



UNIVERSITI PUTRA MALAYSIA

***SEMEN CHARACTERISTICS AND HISTOLOGICAL CHANGES IN TESTES OF
SAANEN CROSSBRED BUCKS FED PALM KERNEL CAKE - BASED DIETS***

SITI AISYAH BINTI SIDIK

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By

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

July 2013

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DEDICATION

This thesis is dedicated to my beloved parents, as always, for being supportive in all aspects, to my beloved grandmother (Uwan), to my sisters and brothers who bring me joy, even on the darkest days and all graduates who suffered from the same struggles as me and never give up in completing their thesis.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

SEMEN CHARACTERISTICS AND HISTOLOGICAL CHANGES IN TESTES OF SAANEN CROSSBRED BUCKS FED PALM KERNEL CAKE - BASED DIET

By

SITI AISYAH BINTI SIDIK

July 2013

Chairman : Halimatun Yaakub, PhD
Faculty : Agriculture

An experiment was conducted to evaluate the effects of different diets based on PKC on sperm quality and spermatogenesis of goats. Fifteen adult Saanen crosses buck were randomly assigned into three formulated diet which are concentrates (Control), 65% Palm Kernel Cake (PKC), 65% Palm Kernel Cake plus ammonium molybdate and sodium sulphate (PKCMS) for six months. Semen characteristics and body weight (BW) were determined fortnightly and dry matter intake (DMI) was measured daily. Blood serums from each buck were collected for copper and testosterone concentration. Testes samples of three bucks from each dietary treatment were assessed for Cu concentration, quantitative histological evaluation and transmission electron microscopy (TEM) viewing. There were no differences in initial and final BW among treatments ($P > 0.05$). The average daily gain (ADG) were highest ($P < 0.05$) in the Control diet followed by PKC and PKCMS groups. The DMI values were not significantly different ($P > 0.05$) among dietary treatment groups. There are no interaction between treatment and time of semen collections. Sperm concentrations and total sperm motility percentage showed significant differences ($P < 0.05$) among dietary treatments. The sperm concentration of PKC was 4% and 16% higher compared to Control and PKCMS, respectively. Total percentage of motility of sperm was consistent with the sperm concentration ($P < 0.05$). Addition of molybdenum sulphate with PKC did not alter the semen quality. However, PKCMS showed the highest mean number among treatment in the percentage of abnormalities ($P < 0.05$). Progressive motility percentage was significantly higher in PKC and Control as compared to PKCMS. Rapid motility was affected by PKC and significantly higher than Control and PKCMS respectively ($P < 0.05$). Other motility parameters as follows sperm motion parameters, VAP, VSL, VCL, ALH, BCF, STR, and LIN did not differ significantly ($P > 0.05$) between dietary treatment groups. Generally, the best semen quality in Saanen crossbred buck was obtained in PKC group. Mean testosterone values under PKC group were significantly higher than PKCMS and Control group at week 4 and 24 ($P < 0.05$). In histological evaluation, there were no significant different ($P > 0.05$) in the mean number of spermatogonia, spermatocyte, spermatid, spermatozoa, Sertoli cells, Leydig cells. However, degenerative cells were significantly higher in goat fed with PKC compared to others ($P < 0.05$). This condition is reflected in the TEM result when there was significant increase in the number of degenerated cell of

the same animal group. Toxic effect on mitotic secondary spermatocytes and cap phase spermatids were observed in buck fed on PKC. In conclusion, the Cu content in PKC diet did not alter the overall spermatogenic cells in buck after feeding for six months. However, TEM evaluation did showed the effect of Cu content in PKC in seminiferous tubules. Further study with larger number of buck and longer period are required.



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

CIRI - CIRI SEMEN DAN PERUBAHAN HISTOLOGI TESTIS KAMBING JANTAN KACUKAN SAANEN YANG DIBERI MAKAN DIET BERASASKAN HAMPAS ISIRONG KELAPA SAWIT

Oleh

SITI AISYAH BINTI SIDIK

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Kajian telah dijalankan untuk menentukan kesan-kesan perbezaan diet berasaskan PKC terhadap kualiti sperma dan spermatogenesis kambing. Lima belas ekor kambing jantan dewasa kacukan Saanen dipilih secara rawak untuk tiga formulasi diet iaitu konsentrat (Kawalan), 65% hampas isirong kelapa sawit (PKC), 65% hampas isirong kelapa sawit ditambah ammonium molibdat dan sodium sulfat (PKCMS) selama enam bulan. Ciri-ciri semen dan berat badan (BW) ditentukan setiap dua minggu dan pengambilan berat kering (DMI) diukur setiap hari. Serum darah setiap kambing jantan dikumpul untuk kepekatan kuprum dan testosteron. Sampel testis daripada tiga kambing jantan bagi setiap rawatan diet dinilai untuk kepekatan kuprum, penilaian histologi kuantitatif dan paparan transmisi electron mikroskopi (TEM). Tiada perbezaan terhadap berat badan (BW) awal dan akhir di antara rawatan diet ($P>0.05$). Purata dapatan harian (ADG) meningkat ($P<0.05$) pada diet Kawalan diikuti dengan PKC dan PKCMS. Nilai DMI tiada perbezaan signifikan ($P>0.05$) antara kumpulan diet. Tiada interaksi antara rawatan diet dan masa pengumpulan semen. Kepekatan sperma dan peratus motiliti sperma total menunjukkan perbezaan signifikan ($P<0.05$) di antara diet rawatan. Kepekatan sperma PKC ialah 4% dan 16% tinggi dibandingkan dengan Kawalan dan PKCMS. Jumlah peratusan motiliti sperma adalah konsisten dengan kepekatan sperma ($P<0.05$). Penambahan molibdenum sulfat dengan PKC tidak mengubah kualiti semen. Walau bagaimanapun, PKCMS menunjukkan nilai purata tertinggi dalam peratus abnormaliti di antara rawatan ($P<0.05$). Peratus motiliti progresif adalah tinggi dengan signifikan pada PKC dan Kawalan dibandingkan dengan PKCMS. Motiliti pantas dipengaruhi oleh PKC dan lebih tinggi signifikan berbanding Kawalan dan PKCMS ($P<0.05$). Parameter motiliti yang lain seperti parameter pergerakan sperma, VAP, VSL, VCL, ALH, BCF, STR, dan LIN tidak berbeza dengan signifikan ($P>0.05$) antara kumpulan rawatan diet. Amnya, kualiti semen terbaik adalah dalam kambing jantan kacukan Saanen adalah diperolehi dari kumpulan PKC. Nilai purata testosteron kumpulan PKC tinggi dengan signifikan berbanding kumpulan PKCMS dan Kawalan pada minggu 4 dan 24 ($P<0.05$). Dalam penilaian histologi, tiada perbezaan signifikan ($P>0.05$) di dalam bilangan purata nombor spermatogonia, spermatisit, spermatid, spermatozoa, sel Sertoli, dan sel Leydig. Walau bagaimanapun, sel degenerasi berbeza dengan signifikan yang tinggi di dalam PKC berbanding yang lain ($P<0.05$). Keadaan ini mencerminkan keputusan TEM apabila

terdapat peningkatan signifikan jumlah sel degenerative pada kumpulan yang sama. Kesan ketoksikan pada spermatosit mitotik sekunder dan kepala spermatid didapati di dalam kambing jantan PKC. Kesimpulannya, kandungan Cu di dalam diet PKC tidak mengubah secara keseluruhan sel spermatogenik di dalam kambing jantan selepas diberi makan selama enam bulan. Walau bagaimanapun, penilaian TEM ada menunjukkan kesan kandungan di dalam PKC ke atas tubul seminiferus. Kajian mendalam dengan kuantiti lebih besar dan tempoh yang lebih panjang adalah diperlukan.



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I certify that a Thesis Examination Committee has met on 9 July 2013 to conduct the final examination of Siti Aisyah binti Sidik on her thesis entitled "Semen Characteristics and Histological Changes in Testes of Saanen Crossbred Bucks Fed Palm Kernel Cake-Based Diets" in accordance with the 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 student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ABP	Androgen Binding Protein
ADF	Acid-detergent fibre
BW	Body Weight
Ca	Calcium
Co	Cobalt
Cu	Copper
CASA	Computer Assisted Semen Analysis
DM	Dry matter
DMI	Dry matter intake
Fe	Iron
FSH	Follicle-stimulating hormone
Gr	Granules
GLM	General Linear Model
H	Hydrogen
HNO ₃	Nitric Acid
H ₂ SO ₄	Sulphric Acid
K	Potassium
kg ^{0.75}	Metabolic Body Weight
LH	Luteinizing Hormone
M	Million
Mg	Magnesium
Mo	Molybdenum
μL	Microliter
μm	Micrometer
ng	Nanogram
nm	Nanometer
NRC	National Research Council
NS	Not Significant
°C	Degree Celcius
P	Phosphorus
PKC	Palm Kernel Cake
PKCMS	Palm Kernel Cake plus Molybdenum Sulphate
ppm	Parts per million
rpm	Revolution per minute
S	Sulphur
Se	Selenium
S.E	Standard Error
TEM	Transmission Electron Microscopy
Zn	Zinc

CHAPTER 1

INTRODUCTION

Malaysia is the second largest producer and exporter of palm oil (World growth, 2009). In Malaysia, palm kernel cake (PKC) is a byproduct that is produced abundantly. Palm kernel cake has high nutritive value and is widely used as feed ingredient for ruminants (Setthapukdee *et al.*, 1991; Salam *et al.*, 1997). Many studies were conducted to evaluate the utilization of PKC as feed in beef and dairy cattle (Chin, 1991), sheep and goat (Hair-Bejo and Alimon, 1995; Yaakub *et al.*, 2009; Jin *et al.*, 1995), pig (Rhule, 1996), poultry (Onwudike, 1986), rabbit (Orunmuyi *et al.*, 2006) and aquaculture (Wan Zahari and Alimon, 2004). However, due to high copper (Cu) content in PKC, it becomes less suitable for sheep (Hair-Bejo and Alimon, 1995; Alimon *et al.*, 2011).

Copper plays important roles in spermatogenesis and fertility (Wong *et al.*, 2001). It has been reported that cows (Underwood, 1977) and ewes (Howell and Hall, 1970) fed with copper deficiency suffered from delayed or depressed oestrus and infertile.

An excess amount of copper in the diet can be toxic (Mc Donald *et al.*, 2002), especially in sheep, it could cause chronic toxicity due to the accumulation of copper in the liver (Abd Rahman *et al.* 1989; Wan Mohamed *et al.*, 1989; Hair-Bejo and Alimon, 1995). However, an addition of molybdenum (Mo) and sulphur (S) in the PKC diet has been reported to overcome the Cu toxicity problems in rams (Abd Rahman *et al.*, 1989; Gooneratne *et al.*, 1989; Kincaid, 1999; Ivan *et al.*, 1999 and Pott *et al.*, 1999; Alimon *et al.*, 2011).

Although the addition of Mo and S in the PKC diets solved the toxicity problems, recent study reported that there was changes occur during the spermatogenesis in ram (Yaakub *et al.*, 2009).

The hypothesis of this study:

The use of 65% PKC in the diet will reduce the semen quality and spermatogenesis of Saanen crossed buck due to high copper content in PKC compared to control diet. Addition of molybdenum sulphate in the PKC formulated diet will prevent the absorption of Cu and will not alter the semen quality and spermatogenesis.

This general objective of the study was:

To examine the effect of copper content in PKC diets on the Saanen crossbred buck reproductive system.

The specific objectives are:

1. to evaluate the semen characteristics of Saanen crossbred buck fed PKC and PKCMS diet.

2. to evaluate the histological structure of testis in Saanen crossbred buck fed PKC and PKCMS diet.
3. to examine the ultrastructure of spermatogenic cells , sertoli cells and leydig cells in the testes using transmission electron microscopy (TEM).



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