



UNIVERSITI PUTRA MALAYSIA

***SERUM BIOCHEMICAL, FATTY ACID PROFILES AND HISTOLOGICAL
CHANGES IN CAPRINE PREGNANCY KETOSIS***

AMIRUL FAIZ BIN MOHD AZMI

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By

AMIRUL FAIZ BIN MOHD AZMI

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

July 2017

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DEDICATION

This thesis is dedicated specially to my beloved parents, Mohd Azmi Othman and Azizah Sahar. A lot of thanks also go to my siblings, lecturers and friends for all the tremendous support, prayers and encouragement during my entire postgraduate life.





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

SERUM BIOCHEMICAL, FATTY ACID PROFILES AND HISTOLOGICAL CHANGES IN CAPRINE PREGNANCY KETOSIS

By

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July 2017

Chair : Hasliza Abu Hassim, PhD
Faculty : Veterinary Medicine

Pregnancy ketosis has been recognized as one of the metabolic diseases that affects goat performance as well as meat and milk products. Indeed, this has resulted in low production and economic losses by the goat farms through culling and mortality. Pregnancy ketosis will further lead in failure to meet the sudden surge of demand for goat milk and meat in Malaysia. Pregnancy ketosis in goat mainly occurs in late stage of pregnancy due to inadequate of energy intake as compared to the demand by fetus development. Indeed, failure to meet this demand will further leads to negative energy balance. The main cause of negative energy balance in pregnancy ketosis is the disturbance of carbohydrate metabolism, characterized by high production of ketone bodies and plasma free fatty acid (FFA), as well as damage to the liver. Thus, this project aimed to assess the serum biochemical, fatty acid profiles and histological changes of liver in pregnancy ketosis of goat. Sixteen pregnant does at third trimester of pregnancy were used and divided into control (n=8) and treatment groups (n=8). The animals in the treatment group were induced with ketosis by restricted the energy intake for up to 50% of the daily requirement. The does in control group were fed on Napier grass and goat concentrate with water *ad libitum*. The ketosis goats were observed for body condition score, subclinical and clinical signs throughout this study. Blood samples were collected and analyzed for glucose, Beta-hydroxybutyrate (BHBA), FFA, calcium, electrolyte (sodium, potassium, chloride), liver enzyme and hormonal levels (cortisol and insulin). Four pregnant does with severe stages of ketosis and four pregnant does in control group were slaughtered according to the Islamic traditions (Halal Slaughter Method) by severing the jugular veins, carotid arteries, trachea and the esophagus. All the liver samples were collected for fatty acid profile and histological study. In addition, blood plasma and liver samples were analyzed for fatty acid composition by using gas chromatography. The type of lesions and histological changes of the liver tissues during pregnancy ketosis also were analyzed under microscopic study. In this study, several clinical signs were observed in pregnancy ketosis goat such as teeth grinding and depressed. The presence of >3+ ketone bodies in urine was also found in pregnancy ketosis goat. In this study, the BHBA, FFA, calcium, amino aspartate transferase (AST), gamma glutamyltransferase (GGT) and

cortisol hormone were significantly higher in pregnancy ketosis goats as compared to control group. Meanwhile, the concentration of glucose, sodium, potassium, chloride, and insulin hormones were lower in pregnancy ketosis goats as compared to control. Fatty acid composition in blood plasma of pregnant goat with ketosis showed higher level of palmitic, stearic and oleic acid whereas the palmitic, oleic and linoleic acid levels was found higher in liver. There are three types of lesion were found in the liver of pregnancy ketosis goat such as fatty liver, congestion and thrombosis. Furthermore, histological study on fatty liver revealed a similar incidence and intensity of mild liver steatosis with lower cellular vacuolation in hepatocyte presence in healthy late pregnant does. Almost all pregnant does with ketosis state had large amount of small lipid droplets in every hepatocyte of whole liver acinus, with higher number of cellular vacuolation. These findings indeed associated with high BHBA and FFA and low glucose levels for pregnancy ketosis goats. In conclusion, liver are involved in the histopathogenesis of caprine pregnancy ketosis as well as pregnancy ketosis can affect the serum biochemical, hormonal, as well as fatty acid profiles in goats.

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

SERUM BIOKIMIA, PROFIL ASID LEMAK DAN PERUBAHAN HISTOLOGI PADA KAMBING KEBUNTINGAN KETOSIS

Oleh

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Kebuntingan ketosis telah diiktiraf sebagai salah satu penyakit metabolik yang menjejaskan prestasi kambing seperti daging dan produk tenusu. Sesungguhnya, ini telah menyebabkan pengeluaran yang rendah dan kerugian ekonomi kepada penternak kambing melalui penakiaian dan kematian. Kebuntingan ketosis akan menyebabkan kegagalan untuk memenuhi peningkatan permintaan yang mendadak bagi susu kambing dan daging di Malaysia. Kebuntingan ketosis pada kambing sering berlaku di peringkat akhir kebuntingan disebabkan kekurangan pengambilan tenaga berbanding dengan keperluan untuk perkembangan janin. Sesungguhnya, kegagalan untuk memenuhi keperluan ini akan membawa berlakunya kepada imbalan tenaga negatif. Punca utama imbalan tenaga negatif dalam kebuntingan ketosis adalah gangguan metabolisma karbohidrat, dikategorikan sebagai pengeluaran badan ketone dan plasma asid lemak bebas yang tinggi, serta kerosakan pada hati. Oleh itu, projek ini bertujuan untuk menilai serumbiokimia, komposisi asid lemak bebas dan perubahan histologi hati semasa kebuntingan ketosis terhadap kambing. Enam belas kambing betina yang bunting pada peringkat trimester ketiga telah digunakan dan dibahagikan kepada dua kumpulan iaitu kumpulan kawalan (n=8) dan kumpulan rawatan (n=8). Haiwan-haiwan dalam kumpulan rawatan telah didorongkan dengan ketosis melalui kaedah terhadap pengambilan tenaga sehingga 50% daripada keperluan harian. Kambing betina dalam kumpulan kawalan pula telah di beri makan dengan rumput Napier dan pelet kambing serta pemberian air secara berterusan. Skor keadaan badan, tanda-tanda subklinikal dan klinikal telah diperhatikan pada semua kambing ketosis sepanjang kajian ini. Sampel darah telah dikumpul dan dianalisis untuk melihat kadar glukosa, Beta-hydroxybutyrate (BHBA), asid lemak bebas, kalsium, elektrolit (natrium, kalium, klorida), enzim hati dan tahap hormon (kortisol dan insulin). Empat kambing betina bunting di dalam kumpulan kawalan dan empat kumpulan rawatan yang mengalami kebuntingan ketosis yang teruk telah disembelih secara kaedah Islam (Kaedah Sembelihan Secara Islam) dengan cara memisahkan urat jugular, arteri karotid, trakea dan esofagus. Kesemua sampel hati telah dikumpulkan untuk kajian profil asid lemak serta histologi. Di samping itu, plasma darah dan sampel hati dianalisis untuk mengkaji komposisi asid lemak dengan menggunakan kromatografi gas. Jenis lesi dan perubahan

histologi tisu hati semasa kebuntingan ketosis juga dianalisis di bawah kajian mikroskopik. Dalam kajian ini, terdapat beberapa tanda-tanda klinikal diperhatikan dalam kambing bunting yang menghadapi ketosis seperti pengisaran gigi dan tertekan. Kehadiran lebih > 3+ badan ketone dalam air kencing juga dijumpai dalam kambing bunting ketosis. Dalam kajian ini, BHBA, asid lemak bebas, kalsium, amino transferase aspartik (AST), gamma glutamyl transferase (GGT) dan hormon kortisol adalah lebih tinggi pada kambing bunting ketosis berbanding kambing bunting yang sihat. Sementara itu, kepekatan glukosa, natrium, kalium, klorida, dan hormon insulin adalah lebih rendah pada kambing bunting ketosis berbanding dengan kambing bunting yang sihat. Komposisi lemak asid dalam plasma darah kambing yang menghadapi kebuntingan ketosis menunjukkan tahap yang lebih pada asid palmitik, stearik dan oleik manakala tahap asid palmitik, oleik dan linoleik didapati lebih tinggi daripada hati. Terdapat tiga jenis lesi yang terdapat pada hati kambing yang ketosis seperti hati berlemak, kesesakan dan trombosis. Tambahan pula, kajian histologi terhadap hati berlemak juga menunjukkan insiden yang sama dan intensiti steatosis hati sederhana dengan selular vakuolasi yang lebih rendah terhadap hati kambing bunting yang sihat. Hampir semua kambing pada kumpulan rawatan mempunyai jumlah besar titisan lipid kecil pada seluruh sel hati, dengan bilangan yang lebih tinggi pada vakuolasi selular. Penemuan kambing bunting ketosis ini sememangnya dikaitkan dengan tahap glukosa yang rendah serta BHBA dan asid lemak bebas yang tinggi. Kesimpulannya, organ hati boleh terlibat dalam histopatogenesis kaprine kebuntingan ketosis serta kebuntingan ketosis juga boleh mempengaruhi profil asid serum biokimia, hormon, dan asid lemak terhadap kambing.

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I certify that a Thesis Examination Committee has met on (17 JULY 2017) to conduct the final examination of Amirul Faiz Bin Mohd Azmi on his thesis entitled “**SERUM BIOCHEMICAL, FATTY ACID PROFILES AND HISTOLOGICAL CHANGES IN CAPRINE PREGNANCY 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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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
LIST OF APPENDICES	xv
LIST OF ABBREVIATIONS	xvi
CHAPTER	
1 GENERAL INTRODUCTION	1
1.1 Background of study	1
1.2 Problem statement	2
1.3 Objectives of the research	2
1.4 Hypothesis	3
1.5 Limitation of the study	3
2 LITERATURE REVIEW	4
2.1 Overview of pregnancy ketosis in ruminant	4
2.2 Pregnancy ketosis in small ruminant	4
2.3 Clinical signs and diagnosis of pregnancy ketosis	5
2.4 Pathophysiology of pregnancy ketosis	7
2.4.1 Dietary changes during pregnancy ketosis	7
2.4.2 Negative energy balance	8
2.4.3 Metabolic changes during pregnancy ketosis	8
2.4.3.1 Lipolysis in adipocyte	8
2.4.3.2 Fatty acid oxidation and ketogenesis in liver	11
2.4.3.3 Gluconeogenesis in liver	16
2.4.4 Serobiochemical changes during pregnancy ketosis	17
2.4.4.1 Serobiochemical and hormonal profiles	17
2.4.4.2 Fatty acid composition	19
2.4.5 Regulation of hormone during pregnancy ketosis	20
2.4.5.1 Insulin	20
2.4.5.2 Cortisol	21
2.4.6 Histological changes in liver tissues during pregnancy ketosis	21
2.5 The importance of early detection of pregnancy	23

ketosis in goat

3	GENERAL MATERIALS AND METHODS	24
3.1	Experimental design	24
3.2	Animal induction and selection of pregnancy ketosis	26
3.3	Measurement of body condition score	27
3.4	Animal and ethical considerations	29
4	SEROBIOCHEMICAL AND HORMONAL PROFILES OF PREGNANCY KETOSIS IN BOER CROSS GOAT	30
4.1	Introduction	30
4.2	Materials and methods	31
4.2.1	Blood collection	31
4.2.2	Serobiochemical assay	31
4.2.3	Hormonal assays	31
4.3	Data analysis	32
4.4	Results	33
4.5	Discussion	37
4.6	Conclusion	39
5	FATTY ACIDS COMPOSITION IN BLOOD AND LIVER OF PREGNANCY KETOSIS IN BOER CROSS GOAT	40
5.1	Introduction	40
5.2	Materials and methods	41
5.2.1	Blood collection	41
5.2.2	Liver collection	41
5.2.3	Fatty acid profile determination	41
5.2.3.1	Chemical and glassware	41
5.2.3.2	Total lipid extraction	42
5.2.3.3	Preparation of fatty acid methyl esters	42
5.2.3.4	Gas liquid chromatography	43
5.3	Data analysis	44
5.4	Results	45
5.4.1	Fatty acid composition in blood	45
5.4.2	Fatty acid composition in liver	48
5.5	Discussion	51
5.6	Conclusion	53
6	HISTOLOGICAL CHANGES IN LIVER TISSUES OF PREGNANCY KETOSIS IN BOER CROSS GOAT	54
6.1	Introduction	54
6.2	Materials and Methods	55
6.2.1	Liver collection	55
6.2.2	Gross examination	55
6.2.3	Histological examination	55
6.2.3.1	Fixation and staining	55
6.2.3.2	Microscopic study	56
6.3	Data analysis	57
6.4	Results	58
6.4.1	Gross pathological findings	58

6.4.2	Histological findings	59
6.4.2.1	Histological scoring	59
6.4.2.2	Extended cellular vacuolation scoring on fatty liver lesion	63
6.5	Discussion	66
6.6	Conclusion	67
7	GENERAL DISCUSSION	68
8	CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH	71
8.1	Conclusion	71
8.2	Recommendation for future research	72
	REFERENCES	73
	APPENDICES	90
	BIODATA OF STUDENT	95
	LIST OF PUBLICATIONS	96

LIST OF TABLES

Table		Page
1	Serobiochemical profiles of pregnancy ketosis in goat	17
2	Clinical signs of pregnancy ketosis in goat	27
3	Body condition score	28
4	Summary of result for induction of pregnant goat with ketosis	34
5	Serobiochemical profiles in control vs treatment groups	35
6	Fatty acid composition in the blood of control and treatment groups	45
7	Fatty acids composition in the liver of control and treatment group	49
8	Grading system on liver according to percentage of lesion distributed and severity of cellular vacuolar degeneration	56
9	Histological lesions scoring of different groups	59
10	Number of goats with and without liver degeneration which corresponding with the percentage of lesion and degree of cellular vacuolation.	65

LIST OF FIGURES

Figure		Page
1	Ketotest strip test	7
2	Adipocyte metabolism	9
3	Lipolysis of adipocyte	10
4	Transportation of free fatty acid in the blood vessels.	11
5	Linkage of metabolism between adipose tissue and liver during pregnancy ketosis in goats	12
6	Transportation of acyl-CoA molecules from cytosol into mitochondria	13
7	Beta-oxidation of fatty acids.	14
8	Ketogenesis pathway during non-availability of oxaloacetate	15
9	Graph association of concentration BHBA and glucose during fasting state	18
10	Histological changes of liver tissues during pregnancy ketosis in goat	22
11	Flowchart diagram summarizing the experimental design and activities	25
12	Animal Grouping	26
13	Body condition score of pregnancy ketosis in goats	33
14	Ketostick strip result	34
15	Concentration of plasma cortisol hormone in goats with pregnancy ketosis and healthy goat	36
16	Concentration of plasma insulin hormone in goats with pregnancy ketosis and healthy goat	36
17	The composition of Monounsaturated fatty acid (MUFA), n-3 Polyunsaturated fatty acid (n-3 PUFA), n-6 Polyunsaturated fatty acid (n-6 PUFA) and Total Polyunsaturated fatty acid (PUFA) in the blood of control vs treatment group	47
18	The composition of Monounsaturated fatty acid (MUFA), n-3 Polyunsaturated fatty acid (PUFA), n-6 Polyunsaturated fatty acid (PUFA) and Total Polyunsaturated fatty acid (PUFA) in the liver of control group vs treatment group	50
19	Liver organ of pregnant goat in control vs treatment groups	58
20	Fatty liver lesions	60
21	Congestion lesions	61
22	Thrombosis lesions	62
23	Histological micrograph of liver tissue in treatment group	63
24	Histological micrograph of liver tissue in control group	64

LIST OF APPENDICES

Appendix		Page
1	Pregnant goat at third trimester	90
2	Laboratory for analyzed serum profiles	90
3	Microtome section for cutting samples	91
4	Sample staining process	91
5	Fatty acid extraction	92
6	History of the animals collected from the farmer	93
7	Animal ethic form	94



LIST OF ABBREVIATIONS

BHBA	Beta Hydroxybutyrate
FFA	Free Fatty Acid
mmol	Millimole
L	Liter
μmol	Micromole
kJ	Kilojoule
ME	Metabolizable Energy
W	Metabolic Body Weight
kg	Kilogram
AST	Amino Aspartate Transferase
GGT	Gamma- Glutamyl Transferase
cAMP	Cyclic Adenosine Monophosphate
CoA	Coenzyme A
VLDL	Very Low Density Lipoprotein
SLC27	Solute Carrier 27
LCFAs	Long Chain Fatty Acids
CPT1	Carnitine Palmitoyltransferase-1
mg	Milligram
dL	Deciliter
ng	Nanogram
ml	Milliliter
TAG	Triacylglyceride
IU	International Unit
nmol	Nanomole
μU	Micro Unit
v	Volume
$^{\circ}\text{C}$	Degree Celsius
min	Minute
g	Gram
SE	Standard Error
PUFA	Polyunsaturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
μm	Micro Meter

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of study

Recently, there has been a sharp increase in the demand for goat milk and meat in Malaysia, particularly in the last three decades due to rapid economic and population growth, with the resultant effects of urbanization, income growth and changing consumer preferences (Bisant, 2010). Nevertheless, the number of goat farms in Malaysia is still insufficient to complement the sudden surge of demand for goat milk and meat. Although the ruminant population particularly goats has indicated positive growth, it has yet to reach its real potential since parameters such as growth rate, breeding performance, feed conversion and losses due to diseases and mortalities have not shown much improvement (Aziz et al., 2011). Regardless of the fact that the livestock industry has evolved in the past decades through research and development (R&D) and innovations in technology, products and services, the majority of ruminant livestock farmers especially smallholders are still practicing traditional farming, owning small herds with improper farm management, low input and minimal application of technology. Among the urgent issues faced by goat farmers include the improper rearing management particularly in feed and feeding (Jamaludin et al., 2012). Indeed, improper and imbalance feeding regime could further affect the body metabolism as well as the performance of animals such as growth and production.

Pregnancy ketosis also known as pregnancy toxemia has been recognized as one of the common metabolic disease affecting goat meat and milk production (Bani Ismail et al., 2009). Pregnancy ketosis commonly occurs in goats or sheep during the late stage of gestation, generally has a low morbidity rate (2-5%) but a high mortality rate (80%) (Brounts et al., 2004; Zamir et al., 2009; Brozos et al., 2011). The main cause of pregnancy ketosis in goats is a disturbance of carbohydrate metabolism due to high demands for glucose by the developing fetuses in the last trimester of pregnancy, resulting in negative energy balance (Schlumbohm and Hameyer, 2004). It is biochemically characterized by hypoinsulinemia, high levels of free fatty acids (FFA) and ketone bodies in the plasma of does diagnosed with pregnancy ketosis (Van Saun, 2000). As a fetal demand of glucose exceeds dietary energy intake, increased lipolysis lead to an augmented synthesis of ketone bodies to maintain metabolic homeostasis (Cal et al., 2009). It was suggested that the fatty liver due to lipolysis interferes with hepatic gluconeogenic capacity, thus ketosis and fatty liver disease would play a central role in pregnancy ketosis (Herdt, 2000).

The histopathological findings in pregnancy ketosis are usually referred to data obtained from liver biopsy or during post mortem examination. Previous studies reported that during ketosis, animal showed various post mortem lesion such as; cerebral and cerebellar neuronal necrosis and vacuolation, early structural maturity of placenta, and liver steatosis (Cal et al., 2009). Although being a rather commonly studied disease, no previous work assessing the evolution of histological changes of the liver tissues during pregnancy ketosis and its relation to serobiochemical changes especially in pregnancy goat.

In the present study, plasma free fatty acid composition, serobiochemical as well as hormonal changes were analyzed in pregnancy ketosis goats. Histological changes of the liver in does with pregnancy ketosis were also analyzed and characterized. Indeed, the relation of these histological changes with other parameters such as free fatty acids (FFA) and serobiochemical levels could be consider as possible prognostic indicator of hepatic damage and the severity of pregnancy ketosis in does.

1.2 Problem statement

The disturbance of carbohydrate metabolism leads to high plasma free fatty acids and changes of serobiochemical and hormonal levels in pregnancy ketosis. This disease may further resulted in low production and economic losses by the goat farms through culling and mortality of goats. It will be challenging as well to complement the sudden surge of demand for goat milk and meat in Malaysia, while the disease is present and failure to return to full production after recovery. Therefore, there is a need to study the development of pregnancy ketosis particularly on the role of plasma free fatty acid and serobiochemical levels, and its potential to be used as biomarkers and as possible prognostic indicator of hepatic damage and severity of pregnancy ketosis on does. Indeed, early detection of this disease in the goat farm could help for a better prevention and treatment of sick animals.

1.3 Objectives of the research

1. To determine the serum biochemical and hormonal profiles in does with pregnancy ketosis
2. To assess the fatty acids composition in the blood and liver of does with pregnancy ketosis
3. To assess the histological changes of liver tissues in does with pregnancy ketosis.

1.4 Hypothesis

Determination of serobiochemical and hormonal profiles, fatty acids composition, and changes of liver metabolisms as well as histological studies could help for better understanding on the developmental stage of pregnancy ketosis in goat.

1.5 Limitation of the study

Although the research has reached its aim, there were some unavoidable limitations. First, this research was conducted only on a small number of animals which provide a poor representation of a larger population. Therefore, to generalize the results for the population, the study should have involved more animals at every group of study (control and treatment group). Apart from that, another limitation which was present is the change of weather. Weather change causes the animals to have higher tendency to get flu. Proper medication was given to infected goat by veterinary officer to ensure constant condition on tested animals.

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