



**UNIVERSITI PUTRA MALAYSIA**

**OVARIAN FOLLICULAR GROWTH AND EMBRYONIC DEVELOPMENT  
IN RABBITS FED PALM KERNEL CAKE BASED-DIET**

**MASNINDAH BINTI MALAHUBBAN**

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**By**

**MASNINDAH BINTI MALAHUBBAN**

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Science**

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fulfilment of the requirement for the degree of Master of Science

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**May 2007**

**Chairman : Halimatun binti Yaakub, PhD**

**Faculty : Agriculture**

An experiment was conducted to evaluate the effect of different diets on the follicular growth and embryo development in the female rabbits. Thirty - six adult rabbits (6-month-old) were randomly assigned into three dietary treatments namely Commercial rabbit pellet (Control); 50% Palm Kernel Cake (50%PKC) and PKC+ Ammonium Molybdate and Sodium Sulphate (PKC+MS) in an individual wire cage, and have access to water and feed *ad-libitum* for eight weeks prior to mating. The changes in body weight and feed intake were recorded weekly. All rabbits were sacrificed at the end of the experiment at different stages (prior to mating (n=2), within 5- 6 hours post mating (n=6) and at 48 hours after mating (n=4) from each treatment). Both ovaries from rabbits



sacrificed prior to or within 5 – 6 hours post mating were prepared for serial sectioning and then stained using Haematoxylin and Eosin (H & E). Slides were examined under microscope (40X magnification) to identify and classify the follicles as either healthy or atretic, measured and either classified as small (<0.1 mm), medium (0.5 mm) and large (>1.0 mm) in diameter. Both oviducts from rabbits sacrificed 48 hours post mating were flushed with PBS supplemented with 10% inactivated fetal calf serum. Embryos were recovered, fixed and stained with Hoechst 33342 and examined under Fluorescent microscope for quality evaluation. Data were analysed using General Linear Model and Chi – square. The results from this study, showed that there were significant difference ( $P<0.05$ ) in terms of feed intake and live bodyweight at 8 weeks, in all the dietary treatments and also there was a significant different in copper (Cu) concentration ( $P<0.05$ ) in the liver, ovary and serum with high concentration of Cu in rabbits fed 50% PKC ( $31.93 \pm 1.20$  ppm,  $5.36 \pm 0.11$  ppm,  $1.63 \pm 0.15$  ppm respectively). However, addition of 0.22 g/kg sulphur dry weight and 0.0013 g/kg molybdenum dry weight to rabbits fed with PKC as basal diet could avoid Cu accumulation in serum, liver and ovary. The number of large follicles on the ovaries from rabbits fed on PKC+MS and Control diets were significantly higher ( $P<0.05$ ) than from rabbits fed on 50% PKC. The numbers of healthy large and medium follicles were significantly higher ( $P<0.05$ ) in Control and PKC+MS compared to 50% PKC. In general, most follicles in the ovaries from rabbits fed on 50% PKC were atretic in small (63%;  $n=109$ ), medium (67%;  $n=36$ ) and large (54%;  $n=24$ ) compared to Control were atretic in small (33%;  $n=76$ ), medium (26%;  $n=50$ ) and large (20%;

n=46) and also PKC+MS were atretic in small (24%; n=126), medium (30%; n=47) and large (21%; n=47). Although, there are not statistically significant between rabbits fed with three different dietary treatments in terms of embryo development, but number of corpus lutea and embryo were decreasing in rabbit fed with 50% PKC ( $8.2 \pm 2.36\%$ ;  $3.0 \pm 0.82\%$ ;  $37.5 \pm 0.20\%$ ) compared to rabbits fed Control ( $10.0 \pm 1.08\%$ ;  $5.0 \pm 0.82\%$ ;  $50.6 \pm 0.13\%$ ) and PKC+MS diets ( $10.8 \pm 1.11\%$ ;  $5.8 \pm 1.31\%$ ;  $53.4 \pm 0.19\%$ ). There was significant difference ( $P < 0.05$ ) in the percentage of embryos at the early morula stage in rabbits under 50%PKC diet (16.7%; 2/12) as compared to Control (40%; 8/20) and PKC+MS diets (47.8%; 11/23). This study suggests that the accumulation of Cu in liver, ovary and serum of rabbits fed on 50% PKC diet were consumed less diet, reduced body weight, healthy, medium and large number of follicles and also negative effect to the development embryo. However, PKC with Ammonium Molybdate and Sodium Sulphate, improved the development of follicle and embryo more compared to control dietary treatment. Therefore, having a balanced Cu content is an important aspect in reproduction.



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**PERTUMBUHAN FOLIKEL OVARI DAN PERKEMBANGAN EMBRIONIK  
ARNAB YANG MENGAMBIL DIET BERASASKAN ISIRONG KELAPA SAWIT**

Oleh

**MASNINDAH BINTI MALAHUBBAN**

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Satu eksperimen telah dijalankan untuk menilai kesan diet yang berbeza ke atas pertumbuhan folikel dan perkembangan embrio pada arnab betina. Tiga puluh enam arnab dewasa (berumur 6 bulan) telah disusun secara rawak kepada tiga rawatan diet iaitu pelet arnab komersil (kawalan); 50% Isirong kelapa sawit (50%PKC) dan PKC+Ammonium molibdat dan Sodium sulfat (PKC+MS) ke dalam satu sangkar dawai secara individu, dan mempunyai kesediaan bekalan air dan makanan selama lapan minggu sehingga sebelum pensenyawaan. Perubahan berat badan dan pengambilan makanan direkodkan secara mingguan. Semua arnab disembelih pada akhir eksperimen pada peringkat yang berbeza (sebelum persenyawaan (n=2), di dalam 5 – 6 jam selepas



persenyawaan (n=6) dan pada 48 jam selepas persenyawaan (n=4) daripada setiap rawatan). Kedua-dua ovari daripada arnab yang disembelih sebelum atau di dalam tempoh 5 – 6 jam selepas persenyawaan telah disediakan untuk potongan bersiri dan kemudian diwarnakan menggunakan Haematoxylin dan Eosin (H & E). Slaid-slaid diperiksa di bawah mikroskop (40X pembesaran) untuk mengenalpasti dan mengklasifikasikan folikel-folikel samada sihat atau atretik, diukur dan samada dikelaskan sebagai kecil (<0.1 mm), sederhana (0.5 mm) dan besar (>1.0 mm) dalam diameter. Kedua-dua oviduktus daripada arnab yang disembelih 48 jam selepas persenyawaan dibilas dengan PBS yang ditambah dengan 10% *fetal calf serum* tidak aktif. Embrio-embrio diambil, ditetapkan dan diwarnakan dengan Hoechst 33342 dan diperiksa di bawah mikroskop fluorescen untuk penilaian kualiti. Data dianalisis menggunakan Model Linear Am dan *Chi-square*. Keputusan daripada kajian mendapati terdapatnya perbezaan yang bererti ( $P < 0.05$ ) pada pengambilan makanan dan berat badan pada 8 minggu pada kesemua rawatan berdiet dan juga terdapat perbezaan bererti pada kepekatan kuprum (Cu) ( $P < 0.05$ ) pada hati, ovari dan serum dengan kepekatan tinggi Cu pada arnab yang mengambil 50% PKC ( $31.93 \pm 1.20$  ppm,  $5.36 \pm 0.11$  ppm,  $1.63 \pm 0.15$  ppm). Walau bagaimanapun, penambahan 0.22 g/kg berat kering sulfur dan 0.0013 g/kg berat kering molibdenum ke atas makanan arnab dengan PKC sebagai asas diet telah menghindar pengambilan Cu ke dalam serum, hati dan ovari. Bilangan folikel besar pada ovari arnab yang mengambil diet PKC + MS dan Kawalan adalah lebih tinggi dengan signifikan ( $P < 0.05$ ) daripada arnab yang mengambil 50% PKC. Bilangan folikel sihat yang besar dan



sederhana adalah lebih tinggi dengan signifikan ( $P < 0.05$ ) pada Kawalan dan PKC + MS berbanding dengan 50% PKC. Pada amnya, kebanyakan folikel dalam ovari arnab yang mengambil 50% PKC adalah atretik pada bersaiz kecil (63%;  $n = 109$ ), sederhana (67%;  $n = 36$ ) dan besar (54%;  $n = 24$ ) berbanding Kawalan yang atretik pada bersaiz kecil (33%;  $n = 76$ ), sederhana (26%;  $n = 50$ ) dan besar (20%;  $n = 46$ ) dan juga PKC + MS adalah atretik pada saiz kecil (24%;  $n = 126$ ), sederhana (30%;  $n = 47$ ) dan besar (21%;  $n = 47$ ). Walaupun, tiada perbezaan yang bererti di antara arnab dari tiga rawatan berdiet dari segi perkembangan embrio, tetapi bilangan *corpus lutea* dan embrio berkurangan pada arnab yang mengambil 50% PKC ( $8.2 \pm 2.36\%$ ;  $3.0 \pm 0.82\%$ ;  $37.5 \pm 0.20\%$ ) berbanding arnab yang mengambil Kawalan ( $10.0 \pm 1.08\%$ ;  $5.0 \pm 0.82\%$ ;  $50.6 \pm 0.13\%$ ) dan diet PKC+MS ( $10.8 \pm 1.11\%$ ;  $5.8 \pm 1.31\%$ ;  $53.4 \pm 0.19\%$ ). Terdapat perbezaan yang beerti ( $P < 0.05$ ) pada peratusan embrio pada peringkat awal morula pada arnab yang mengambil diet 50% PKC (16.7%; 2/12) berbanding Kawalan (40%; 8/20) dan diet PKC + MS (47.8%; 11/23). Kajian ini mencadangkan pengambilan Cu di dalam hati, ovari dan serum arnab yang mengambil diet 50% PKC telah kurang mengambil diet, mengurangkan berat badan, folikel yang sihat bersaiz sederhana dan besar dan juga kesan negatif ke atas perkembangan embrio. Walau bagaimanapun, PKC dengan Ammonium molibdat dan Sodium sulfat, telah memulihkan perkembangan folikel dan embrio lebih banyak berbanding rawatan diet kawalan. Dengan itu kandungan Cu yang seimbang adalah aspek penting dalam reproduksi.

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I certify that an Examination Committee has met on 3 May 2007 to conduct the final examination of Masnindah Binti Malahubban on his Master of Science thesis entitled "Ovarian Follicular Growth and Embryonic Development in Rabbits fed Palm Kernel Cake based-diet." in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the Master of Science.

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## 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, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

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**MASNINDAH BINTI MALAHUBBAN**

Date: 13 June 2007



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## LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ADF	Acid-detergent fibre
CRD	Complete randomized design
Cu	Copper
DM	Dry matter
Fe	Iron
G	Gram
GLM	General Linear Model
H	Hydrogen
HCl	Hydrochloric acid
HNO <sub>3</sub>	Nitric Acid
H <sub>2</sub> SO <sub>4</sub>	Sulphuric Acid
Kg	Kilogram
Kg <sup>0.75</sup>	Metabolic Body weight
L	Litre
LSD	Least Square Difference
Min	Minute
mL	Milliliter
mm	Millimeter
Mo	Molybdenum
MPOB	Malaysian Palm Oil Board



µm	Micrometer
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NDF	Neutral-detergent fibre
NRC	National Research Council
O	Oxygen
°C	Degree Celsius
PBS	Phosphate Buffer Saline
PKC	Palm Kernel Cake
PKC+SM	Palm Kernel Cake + Sulphur and Molybdenum
PPM	Parts per million
RPM	Revolutions per minute
S	Sulphur
SD	Standard Deviation
SDW	Sample Dry Weight
SE	Standard Error
S-Mo	Sulphur molybdenum complex
SPSS	Statistical Package for Social Science
X <sup>2</sup>	Chi-squared
Zn	Zinc



## CHAPTER 1

### INTRODUCTION

In Malaysia, palm kernel cake (PKC) by-products from the palm oil industry, is a popular and widely accepted animal feed. The nutrient content of PKC makes suitable for most ruminant and non-ruminant animals. PKC is good source of energy and protein (Salam *et al.*, 1997).

Studies showed that PKC can be utilise by a wide range of animal species such as chicken (Onwudike, 1986), goat (Jin *et al.*, 1995), sheep (Hair-Bejo and Alimon, 1995), and cattle (Abdullah and Hutagalung, 1988), pig (Rhule, 1996) and also in rabbit (Adenaji and Omonijo, 2004). Akinfala *et al.* (2003) reported that rabbit can consume diets containing up to 30% PKC with 70.9% digestibility of the crude protein. However, the high copper (Cu) content in PKC may cause toxicity. In sheep, adding molybdenum sulphate in a PKC-based diet will overcome the toxicity problem (Hair-Bejo and Alimon, 1995). Both molybdenum and sulphur were effective in reducing the Cu concentrations in the liver and kidneys of Santa Ines sheep fed a PKC-based diet for 6 months (Al-Kirshi, 2004).

Copper plays an important role in male and female reproduction (Wong *et al.*, 2001). In cows and ewes, Cu deficiency (0.5µg/g) can cause infertility and delayed oestrus (Underwood, 1977; Puls, 1994). In the rams fed a Cu-deficient diet they become successful breeders (Weiner and Sales, 1976).

This may probably due to the abnormal levels of Cu interfering with spermatogenesis – the production, maturation and fertilizing capacity of the spermatozoa (Skandhan, 1992; Yaakub *et al.*, 2005).

Most of the studies on feeding by-products have focussed on production by the animals, not on their reproductive performance. The ovarian follicle plays a major role in controlling the oestrous cycle, ensuring oocyte competency and the subsequent embryo development and determining the post ovulation *corpus luteum* function (Diskin *et al.*, 2003). The ability to manipulate the ovulation rate in animals has commercial significance because of the potential to improve reproductive performance (Findlay *et al.*, 1996).

In rabbits, there was an increase in the overall number of ovulatory follicles following coital stimulation, suggesting that the ovulation rate can be raised (Mattaraia *et al.*, 2005). The ultimate goal of folliculogenesis is to ovulate a mature egg ready for fertilization which takes place in the ampulla of the oviduct, usually within 48 hours of ovulation (Robert and Edward, 2000). The rabbit is an important species for reproductive research because it is prolific and adapts easily to most environments. It does not exhibit an oestrous cycle *per se*; rather, the mature ovary at all times has follicles to be released, i.e. ovulated, in response to the stimulus of mating or injection of Human chorionic gonadotropin (hCG) or luteinizing hormone (LH) (Miller, 1999).

Comprehension of folliculogenesis will enable a better understanding of factors influencing the follicular behaviour due to nutritional challenges.

Follicular development is dependent on the nutritional state, age of the animal and genotype (Nalbandov, 1976, Findly *et al.*, 1996).

This study was undertaken to evaluate the effect of three diets (Control, 50%PKC and PKC+MS) on follicular growth and embryo development in domestic rabbits. Therefore the objectives of this study are:

- To determine the levels of copper in blood serum, liver and ovaries in rabbits fed on PKC.
- To assess the effects of PKC on the distribution and diameter of ovarian follicles.
- To evaluate the effect of PKC on the ovulation rate and numbers embryonic cells.



## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Palm Kernel Cake

Palm Kernel Cake (PKC) is an important by-product of the oil palm industry. It is the residue after extraction of oil from the kernel of the oil palm fruit. Palm Kernel Cake (PKC) is a source of protein and energy and is commonly used to feed ruminants. It is not so suitable for monogastric animals because of its high fibre content making it less digestible without the aid of rumen bacteria, apart from having a low palatability (Ravindran and Blair, 1992).

Although PKC is cheap (Aduku *et al.*, 1988), it has a high crude fibre content of about 14.5% which rather restricts its use in monogastric diets. The rabbit, however, although a non-ruminant, has a long caecum, or blind gut, which acts like a rumen. Like the horse, therefore, it is a blind gut digester and can consume high fibre diets (Ehrlein *et al.*, 1983).

A problem with using PKC is its high Cu content (Table 2.1). Fed to sheep, it has caused chronic Cu toxicity (Rahman *et al.*, 1989; Wan Mohammad *et al.*, 1989). The excess Cu accumulates in the liver, beyond a threshold of which chronic toxicity occurs (Ivan *et al.*, 1999).