



**EFFECTS OF RUMEN-PROTECTED FAT ON CHANGES OF
METABOLITES AND REPRODUCTIVE GENES IN TESTES OF MALIN
RAMS**

By

ALIFAH BINTI ISMAIL

**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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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 RUMEN-PROTECTED FAT ON CHANGES OF METABOLITES AND REPRODUCTIVE GENES IN TESTES OF MALIN RAMS

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June 2022

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An energy-based diet is necessary for male animals especially during breeding activities to improve most of the reproduction parameters. In addition to grains, the consumption of fat can also contribute to the activity. A previous study reported that RPF did improve reproductive performance by increasing sperm quality in Malin ram. The present research was conducted to understand the RPF influence on the blood biochemistry profiles, metabolites changes and genes associated to the male reproduction organ at the molecular level that can reduce the knowledge gap in this area. Twenty Malin rams with average bodyweight of $36.6\text{kg} \pm 5.57\text{ kg}$, aged 10 to 14 months reared at The National Institute Animal Biodiversity Jerantut, Pahang were assigned randomly into four experimental groups. Each treatment group was fed with different lipid types that composed with different fatty acids property: A; basal diet without rumen-protected fat (RPF), B; basal diet with 2% prilled fat, C; basal diet with 2 % calcium salt and D; basal diet with 2% canola oil for comparison. The period of the experiment was 12 weeks the animals were allowed for 2 weeks of adaptation period and 10 weeks of feeding trials. Blood was taken via jugular vein at every fortnight and four out of five animals from each group were slaughtered at the end of the experiment. The testes were excised from the slaughtered animals for metabolites studies using LC-MS analysis and gene expression analysis using quantitative Real-Time PCR (q-PCR). In this study, the blood biochemistry test was also done to determine the effects of RPF on glucose, cholesterol, triglycerides and low-density lipoprotein (LDL), high-density lipoprotein (HDL) level. The genes tested in the present study were associated to testes development and spermatogenesis (reproductive genes in testes: ODF1, SERPINA10, CatSper4 AdipoR2 and DAZL). The blood biochemistry result showed the cholesterol, triglycerides, HDL and LDL did not differ in all groups. Feeding RPF with calcium salt (Treatment C) has resulted in the up-regulation effects, which are more than two folds in all reproductive genes that have been studied in the testes. The present study showed there were metabolites changes occurred between the groups and identified 44 important putative metabolites present in the testes via preliminary screening of LC-MS. The identified

putative metabolites in the treatment groups demonstrate the findings of 10-hydroxy-(2E,8E)-decadien-4-ynoic acid in the control group, 1-Heneicosanoyl-2- docosanoyl-3-sn glycerol in the prilled fat group and the same putative metabolites which are Adenosine 5' pentaphosphate and PE(24:0/24:1(15Z)) in calcium salt and canola oil group. In conclusion, feeding of RPF to the animals as a source of energy has no impact on the blood lipid profiles which could be due to the metabolic processes efficiency since there were metabolites changes that occurred according to the type of fat given. The metabolism efficiency of the RPF especially with calcium salt to produce fatty acids as a source of energy indirectly expressed the genes related to reproduction performances in the testes.



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

KESAN PENGGUNAAN LEMAK RUMEN TERLINDUNG (RPF) KEPADA PERUBAHAN METABOLIT DAN GEN PEMBIAKAN DALAM TESTIS BIRI-BIRI MALIN JANTAN

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Diet yang mengandungi tenaga diperlukan oleh haiwan jantan terutamanya semasa aktiviti pembiakan kerana ia dapat meningkatkan sebahagian besar parameter pembiakan. Selain bijirin, penggunaan lemak dalam makanan juga dapat menyumbang kepada aktiviti itu. Kajian terdahulu melaporkan bahawa pemberian lemak terlindung rumen (RPF) telah meningkatkan prestasi pembiakan dengan meningkatkan kualiti sperma dalam Malin jantan. Kajian ini pula dijalankan untuk memahami kesan RPF pada tahap molekul iaitu perubahan pada metabolit di dalam testes dan gen pembiakan yang boleh mengurangkan jurang pengetahuan dalam bidang ini. Dua puluh ekor biri-biri jantan Malin ($36.6\text{kg} \pm 5.57\text{ kg}$) yang berumur 10-14 bulan dengan skor badan 3 hingga 3.5 yang ditenak di Institut Biodiversiti Haiwan Nasional Jerantut, Pahang dibahagikan secara rawak kepada empat kumpulan eksperimen yang berbeza. Setiap kumpulan diberi makan dengan lemak yang mempunyai komposisi asid lemak yang berbeza: A; diet asas tanpa lemak terlindung rumen (RPF), B; diet basal dengan 2% butiran lemak, C; diet basal dengan 2% garam kalsium asid lemak dan D; diet basal dengan 2% minyak canola untuk perbandingan dibuat. Tempoh eksperimen adalah 12 minggu yang mana 2 minggu adalah tempoh penyesuaian dan 10 minggu ujian pemakanan. Darah diambil setiap dua minggu disepanjang eksperimen dan pada akhir eksperimen empat daripada lima haiwan dari setiap kumpulan disembelih. Testis diambil dari haiwan yang disembelih untuk kajian metabolit menggunakan analisis LC-MS dan analisis ekspresi gen menggunakan Quantitative Real Time PCR (q-PCR). Dalam kajian ini, ujian biokimia darah juga dilakukan untuk menentukan kesan RPF pada kolesterol, LDL, HDL, trigliserida dan tahap glukosa. Gen yang diuji dalam kajian ini adalah yang berkaitan dengan pertumbuhan testis dan spermatogenesis (gen pembiakan di dalam testis: ODF1, SERPINA10, CatSper4 AdipoR2 dan DAZL). Keputusan biokimia darah menunjukkan kolesterol dan lipoprotein berketumpatan rendah (LDL) tidak meningkat secara signifikan dalam semua kumpulan. Kumpulan yang diberi makan RPF dengan garam kalsium (Rawatan C) menunjukkan kesan keatas ekspresi gen, yang lebih dari dua kali

ganda pada semua gen reproduksi yang telah dikaji. Kajian kami mendapati terdapat perubahan metabolit yang berlaku diantara kumpulan yang diuji dan telah mengenalpasti 44 metabolit putatif penting yang terdapat di dalam testis melalui pemeriksaan awal LC-MS. Metabolit putatif yang dikenali pasti adalah asid 10-hidroksi- (2E, 8E) -decadien-4-ynoic dalam kumpulan kawalan, Gliserol 1-Heneicosanoyl-2-docosanoyl-3-sn dalam kumpulan lemak prilled dan metabolit putatif yang sama iaitu Adenosine 5' pentaphosphate dan PE (24: 0/24: 1 (15Z) dalam garam kalsium dan kumpulan minyak canola. Kesimpulannya, pemberian RPF kepada haiwan sebagai sumber tenaga tidak memberi kesan pada profil lipid darah yang mungkin disebabkan oleh kecekapan proses metabolisma di dalam testis memandangkan terdapat perubahan metabolit mengikut jenis lemak yang diberikan. Kecekapan metabolisme RPF terutamanya dengan garam kalsium untuk menghasilkan asid lemak sebagai sumber tenaga secara tidak langsung telah meningkatkan gen yang berkaitan dengan prestasi pembiakan di dalam testis.

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
DVS	Department of Veterinary Services
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
PCR	Polymerase chain reaction
PUFA	Polyunsaturated fatty acid
RNA	Ribonucleic acid
RPF	Rumen protected fat
RPM	Round per minute

CHAPTER 1

INTRODUCTION

Animal feeding is one of the main factors that can alter the performance of male animals. In the reproduction activities, rams that are not allowed to have proper feeding and nutrition will impact the breeding performance such as undeveloped testes, low sperm production and quality which in turn will lead to reducing the fertility of the animals (Guan *et al.*, 2014). To overcome these limitations, the farmers need to provide the rams with proper feeds especially during breeding seasons. Grains that were incorporated in the pellet is the common practice to overcome these issues. However, as the price of the grains is getting much higher these days, it would affect the farmers in terms of their finances. Alternatively, the use of fats as feed supplements during breeding seasons will help to resolve these issues.

Nowadays, there was a technology to produce insoluble fats from microbial fermentation and biohydrogenation which is called rumen-protected fats (RPF). This helps the RPF to escape the rumen microbial fermentation, absorbed through the small intestine and then converted to a source of energy.

Application of rumen protected fats in animal feed also can stimulate various metabolic functions in the animals and increases their productivity. Supplements made of calcium salts of fatty acids from palm oils are the most effective due to fatty acids content (84%) that act as a metabolic fuel to supply the energy requirement to the animal (Abd El-Hamid *et al.*, 2016; Pavkovych *et al.*, 2015). In female animal, Hashem *et al.*, (2014) reported that supplementation the Rahmani ewes with RPF can improve the metabolism which resulted higher conception and lambing rates.

In male animals, Wathes *et al.*, (2007) reported that spermatogenesis could be promoted by PUFA through the regulation of gene expression. Furthermore, the sperm membrane lipid composition can be considered as a major factor of motility, overall viability and cold sensitivity, lipidmetabolism and the sperm fusion capacity (Jafaroghli *et al.*, 2014). Ahmad *et al.*, (2018), described that RPF did improve the reproduction performance by increasing the sperm quality in Malin ram. The current study was conducted to increase our knowledge on the effects and responds of RPF supplementation to see if any changes on the gene level that correspond to the reproduction performance of the Malin ram.

1.1 Hypothesis

Supplementation of RPF in the diet of the animal will up-regulate the reproductive gene expression, and changes in metabolites in the testes. Apart from that, changes in blood parameters such as glucose, triglyceride, cholesterol, LDL and HDL is noticeable in Malin ram between supplemented and non-supplemented group.

1.2 General objective

To increase our knowledge on the effects of rumen-protected fat on blood profiles, metabolites and expression of the reproductive genes in the Malin ram.

1.3 Specific objectives

1. To determine the effects of rumen-protected fat (RPF) on blood biochemistry parameters and metabolites changes in testes of Malin Ram.
2. To determine the effects of rumen-protected fat (RPF) on gene which is related to testes development and spermatogenesis (reproductive) in testes of Malin ram.



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