UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF PREGNANCY KETOSIS IN GOAT FARMS, BLOOD PROFILING AND HISTOPATHOLOGICAL CHANGES IN LATE GESTATION AND POST-PARTUM GOATS WITH KETOSIS

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By

MUHAMAD AFFAN BIN AB AZID

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

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DEDICATION

This thesis is wholeheartedly dedicated to my beloved parents, Ab Azid bin Mat Jusoh and Nazrini binti Mat Zain who have been my source of inspiration that gave me strength when I thought of giving up and who continually provide their moral, spiritual and financial supports to me throughout this journey.

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

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Chairman : Hasliza Abu Hassim, PhD
Faculty : Veterinary Medicine

Pregnancy ketosis is one of the common metabolic diseases that affect goats' meat and milk production, resulting in low productivity and hence, high economic losses. Thus, this study aimed to evaluate the prevalence of natural ketosis in selected dairy goat farms and its associated risk factors, and also to study the serum biochemical profiles and the histopathological changes in late gestation and post-partum goats with experimental ketosis. A total of 255 pregnant goats from ten farms from Terengganu (n=5) and Selangor (n=5) were screened for ketosis, which comprised of 20 to 30 random pregnant goats for each farm. Chi square analysis was done to determine the risk factors that associated with the prevalence of ketosis in the farms. For experimental trial, twelve (n=12) pregnant goats were divided into control (n=3) and treatment (n=9) groups. The control group was fed diet with adequate energy, while the treatment group was exposed to 50% reduction in the energy intake to induce ketosis. Blood was collected at weekly intervals for biochemical analysis, which included glucose, Beta-hydroxybutyrate (BHBA), free fatty acid (FFA), calcium, sodium, potassium, chloride, cortisol and insulin. On days 20 (PK20) and 40 (PK40) post-induction, 3 induced and 1 control goats were slaughtered while the remaining 3 ketosis-induced goats were provided with normal balanced diet to allow for recovery (PKRD). At slaughter, the liver, heart and brain were collected for histopathological study. Following ketosis screening, Selangor farms showed high prevalence (47.33%) of ketosis as compared to Terengganu farms (20.97%), which was characterized by high BHBA level (≥0.08 mmol/L) and presence of clinical signs. The survey revealed that all the nutritional risk factors, namely type, composition, nutritive value and quality of feed were significantly associated ($p<0.05$) with the prevalence of ketosis. Moreover, the experimental induction resulted in acute ketosis after 20 days with clinical signs including weakness, low body condition score and recumbency. Chronic ketosis was observed at 40 days, showing incoordination and abortion. Following re-introduction of adequate energy supply, the recovered PKRD goats showed no clinical sign. There were significant ($p<0.05$) decrease in the glucose,

insulin, calcium and potassium levels among the induced goats while the concentrations of BHBA, FFA and cortisol were significantly ($p<0.05$) higher. Histopathological examinations revealed that the liver of goats with ketosis showed fatty degeneration and congestion with polymorphonuclear leukocytes and mononuclear cells infiltrations. The heart of ketotic does were congested while the brain had congestion and cerebellar spongiosis. Indeed, pregnancy ketosis has been associated with insufficient diet during the late stage of pregnancy which the severity of the disease caused changes in serum biochemical and hormonal profiles during the late gestation and post-partum period of the goats as well as the histopathological changes of various organs such as liver, heart and brain.

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

PREVALENS KETOSIS KEBUNTINGAN DI LADANG-LADANG KAMBING, PROFIL DARAH DAN HISTOPATOLOGI PADA KAMBING KETOSIS SEMASA PERINGKAT AKHIR KEBUNTINGAN DAN POSTPARTUM

Oleh

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Ketosis kebuntingan merupakan salah satu penyakit metabolik yang sering kali mempengaruhi pengeluaran daging dan susu kambing iaitu boleh menyebabkan pengeluaran yang rendah dan kerugian yang tinggi pada ekonomi. Oleh itu, kajian ini bertujuan untuk menilai prevalens ketosis secara semula jadi di ladang-ladang kambing tenusu terpilih dan faktor-faktor risiko yang berkait dan juga untuk mengkaji perubahan-perubahan pada profil serum biokimia dan histopatologi semasa tempoh akhir kebuntingan dan postpartum pada kambing-kambing melalui eksperimen ketosis. Sebanyak 255 ekor kambing bunting dari sepuluh ladang dari Terengganu (n=5) dan Selangor (n=5) telah disaring untuk ketosis kebuntingan yang mana telah melibatkan 20 ke 30 ekor kambing bunting yang rawak bagi setiap ladang. Analisis Khi kuasa dua telah dijalankan bagi mengenalpasti faktor-faktor risiko yang berkait rapat dengan kadar prevalens ketosis di ladang-ladang tersebut. Untuk ujikaji percubaan, dua belas (n=12) ekor kambing bunting telah dibahagikan kepada kumpulan kawalan (n=3) dan rawatan (n=9). Kumpulan kawalan telah diberi makan dengan tenaga yang mencukupi manakala kumpulan rawatan telah dihadkan pengambilan tenaga sehingga 50% daripada keperluan harian untuk mendorong ketosis kebuntingan. Sampel-sampel darah telah dikumpul setiap minggu untuk analisis biokimia yang melibatkan glukosa, Beta-hidroksibutirat (BHBA), asid lemak bebas (FFA), kalsium, elektrolit (natrium, kalium, klorida), kortisol dan insulin. Pada hari ke 20 (PK20) dan 40 (PK40) pasca induksi, tiga (n=3) kambing rawatan dan satu (n=1) kambing kawalan telah disembelih manakala tiga (n=3) kambing rawatan yang selebihnya telah diberi diet normal yang seimbang untuk pemulihan (PKRD). Semasa penyembelihan, organ hati, jantung dan otak telah diambil untuk kajian histopatologi. Dalam saringan ketosis, ladang di Selangor menunjukkan prevalens ketosis yang tinggi (47.33%) berbanding dengan ladang di Terengganu (20.97%) yang mana bercirikan dengan kepekatan BHBA yang tinggi (≥0.8 mmol/L) dan kewujudan tanda-tanda klinikal ketosis. Tinjauan menunjukkan yang semua faktor-faktor risiko iaitu jenis, komposisi, kadar nutrisi dan kualiti makanan adalah berkait ($p<0.05$) dengan prevalens ketosis. Seterusnya, induksi ujikaji telah mengakibatkan ketosis kebuntingan akut selepas 20 hari dengan tanda-tanda klinikal termasuk kelemahan, kemerosotan

keadaan badan dan sentiasa terbaring. Ketosis yang kronik telah dikesan pada hari ke-40, iaitu menunjukkan tanda-tanda seperti pergerakan tidak seimbang dan juga keguguran. Hasil daripada pemberian semula bekalan tenaga yang mencukupi, kambing pemulihan PKRD tidak menunjukkan sebarang tanda klinikal. Terdapat penurunan yang signifikan ($p<0.05$) pada kadar glukosa, insulin, kalsium dan kalium dekat kambing yang telah didorong dengan ketosis manakala terdapat pertambahan yang signifikan ($p<0.05$) bagi kepekatan BHBA, FFA dan kortisol. Hasil daripada pemerhatian histopatologi menunjukkan yang tisu hati bagi kambing ketosis telah dikenalpasti dengan kewujudan hati berlemak, pengumpulan darah, leukosit polimorfonuklear dan penyusupan sel mononuklear. Tisu hati pada kambing ketosis telah dikesan dengan lesi pengumpulan darah manakala tisu otak juga telah dikesan dengan lesi pengumpulan darah dan serebelum spongiosis. Sesungguhnya, ketosis kebuntingan telah dikenalpasti sebagai penyakit yang berkait dengan kekurangan diet semasa peringkat akhir kebuntingan yang mana penyakit itu telah menyebabkan perubahan pada profil serum biokimia dan hormon semasa tempoh akhir kebuntingan dan postpartum pada kambing serta menyebabkan perubahan pada pelbagai organ seperti hati, jantung dan otak.

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I certify that a Thesis Examination Committee has met on 29th June 2021 to conduct the final examination of Muhamad Affan bin Ab Azid on his thesis entitled "Prevalence of Pregnancy Ketosis in Goat Farms, Blood Profiling and Histopathological Changes in Late Gestation and Post-partum Goats with Ketosis" 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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Declaration by graduate student

I hereby confirm that:

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LIST OF ABBREVIATIONS

AcAc	Acetoacetic acid
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BHBA	Beta-hydroxybutyrate
cAMP	Cyclic adenosine monophosphate
CoA	Coenzyme A
DM	Dry matter
DPX	Distyrene Plasticiser Xylene
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FFA	Free fatty acid
GTP	Guanosine triphosphate
HSL	Hormone sensitive lipase
H&E	Haematoxylin and eosin
kJ	Kilo joule
ME	Metabolizable energy
mmol	Millimole
MJ	Megajoule
PMN	Polymorphonuclear leukocytes
PK	Pregnancy ketosis
PKC	Palm kernel cake
PKRD	Pregnancy ketosis reversed diet
SEM	Standard error mean
SPSS	Statistical package for the social sciences

W	Metabolic body weight
μm	Micrometre
$\chi 2$	Chi square

CHAPTER 1

INTRODUCTION

1.1 Background of study

Goat rearing plays a vital role for the economics of farming community. Goats are reared for meat, milk and hide. However, morbidity and mortality among goats, including pregnant goats have caused bad economic impact on the livelihood of marginal farmers. Pregnancy ketosis, also known as pregnancy toxaemia, is one of the common metabolic diseases in pregnant ruminant around the world that affect livestock's meat and milk productions (Bani Ismail et al., 2008). The disease is commonly occurring in ruminants such as goat, sheep and cattle during the late stage of pregnancy, which generally has a low morbidity rate (2-6%) but a high mortality rate (80%). It is caused by abnormal metabolism of carbohydrates and fats, as a result of negative energy balance that occurs during the late stage of gestation. It is characterized by relatively high concentrations of the ketone bodies specifically acetoacetate, Beta-hydroxybutyrate (BHBA) and acetone as well as low concentration of glucose in blood circulation (Brozos et al., 2011). According to Schlumbohm and Harmeyer (2014), obese ewes or does carrying multiple foetuses are at higher risk of developing the disease due to the high demand of feed intake.

In Malaysia, pregnancy ketosis has been observed but not been thoroughly studied such as the information regarding the detailed serum biochemical changes and disease development, risk factors and impact of ketosis on dairy goats. Information regarding the predisposing risk factors such as the nutritional and non-nutritional aspects that contributed to the outbreak of pregnancy ketosis in goat farms would help goat farmers as an early measure to prevent the occurrence of the disease. Diagnosis of clinical pregnancy ketosis is based on history, clinical signs (e.g.: teeth grinding, weakness, recumbency) and the serum biochemical profiles (Lima et al., 2012). In addition, the use of these serum biochemical profiles as biomarkers in developmental stage of pregnancy ketosis offers a promising opportunity to develop rapid and accurate test kit that could be used by dairy farmers or veterinarians to screen and diagnose herds for pregnancy ketosis.

Histopathological reports related to any disease are usually referred to data obtained from internal organ biopsy or during post-mortem examination. Many researchers claimed that during ketosis changes occur in some tissues, particularly the cerebral and cerebellar neuronal necrosis and vacuolation, early structural immaturity of placenta, and liver steatosis (Andrews, 2017; Jeffrey and Higgins, 2012). Thus, it is believed that understanding the pathogenesis of ketosis could highlight critical points that can be used to prevent the development of the disease from becoming more severe which will affect the dairy goats that may further affect various internal organs such as liver, heart and brain which can be observed through development of lesions.

In the present study, the prevalence of pregnancy ketosis from selected goat farms in Malaysia was determined. Apart from that, the serum biochemical profiles and histopathological changes also were analyzed during the late gestation and post-partum period of experimental pregnancy ketosis does.

1.2 Problem statement

Although pregnancy ketosis is frequently observed among sheep and goats, the prevalence of the disease in goat farms in Malaysia has not been studied thus far. Hence, the predisposing risk factors in terms of nutritional and non-nutritional aspects that may cause the pregnancy ketosis in animal farms remained unknown. Furthermore, clinical ketosis could be classified as acute or chronic. Therefore, the serum biochemical profiles and histopathological changes in organs could be used to classify the stage of ketosis in pregnant animals. In facts, the observation of serum biochemical profiles can be used as an early prevention for subsequent attempt to treat or control pregnancy ketosis in dairy goat farms while the histopathological changes in various organs of affected animals can be used as a guideline or reference in pregnancy ketosis study. Therefore, understanding the risk factors on the prevalence of pregnancy ketosis in dairy goat, the serum biochemical profiles and the histopathological changes in experimental trial warrant further investigation.

1.3 Objectives

a) To evaluate the prevalence of subclinical and clinical ketosis among dairy goats in Terengganu and Selangor farms.
b) To evaluate the association between the risk factors and prevalence of subclinical and clinical ketosis among dairy goats in Terengganu and Selangor farms.
c) To evaluate the serum biochemical profiles among pregnant goats with subclinical and clinical ketosis at late gestation and post-partum period.
d) To observe the gross and histopathological changes in selected organs of pregnant goats with experimental ketosis at late gestation and post-partum period.

1.4 Hypotheses

High prevalence of pregnancy ketosis in Selangor farms as compared to Terengganu farms are associated with the risk factors (nutritional and non-nutritional) that contributed to occurrence of the disease in each state. The development of pregnancy ketosis in affected animals without proper treatment will cause severe changes in serum biochemical profiles and histopathological in various organs (e.g.: liver, heart, brain) during late gestation as well as postpartum period.

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