015. 1-3 June, Port Dickson, Negeri Sembilan, Malaysia.

Odhaib K.J., Adeyemi, K. D., Ahmed, M.A., Jahromi, M.F., Jusoh, S., Samsudin, A. A., Alimon, A.R., Yaakub, H., & Sazili, A Q. Influence of *Nigella sativa* Seeds, *Rosmarinus officinalis* Leaves and their combination on Growth Performance, Immune Response and Rumen Metabolism in Dorper Lambs. *Tropical Animal Health and Production*. Accepted for Publication.

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