



UNIVERSITI PUTRA MALAYSIA

***LONG TERM DIETARY SUPPLEMENTATION OF SOYBEAN OIL AND
VITAMIN E AND THEIR EFFECTS ON REPRODUCTIVE FUNCTIONS OF
SPRAGUE-DAWLEY RATS***

NASEEM BIBI BT. MALIK KHUSHI MUHAMMAD

FPV 2012 13

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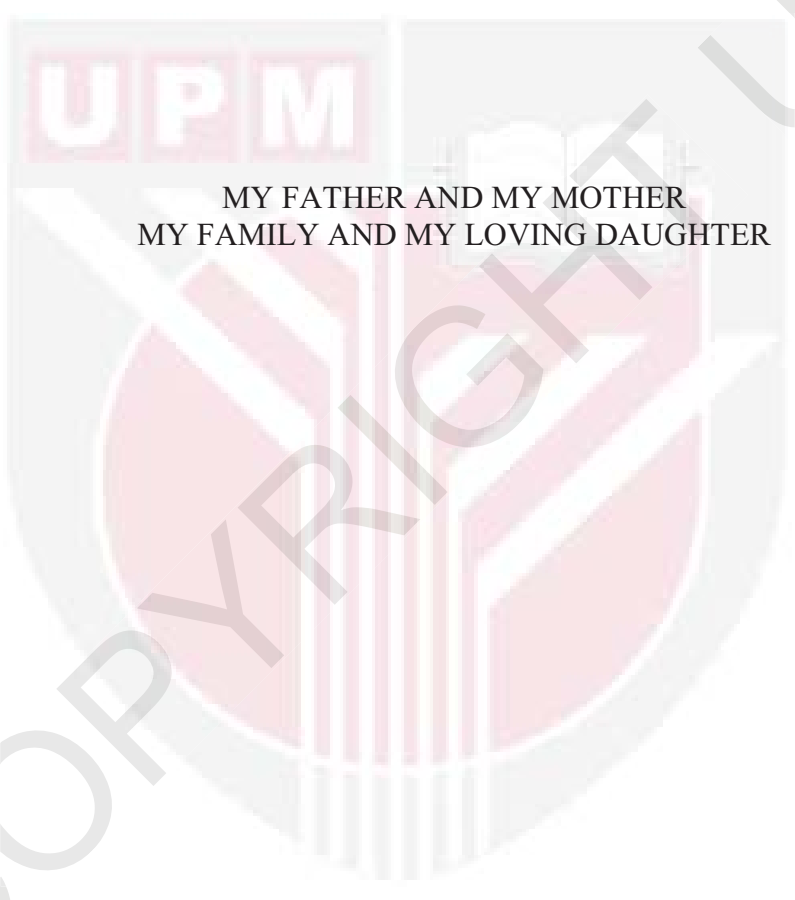
**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science**

December 2012

DEDICATION

...To my beloved family and especially my wonderful daughter,

Thank you for your patience, understanding and continual motivation...

The image features a large, faint watermark of the UPM logo, which is a shield-shaped emblem with a red and white color scheme. The letters 'UPM' are prominently displayed in the upper left corner of the shield. The watermark is oriented diagonally across the page.

MY FATHER AND MY MOTHER
MY FAMILY AND MY LOVING DAUGHTER

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

LONG TERM DIETARY SUPPLEMENTATION OF SOYBEAN OIL AND VITAMIN E AND THEIR EFFECTS ON REPRODUCTIVE FUNCTIONS OF *SPRAGUE-DAWLEY* RATS

By

NASEEM BIBI BT. MALIK KHUSHI MUHAMMAD

December 2012

Chairman: Associate Professor Dr. Goh Yong Meng, PhD

Faculty: Veterinary Medicine

An investigation was carried out to determine the effects of long term dietary fat and vitamin E supplementation on the reproductive functions of *Sprague-Dawley* rats. A total of 120 *Sprague-Dawley* 8-weeks old male and female rats were used in this 64-week trial inclusive of one week of adaptation period. The animals were assigned randomly into five groups of 24 animals each, comprising of 12 males and 12 females per group. The dietary treatment groups were CTRL (Base Diet + 5 % soybean oil + 1250 mg/kg Vitamin E), BDOOnly (Base Diet only), BDVitE (base diet + 1250 mg/kg vitamin E Only), BDSBO (base diet + 5 % soybean oil only), and commercial pellet (COMM). Vitamin E supplements were given as natural α -tocopherol only. Of the five treatment diets, the CTRL and COMM diets were balanced diets. Six males and six female animals from each of the treatment group were randomly selected and sacrificed after 16 weeks of feeding (actual age = 24 weeks old). Of the remaining animals, 30 females were sacrificed after 32 weeks of treatment (actual age = 40 weeks old) to evaluate changes in the ovaries and uterine horn tissues. The remaining males were terminated at the end of the trial after 63 weeks of dietary intervention, when they are about 71 weeks old for sperm quality

assessments and testicular histological studies. The samples were used for plasma fatty acid (FA) and vitamin E determination, sperm quality and testicular histological evaluation, as well as morphological evaluation of the ovarian and uterine horns accordingly. All protocols and procedures of this study were approved by the Animal Care and Use Committee, Ministry of Health Malaysia, Approval No. ACUC/KKM/02(3/2006).

Vitamin E and *n-6* PUFA supplementation when carried out in tandem resulted in significantly higher plasma *n-6* PUFA enrichment, and plasma vitamin E in both male and female animals. This is very much evident in the CTRL and BDSBO groups which had higher *n-6*FA compared to others. However, there is probable upper limit of vitamin E accumulation, beyond which it becomes more difficult to further enrich blood plasma with vitamin E. This was clearly evident by the fact that the level of plasma alpha-tocopherol in the COMM group which had about 110 mg/kg vitamin E added to the diet, was only about half of those from CTRL, whose diet was enriched with 1250 mg/kg of vitamin E, or more than ten times the amount in the COMM diet. Supplementing vitamin E and *n-6* PUFA fat together in the CTRL and COMM also created synergism which helped to preserve the testicular morphology and reduce the percentage of sperm abnormalities. In fact, the unsupplemented BDOnly group registered almost 60 % spermatozoa abnormalities at the age of 71 weeks compared to approximately 30 % for all other supplemented groups. This would contribute significantly towards ensuring male animals had a longer and more productive breeding expectancy. Similar effects were also noted for the female animals where both vitamin E and *n-6* PUFA supplementation not only helped in the development of ovarian follicles, but also ensured significantly more

mature follicles available both after 16 and 32 weeks of dietary intervention. It was also noted that for the females *n-6* PUFA or fat supplementation is more potent as compared to vitamin E when given singly.

In summary, both fats (*n-6* PUFA) and vitamin E are essential in maintaining the normal reproductive functions of male and female rats. This study has conclusively shown that sustained dietary *n-6* PUFA and Vitamin E supplementation can be used to improve semen quality, as well as ovarian activity and thus extend the breeding expectancy in both male and female *Sprague-Dawley* rats.

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

**SUPLEMENTASI MINYAK KACANG SOYA DAN VITAMIN E JANGKA
MASA PANJANG DAN KESANNYA KE ATAS FUNGSI PEMBIAKAN
TIKUS SPRAGUE DAWLEY**

Oleh

NASEEM BIBI BT. MALIK KHUSHI MUHAMMAD

Disember 2012

Pengerusi : Profesor Madya Dr. Goh Yong Meng, Ph.D

Fakulti: Perubatan Veterinar

Satu kajian telah dijalankan untuk menentukan kesan suplementasi lemak (Asid Lemak Politaktepu *n-6*, PUFA *n-6*) dan Vitamin E dalam jangka masa panjang, ke atas fungsi pembiakan tikus Sprague-Dawley. Seratus dua puluh ekor tikus yang berumur 8 minggu telah digunakan dalam kajian selama 64 minggu ini (termasuk seminggu untuk adaptasi). Haiwan ini telah diagih secara rawak ke dalam lima kumpulan yang terdiri daripada 24 tikus setiap kumpulan, yakni 12 jantan dan 12 betina untuk setiap kumpulan. Kumpulan diet rawatan terdiri daripada kumpulan CTRL (Diet Asas + 5 % minyak kacang soya + 1250 mg/kg Vitamin E), BDOnly sahaja (Diet Asas sahaja), BDVitE (Diet Asas + 1250 mg/kg Vitamin E sahaja), BDSBO (Diet Asas + 5 % minyak kacang soya sahaja) dan COMM (pelet komersial). Suplementasi vitamin E diberikan sebagai alfa-tokoferol asli sahaja. Daripada kelima kumpulan tersebut, diet CTRL dan COMM merupakan diet seimbang. Enam tikus jantan dan enam tikus betina daripada setiap kumpulan rawatan dipilih secara rawak untuk tujuan pensampelan selepas menjalani rawatan diet untuk 16 minggu (umur sebenar = 24 minggu). Daripada bilangan haiwan yang berbaki, 30 tikus betina telah disampel pada minggu ke-32 eksperimen (umur sebenar = 40

minggu) untuk menilai perubahan histologi ovari dan uterus. 30 ekor tikus jantan yang terakhir pula dikorbankan di penghujung eksperimen di minggu ke-63 (umur sebenar 71 minggu) untuk penilaian kualiti semen dan histologi testis. Sampel yang telah diambil digunakan untuk analisis profil asid lemak plasma dan kandungan vitamin E plasma, kualiti semen dan histologi testis, serta penilaian histologi struktur ovari dan uterus. Kesemua protokol yang dilaksanakan dalam kajian ini telah diluluskan oleh Jawatankuasa Penjagaan dan Penggunaan Haiwan, Institut Penyelidikan Perubatan, Kementerian Kesihatan Malaysia, Kelulusan No. ACUC/KKM/02(3/2006).

Keputusan menunjukkan bahawa suplementasi vitamin E dan PUFA *n-6* mengakibatkan peningkatan kuantiti PUFA *n-6* yang banyak dalam plasma tikusjantan dan betinaapabila keduanya diberikan bersekali dalam diet.Keadaan ini amat ketara bagi kumpulan CTRL dan BDSBO yang mencatatkan pengayaan PUFA *n-6* yang tertinggi dikalangan semua kumpulan rawatan. Keputusan juga menunjukkan terdapatnya had tahap pengayaan vitamin E dalam plasma, di mana pengayaan vitamin E selepas tahap ini adalah sukar. Ini jelas memandangkan tahap alfa-tokoferol plasma pada kumpulan COMM yang menerima diet rawatan mengandungi 110 mg/kg vitamin E hanya merupakan separuh daripada tahap alfa-tokoferol pada plasma darah haiwan CTRL. Sedangkan haiwan CTRL diberi diet yang mengandungi 1250 mg/kg vitamin E, yakni lebih daripada sepuluh kali ganda kuantiti vitamin E dalam diet COMM. Suplementasi vitamin E dan PUFA $n-6$ secara bersekali juga menjana sinergi yang membantu pengekalan morfologi testis dan mengurangkan peratusan spermatozoa yang normal. Malah kumpulan BDOOnly yang tidak menerima sebarang suplementasi mencatatkan hampir 60 % keabnormalan

spermatozoa apabila mencapai umur 71 minggu, berbanding hanya sekitar 30 % untuk kesemua kumpulan yang yang diberi vitamin E atau/dan PUFA *n-6*. Ternyata jelas suplementasi ini mampu melanjutkan tempoh pembiakan efektif tikus jantan dalam hal ini. Kesan yang sama turut dicerap di kalangan tikus betina di mana, suplementasi vitamin E dan lemak PUFA *n-6* bukan sahaja bertanggungjawab dalam perkembangan folikel, malah menjana lebih banyak folikel ovari yang matang selepas rawatan diet selama 16 dan 32 minggu. Dalam masa yang sama, lemak PUFA *n-6* juga didapati lebih berkesan berbanding vitamin E apabila digunakan berasingan pada tikus betina.

Secara keseluruhannya, lemak makanan membekalkan tenaga dan metabolit perlu untuk perkembangan dan fungsi sistem pembiakan, sementara vitamin E melindungi tisu daripada mudarat pengoksidaan. Kesimpulannya, lemak PUFA *n-6* dan vitamin E sama pentingnya dalam menjamin keutuhan fungsi dan struktur sistem pembiakan tikus. Kajian ini telah menunjukkan bahawa kedua-dua bahan ini boleh digunakan untuk meningkatkan kualiti semen serta aktiviti ovary, dan seterusnya melanjutkan usia pembiakan tikus *Sprague-Dawley*.

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To my family and especially my daughter, thank you for the patience, understanding and sacrifices made. Last but not least to everyone who had contributed directly or indirectly to the completion of this dissertation, thank you all and may the best of things be with all of you always.

I certify that a Thesis Examination Committee has met on XXXX to conduct the final examination of Naseem Bibi Bt. Malik Khushi Muhammad on her Master of Science thesis entitled 'The Effects of Long Term Fat and Vitamin E Supplementation on The Reproductive Performance Of *Sprague- Dawley* Rats' in accordance with 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 candidate be awarded the relevant degree of Master of Science.

Members of the Examination Committee are as follows:

Md Zuki Abu Bakar, PhD

Professor
Faculty of Veterinary Medicine,
Universiti Putra Malaysia
(Chairman)

Mohamed Ali Rajion, PhD

Professor
Faculty of Veterinary Medicine,
Universiti Putra Malaysia
(Internal Examiner)

Engku Azahan Engku Ahmed

Associate Professor,
Faculty of Veterinary Medicine,
Universiti Putra Malaysia
(Internal Examiner)

SEOW HENG FONG, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science. The Members of the Supervisory Committee were as follows:

Goh Yong Meng, Ph D

Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Fauziah Bt. Othman, PhD

Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)



BUJANG BIN KIM HUAT, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 February 2013

DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree at Universiti Putra Malaysia or any other institutions.

NASEEM BIBI BT. MALIK KHUSHI MUHAMMAD

Date:13 December 2012

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LIST OF ABBREVIATIONS

$^{\circ}\text{C}$	-	degree Celsius
cal	-	calorie
d	-	day
DM	-	dry matter
EFA	-	Essential Fatty acids
g	-	gram
h	-	hour
kcal	-	kilo calories
kg	-	kilogram
KJ/g	-	kilo Joules per gram
L	-	litre
min	-	minute
mmol/L	-	millimoles per litre
M	-	Molar
mM	-	millimolar
mg	-	milligram
mo	-	month
MUFA	-	Monosaturated FA/ Monoenoic FA
N	-	Normal
<i>N-3: n-6</i>	-	Total <i>n-3</i> PUFA to Total <i>n-6</i> PUFA ratio
PUFA	-	Polyunsaturated Fatty acids
SD	-	standard deviation
SE	-	standard error

sec	-	second
SFA	-	Saturated Fatty acids
UFA	-	Unsaturated Fatty acids
U: S ratio	-	Total UFA to Total SFA ratio
wk	-	week



CHAPTER I

GENERAL INTRODUCTION

Fatty acids (FA) are essential components of the diet and an important source of dietary energy (Rudin *et al.*, 1987). FA had been known to elicit significant physiological effects in living organisms. They can be classified as saturated or unsaturated, based on the presence of chemically inert or reactive carbon-carbon bonds in the carbon chain (Gunstone, 1996). The saturated FA are usually chemically inert, while FA molecules with unsaturated bonds are known to be more biologically reactive, for the example the *n-3* and *n-6*FA. The *n-3* and *n-6*FA are essential for the growth and functional development of the infant and during early life (Uauy *et al.*, 2000). Researchers in the 1930 have first acknowledged that specific components of FA might be necessary for the proper growth and development of animals and possibly humans (Burr and Burr, 1930). These findings were reported many years before the essentiality of FA to the dermal, nervous, reproductive, and other organ systems are established (Sardesai, 1992). In mammals, FA have been recognized as important factors for reproductive performance in both males and females. FA have been identified as key nutrients in sustaining reproductive performance (Burr and Burr, 1930; Amira *et al.*, 2010). Studies showed that nutrient supplementation of FA stimulate the ovary and causes the development of large follicles (Lucy *et al.*, 1992). FA are also reported to have a significant impact in improving reproductive performance, and these include the stimulation of prostaglandin synthesis and secretion, as well as enhanced utilization of blood cholesterol for progesterone synthesis (Petit, 1998).

Human populations with high fat intake are at risk of developing metabolic syndromes (Buettner *et al.*, 2007). Prominent clinical dysfunctions associated with metabolic syndromes included polycystic ovary, diabetes, hypercholesterolaemia, coronary heart disease (CHD) and other debilitating disorders (Mensink *et al.*, 2003). These negative changes are associated with both dietary fat and their oxidative by-products from unregulated lipid metabolism. These therefore justifies the inclusion of antioxidants in diets, as it is known since the 1920's that vitamin E in vegetable oils is required in female rats (Evans and Bishop, 1922). It is known that this vitamin is effective in preventing lipid peroxidation and other radical-driven oxidative events (Allard *et al.*, 1997) and acts as a potent antioxidant (Das and Ghosh, 2004) and androgenic stimulant (Ghosh, 2002).

In view of the potential hazards associated with high saturated fat intake as in the Malaysian diet (Ng, 1995), it is important to determine whether the presence of significant amounts of *n-6* and vitamin E antioxidant would mitigate these hazards. The rat model was chosen for this study as it has a short generational gap (Krinke, 2000). Furthermore, it is also known that age and dietary factors had profound effects on the rat's reproductive functions, even more so in the case of male rats where their reproductive life is twice as long as females (Hilakivi-Clarke *et al.*, 1997). Therefore, this study focused on both morphological and functional changes in female rats at 16 and 32 week of age, which corresponded to puberty, maturation and decline of reproductive cycle, respectively in female rats, and at 16, 32 and 63 weeks in males which had a much longer reproductive cycle. This study aimed to elucidate both the age related changes in the reproductive tract and functions, as well as whether the

types of fats played a significant role in modulating reproductive functions over a long term supplementation period.

General Objectives and Organization of the study

The thesis is organized around 6 chapters. Chapter I explained the aspects that are relevant to the project and provided justification for the work carried out in this thesis. Chapter II covered an in-depth literature review that summarizes the present knowledge on the importance of PUFA in animals, with special emphasis on lipid metabolism, vitamin E, male and female reproductive systems. Chapters III and IV focused on the effects of vitamin E and FA on male and female reproductive parameters, respectively, before the general discussion in Chapter V. The general conclusion in Chapter VI, aimed to provide a condensed view and summary of what had been achieved by the present study.

Hypothesis

It was hypothesized that dietary *n-6* PUFA and vitamin E supplementation improved both male and female reproductive performance. It is expected that the sperm concentration, sperm motility and overall sperm quality, as well as indicators of the female reproductive functions will be enhanced in the presence of *n-6*FA and vitamin E.

Research Objectives

1. To determine the effects of sustained dietary *n-6* PUFA and Vitamin E supplementation on sperm concentration, sperm quality and sperm motility in male *Sprague-Dawley* rats.
2. To determine the effects of sustained dietary *n-6* PUFA and vitamin E supplementation on the morphology, functions of uterus and ovaries in female *Sprague-Dawley* rats.
3. To identify possible undesirable effects of prolonged and sustained dietary *n-6* PUFA and vitamin E supplementation in *Sprague-Dawley* rats

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