UNIVERSITI PUTRA MALAYSIA

HISTOLOGICAL CHANGES IN THE LIVER OF GOATS WITH KETOSIS

SHOGASHAN BALAN

HISTOLOGICAL CHANGES IN THE LIVER OF GOATS WITH KETOSIS

SHOGASHAN BALAN

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
in partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan.

MARCH 2017

It is hereby certified that we have read this project paper entitled "Histological changes in the liver of goats with ketosis" by Shogashan Balan and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project.

_____

PROFESSOR DR MOHD ZAMRI SAAD
DVM (UPM), PhD (LIVERPOOL)
Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

_____

DR HASLIZA ABU HASSIM
DVM, MSc (UPM), PhD (Ghent, Belgium)
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-Supervisor)

DEDICATION

This thesis is dedicated to my family, my best friends and the one I love, who had accompanied me in my journey of becoming a veterinarian.

ACKNOWLEDGEMENTS

Special thanks for those who have contributed time, effort and aid to the completion of this project paper:

Prof Dr Mohd Zamri Saad,

Dr Hasliza,

Amirul Faiz,

Pn Latifah,

Dr Alif,

Jaizurah,

Family and friends.

CONTENTS

		Page
TITLE		i
CERTIFICATION		ii
DEDICATION		iii
ACKNOWLEGDEMENTS		iv
CONTENTS		v
LIST OF ABBREVIATIONS		vii
LIST OF TABLES		viii
LIST OF FIGURES		ix
ABSTRAK		x
ABSTRACT		xii
1.0	**INTRODUCTION**	1
2.0	**LITERATURE REVIEW**	3
	2.1 Reproductive Cycle	3
	2.2 Causal Factor of Pregnancy Toxaemia	5
	2.3 Diagnostic Method	7
	2.4 Clinical Signs	8
	2.5 Biochemical Parameter	8
	2.6 Nutritional Feeding	9
3.0	**MATERIALS AND METHODS**	11
	3.1 Study Design	11
	3.2 Study Animal	11

3.3 Histology Procedure 12

3.4 Histological Examination 12

3.5 Statistical Analysis 13

4.0 RESULTS 15

4.1 Histological Scoring 16

4.2 Clinical Signs and Blood Parameter 17

5.0 DISCUSSION 18

6.0 CONCLUSION 19

7.0 REFERENCES 20

LIST OF ABBREVIATIONS

BHBA	Serum β- hydroxybutyric acid
NEFAs	Nonesterified Fatty Acids
SEM	Standard Error of Mean
°C	Celsius
%	Percentage
SD	Standard Deviation
AlT	Alanine Transaminase
AST	Aspartate Transaminase
FFA	Free Fatty Acids
Ph	Potential of Hydrogen

LIST OF TABLES

		Page
Table 1:	Histological lesions scoring of different cases	14
Table 2:	The frequency of clinical signs with ketosis and control group	15
Table 3:	The Glucose and BHBA (mean ± SD) for ketosis and control group	16

LIST OF FIGURES

		Page
Figure 1:	Arrows pointing fatty liver lesion	11
Figure 2:	Fatty liver infiltration under a lower magnification.	12
Figure 3:	Congestion is indicated by the arrow.	12
Figure 4:	Thrombosis is observed as blood clot in the blood vessel as indicated by the arrow	13
Figure 5:	Histological lesions scoring of different cases	14

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Ilmiah Tahun Akhir.

PERUBAHAN HISTOLOGI PADA HATI KAMBING BERPENYAKIT KETOSIS

Oleh:

Shogashan Balan

2012

Penyelia: Prof. Dr Mohd Zamri Saad

Penyelia bersama: Dr Hasliza Abu Hassim

Tuntutan untuk susu kambing dan daging di Malaysia dilaporkan semakin meningkat dan faktor utamanya adalah kerana populasi penduduk yang semakin meningkat. Oleh itu, industri tenusu di Malaysia terutamanya industri ruminan kecil harus meningkatkan produksi tenusu. Oleh kerana masalah ketosis, pengeluaran susu tidak dapat ditingkatkan. Kajian kes-kawalan telah dilakukan untuk mengenal-pasti lesi histopatologi pada hati kambing yang mengalami ketosis. Kajian ini dijalankan tanpa pengetahuan latar belakang kes klinikal. Sembilan sampel hati termasuk kes kawalan diawetkan dalam formalin 10%. Prosedur histologi membolehkan irisan tisu setebal 4µm sebelum diwarnakan dengan haematoxylin dan eosin. Pemeriksaaan histologi dijalankan dan keparahan lesi digred menggunakan teknik peratusan lesi teragih. Lesi yang ditemui dalam hati adalah perlemakan hati, kongesi dan trombosis. Lesi

tersebut digredkan kepada lemah, sederhana dan teruk dan dianalisa dengan ujian Mann-Whitney U. Lesi perlemakan hati menujukkan perbezaan bermakna ($p<0.05$) berbanding dengan kongesi dan trombosis. Tanda klinikal dan parameter berkaitan dengan kes tersebut dicatatkan. Petanda klinikal yang dilihat pada kambing adalah pudar, depresi, pengertakan gigi dan terbaring. Parameter yang dipilih adalah kadar glukos darah dan asid betahidroksibutirat (BHBA) pada serum. Analisa frekuensi data menunjukkan petanda klinikal dilihat hanya pada kes ketosis. Kadar glukosa darah dan asid betahidroksibutirat dianalisis mengunakan ujian bebas-T. Paras glukosa darah untuk kes ketosis menunjukkan rendah yang bermakna lebih rendah ($p<0.05$) berbanding dengan kes kawalan. Asid betahidroksibutirat dalam kes ketosis pula menunjukkan paras lebih tinggi ($p<0.05$) berbanding kes kawalan. Analisa korelasi menunjukkan BHBA ada korelasi negatif dengan glukosa tetapi korelasi positif pada pemarkahan histologi. Pemarkahan histologi juga mempunyai korelasi negatif dengan paras glukosa. Kesimpulanya, perlemakan hati berkait rapat dengan ketosis terutamaya kes ketosis larat dengan petanda klinikal. Perbezaan penting ditemui antara kes ketosis dan biokimia serum, iaitu BHBA dan glukoas. Oleh itu, kambing yang mempunyai lesi perlemakan hati kemungkinan besar mengalami ketosis teruk dengan petanda klinikal.

Kata kunci: Ketosis, Perlemakan hati, Kongesi, Trombosis, asid betahidroksibutirat

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project

HISTOLOGICAL CHANGES IN THE LIVER OF GOATS WITH KETOSIS

By

Shogashan Balan

2017

Supervisor: Prof. Dr Mohd Zamri Saad

Co-Supervisors: Dr Hasliza Abu Hassim

The demand for goat milk and meat in Malaysia has been reported to increase due to rapid population growth, urbanisation together with changing consumer preference. However, the dairy production of small ruminants is not fulfilling the demand. One of the major factors that restrict the production is the metabolic disorder known as ketosis. This case-control study is done to identify the histopathological lesions in liver of goats clinically diagnosed with ketosis and to associate the histopathological changes with the clinical features. The study was carried out without knowing the clinical background of the cases. A total of 9 liver samples including control cases were fixed in 10% buffered formalin. Histological procedure was done with sectioning, processing and sectioning at 4µm before staining with haematoxylin and eosin. Histological examination was carried out and severity was graded using the

percentage of lesion distributed. The lesions found were fatty liver, congestion and thrombosis. These lesions were graded into mild, moderate and severe and further analysed using Mann-Whitney U test. The fatty liver lesion was statistically significant ($p<0.05$) compared to congestion and thrombosis, which were not significant ($p>0.05$). The clinical signs observed included dull, depression, teeth grinding and recumbency while the blood glucose and serum β- hydroxybutyric acid (BHBA) were measured. Frequency data analysis revealed that clinical signs were observed only in ketosis group. Blood glucose level of ketosis group was significantly ($p<0.05$) lower than control group while BHBA of ketosis group was significantly ($p <0.05$) higher than control group. Correlation analyse revealed that the BHBA have negative correlation with glucose but positive correlation with histological score, similarly histological scoring showed negative correlation with glucose level. In conclusion, the fatty liver is strongly associated with ketosis, particularly severe ketosis with clinical signs. Significant differences were detected between ketosis group and biochemistry parameters, which were BHBA and glucose. Therefore, there is high likely that a goat exhibiting fatty liver lesion also suffering from severe clinical ketosis with clinical signs.

Keywords: Ketosis, Fatty Liver, Congestion, Thrombosis, B- Hydroxybutyric acid

1.0 INTRODUCTION

Metabolic disorder is a disorder, which normally associated with nutritional aspects especially in ruminants. It is known as ketosis. Ketosis or pregnancy toxaemia is a metabolic disease that occurs during the last 3 to 6 weeks of gestation (Abba et al., 2015). Ketosis occurs when there is negative energy balance resulting from increased energy demand for rapid fetal growth in late gestation. This would lead to increased glucose required by the doe during late gestation and also for lactation. Doe would then have energy deficit giving rise to mobilization of lipid reserve. Ketone bodies such as acetone, acetoacetate and B-hydroxybutyrate surge up proportional to the sudden increased of nutritional demands of rapid fetus development.

Ketosis is divided into clinical and subclinical ketosis. Subclinical ketosis is defined as a condition of high serum ketone body concentration without clinical signs while clinical ketosis is presented with the clinical signs. Classical signs of ketosis include dullness, depression and inappetance. Ketosis is usually observed in small ruminants due to inefficient feeding management causing metabolic disorder. Ketosis resulted in huge economic loss to the farmers, thus posing a threat to the farm through the high rate of mortality (Cal et al., 2009). Frequently, affected does with high level of ketone body in blood exhibit sweet or foul smelling breath.

Therefore, the objectives of this study were:

1. to describe detailed histopathological changes in liver of goats with ketosis
2. to determine the correlation between the histological changes and clinical parameters of ketosis

2.0 LITERATURE REVIEW

2.1 Reproductive Cycle

Goats are polyestrous cycle, which means that they ovulate spontaneously. However, Albay et al. (2014) considered reproduction of goats to be seasonal and is associated with various factors such as latitude, climate, breed, physiological stage of the animal, presence of male and breeding system. Since breeding is seasonal, strategies to synchronize the oestrus cycle using hormonal treatment has been attempted for better manage of the breeding system.

Oestrous cycle, which comprises of proestrus, oestrus, metoestrus, anoestrus and dioestrus was recorded to be between 17 and 25 days (Fatet et al., 2011). Copulation occurs during oestrus and usually before ovulation. Depending on farm management whether artificial insemination or natural breeding, the inseminated sperm remains for up to 3 days and the implantation of embryo is observed at 18-22 days after oestrus (Fatet et al., 2011). The gestation period for goats is 150 days but many factors affect the success of pregnancy. Nutrition is the most important factor since it provides significant effect throughout the gestation period (Fatet et al., 2011). Therefore, metabolic disorder following inefficient feeding management is a common issue in does that leads to ketosis (Us & Offices, 2015).

2.2 Causal factor of ketosis

Pregnancy ketosis is commonly known as pregnancy toxaemia or twin lamb disease (Saun, 2015). It is a metabolic disease that occurs commonly in either goat or

sheep and is usually affects goats at the last 6 weeks of gestation period (Hassim et al., 2016). Since approximately 80% of the fetal growth occurs in the last 6 weeks of gestation, this increases the risk of getting ketosis (Lima et al, 2012). There are few major factors in the development of this disease, which include the presence of multiple foetuses, usually two or more. In addition, it involves undernourishment during late pregnancy. Furthermore, additional stress factors could affect the physiological state of doe such as severe weather, sudden change in feed or other disease or transportation (Saun et al., 2015; Hassim et al., 2016). Reason behind doe with multiple foetuses have higher risk is very much related to the importance of glucose in the pregnant goat. According to Lima et al. (2012), the energy requirements of the pregnant goat increases about 1.5 times when she carries one fetus but increases exponentially with the number of foetuses. High rate of fetal demand for energy therefore increases the glucose demand for the doe resulting in hyperglycaemia in which occurs due to effect of foetus on hepatic gluconeogenesis (Lima et al., 2012).

Undernourishment plays a role in ketosis, which is proven through experimentally induced pregnancy toxaemia either through a case of chronic under nourishment during pregnancy or an acute but nearly complete fast in well constant environmental and physiological stress (Cal et al., 2009; Us & Offices, 2015). Undernourishment factor also bring in together the fact that glucose plays an important role to provide sufficient energy for metabolism. Since ketosis associates with negative energy balance, providing high-energy concentrate taking into care the body condition score of the doe is very important to maintain the health status of the

animal. Does that are fat are also subjected to ketosis due to the fact that it causes physiological stress and might disrupt the metabolism (Cal et al., 2009).

Stress factor is a variable factor since it elevates and disrupts the homeostasis of the animal together with the latter factors. Besides that, stress factor changes accordingly with climate, breed and etc. Stress factor comes into place when it disturb the biochemistry profile of the pregnant doe (Barakat et al., 2007).

2.3 Diagnostic Method

There are several ways to diagnose ketosis either through clinical manifestations and/or biochemical parameters via haematological analysis (Hefnawy et al, 2011). A standard clinical examination comprising physical examination together with proper history are needed to diagnose ketosis cases. However, the definitive diagnosis is through blood β- hydroxybutyric acid (BHBA). According to Mahmoud (2014), ketone body is more stable than other ketone body such as acetone or acetoacetate. Diagnosing a ketosis case is difficult quantitatively because clinical ketosis rate value is extremely limited. Furthermore, there are subclinical and clinical ketoses. Subclinical ketosis poses a problem since affected does in most cases do not show any clinical signs (Bousquet et al., 2005; Oetzel, 2007). On top of that, ketosis cases are self-limiting provided the causal factor is removed and supplement is given. This goes the same for clinical and subclinical cases (Vsava et al, 2016).

2.4 Clinical Signs

Clinical signs commonly observed in clinical cases involve several systems, including the nervous and gastrointestinal systems. A research done by Vasava et al. (2016) revealed that the clinical findings observed in naturally affected goat with ketosis are anorexia (100%), recumbency (100.00%), lethargy (86.67%), opisthotonos (73.33%), dropped head (62.22%), periodic convulsion (57.78%), sweetish fruity odor from breath (51.11%), apparent blindness (42.22%), bloat (40.00%), grinding of teeth (37.78%), and frothy salivation (24.44%). The total number ketosis goats used were 45 goats (Vasava et al., 2016). Study has shown that some of the clinical signs have direct involvement of the central nervous system.

In clinical ketosis, the pathophysiology of this metabolic disorder starts with does isolating themselves from each other. Dullness and depression then becomes severe together with blindness in acute cases. Soon, the affected animal is recumbent with sternal first and lateral with little response to environmental stimuli. Eventually, the does become comatose and die (Oetzel, 2007; Saun, 2015). In a chronic ketosis case, other clinical signs observed include quivering, twitching of the ear, muzzle, eyelid and muscle spasms. Incoordination was also observed with chewing, teeth grinding or vigorous licking. Drooling saliva was also observed together with the typical sign of sweet acetone smell on the breath (Saun, 2015; Hassim et al., 2016).

2.4.2 Biochemical Parameter

Biochemical parameter that are used in diagnose ketosis include concentrations of glucose, calcium, liver enzyme AlT, AST and triglyceride. Besides that, blood concentration of D-3-hydroxybutyrate level BHBA is also used as a parameter to determine ketosis case. Beside those parameters, blood pH and urine test strip could also be used to detect ketone body (Albay et al., 2014). The significant parameters that have been proven useful are he BHBA, glucose concentration and serum lipids. Changes in blood BHBA, glucose concentration and serum lipids are interrelated and all these happen during ketosis.

During ketosis, the doe that uses up glucose in a state of deficit and to replace the glucose, adipocyte undergo lipolysis and produces more free fatty acid (FFA) in plasma. Due to the incomplete breakdown of fatty acid in the liver, the level of plasma BHBA concentration increases. Therefore, BHBA is used as a tool to detect does that suffer under nutrition an a sign of ketosis (Amirul et al., 2016). Study by Ramin et al. (2009) shows that, healthy animal has BHBA of below 0.8 mmol/l while concentration of more than 3.0 mmol/l indicates that the animal is suffering from pregnancy ketosis.

Glucose serves as an important parameter for pregnancy ketosis since foetus uses up glucose and metabolites from does and this would lead to carbohydrate, fat, and protein metabolisms (Lima et al., 2012). There are several other parameters that serve significant roles such as blood pH. Large quantity of metabolic substrate needed by the foetus overwhelm the does' body function resulting in hypoglycaemia. This hypoglycaemia could then lead to metabolic acidosis due to the fact that

hydrogen ions are being produced in the plasma with ketone bodies causing a drop in blood pH (keto-acidosis) (Lima et al., 2012).

2.6 Nutritional Feeding

Since this is a metabolic disorder, the appropriate action that could be taken to overcome the problem is to provide proper management and nutrition. Barakat (2007) concluded that total metabolic rate increases by at least 50% during late trimester. Increasing amount of feed exceeding the digestive capacity can be achieved by using grain as substitution for part of the feed. Since foetus is increasing in size, it would take up space in the digestive tract. Study by Lima et al, (2016) has shown that by incorporating concentrates, the case of ketosis could be reduced with no clinical manifestations. The concentrates should be fed daily during the last 6 weeks of pregnancy (Mahmoud & Azab, 2014).

3.0 MATERIALS AND METHODS

3.1 Study Animals

A total of 9 animals were induced to develop ketosis through feed alteration at the last month of trimester. The nine animals were then divided into 3 groups with group A kept as control unaffected, group B with mild feeding alteration and group C with significant feeding alteration. The animals were then kept for few months to develop ketosis and were slaughtered after parturition. The organs were collected and fixed in 10% buffered formalin.

3.2 Study Design

The investigation was done in a blinding method in which organs that were obtained was picked under the category of ketosis, however clinical background of the individual case was not revealed to eliminate biasness. The information were only provided after the process of histological examination and scoring was completed.

3.3 Histology Procedure

After fixation, liver organs are trimmed using a scalpel blade approximately 1cm to enable them to fit appropriately into cassette. The cassette was then stored in formalin before proceeding, which included dehydration by using alcohol and clearing by using xylene. Embedding was then done by infiltrating the cassette with paraffin wax. The wax allowed tissues to be sectioned at 4 μm using a microtome. The sectioned tissue was mounted on the slides and incubated at 37℃ overnight. The slide was stained with hematoxylin and eosin for histological examination.

As for staining, the slide was first dipped in xylene for 5 minutes, followed by 100% alcohol and 70% alcohol, each for 5 minutes. After that, the slides were rinsed with tap water and dipped in Haematoxylin for 5 minutes before being rinsed again under running tap water for 5 minutes. The slides were then dipped in Eosin for 1 minute before spraying 95% alcohol directly on the slides and then were rinsed with tap water for about 5 seconds. The slides were then sprayed with 95% alcohol for a last time and left to dry. Distyrene Plasticiser Xylene (DPX) a mounting medium was used to mount cover slip on the slide before proceeded for viewing.

3.4 Histological examination

Stained slides were viewed under the microscope for each organ. The whole slide was viewed before being narrowed down on the lesion in the slide. Concurrently, the lesions that were observed were noted. Then the type of lesion, the number lesion encountered and the number microscope field that have been viewed were recorded. Histological grading was then done. Lesions were graded according to score 1, when lesion expression was less than or equal to 20% throughout the slide field. Graded when lesion expression was 20%-60% throughout the total field and grade 3 when lesion expression was more than 60% throughout the slide.

3.5 Statistical Analysis

Data collected from histological scoring were analysed statistically using Mann-Whitney-U test, a non-parametric one-way analysis of variance. The clinical background was retrieved and frequency data was compared between ketosis and control group with clinical signs observed in goats. As for the biochemistry

parameter, independent t test was used to statistically analyse the difference of mean value of glucose (mmol/L) and BHBA (mmol/L) with ketosis and control group. Correlation analysis was also done to interpret the relationship between glucose and BHBA with ketosis and control group similarly with histological score.

All statistical tests were conducted with Statistical Package for the Social Sciences (SPSS) V. 22 at 95 % confidence level.

4.0 RESULTS

4.1 Histological Scoring

The lesions observed were fatty liver, congestion and thrombosis. They lesions were then graded and the results for the lesion scoring are shown in Table 1 and Figure 1. There was no statistical difference ($p>0.05$) between lesions of congestion and thrombosis but significant ($p < 0.05$) different when compared with fatty liver. All fatty liver lesions were graded as severe.

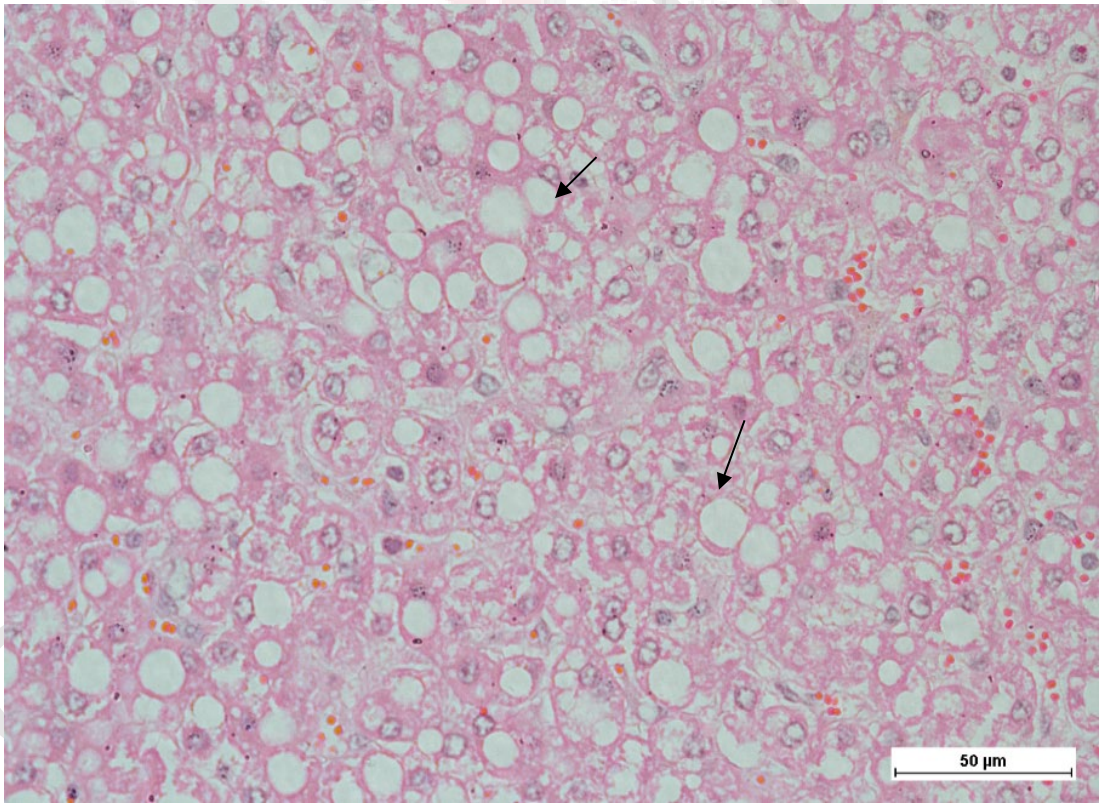

Figure 1: Arrows pointing fatty liver lesion

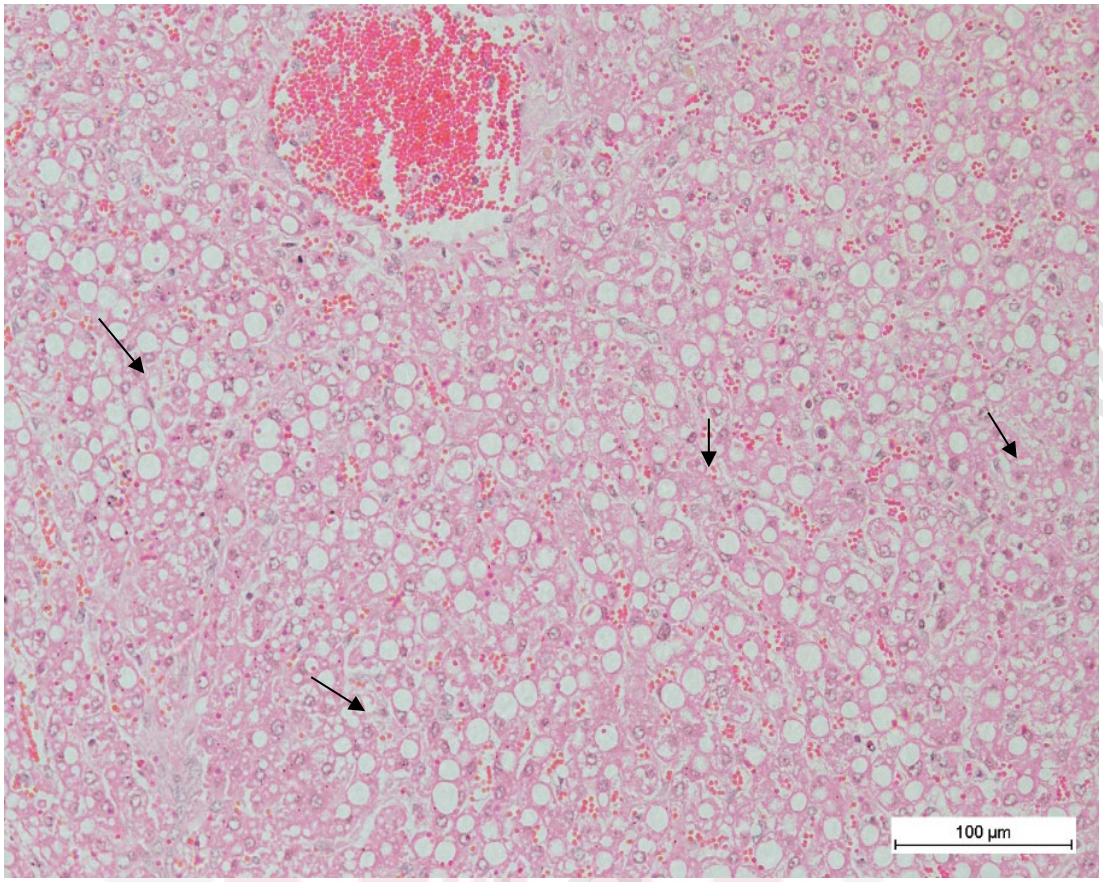

Figure 2 : Fatty liver infiltration under a lower magnification.

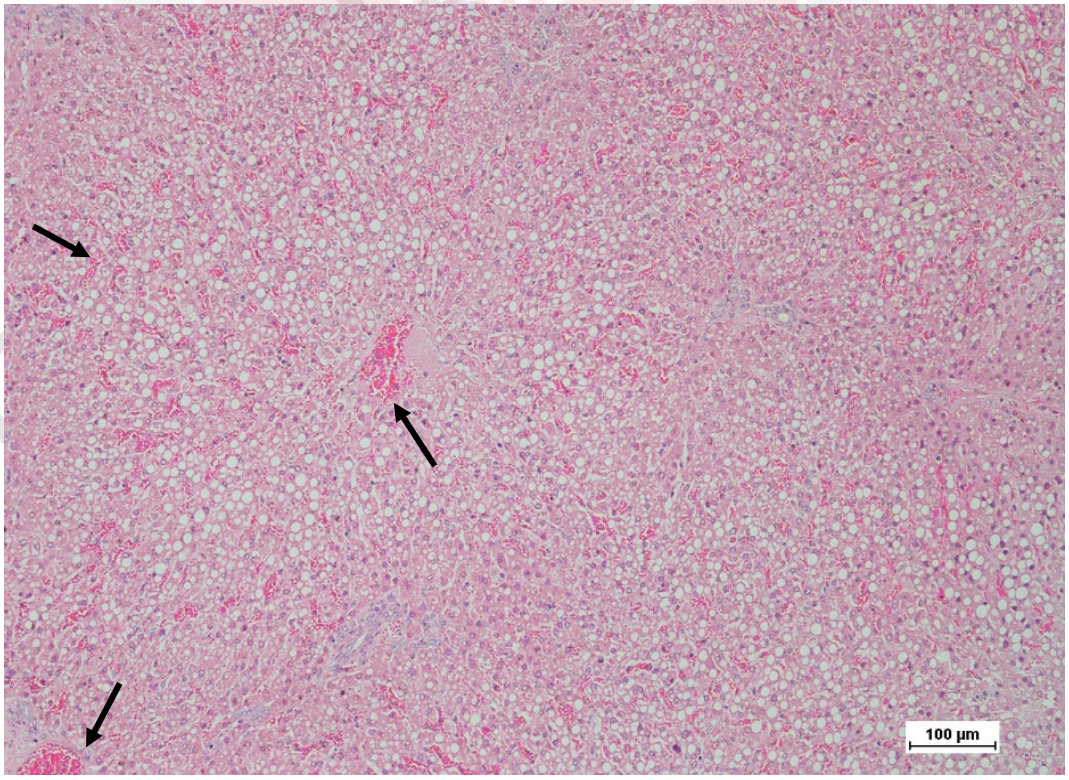

Figure 3: Congestion is indicated by the arrow.

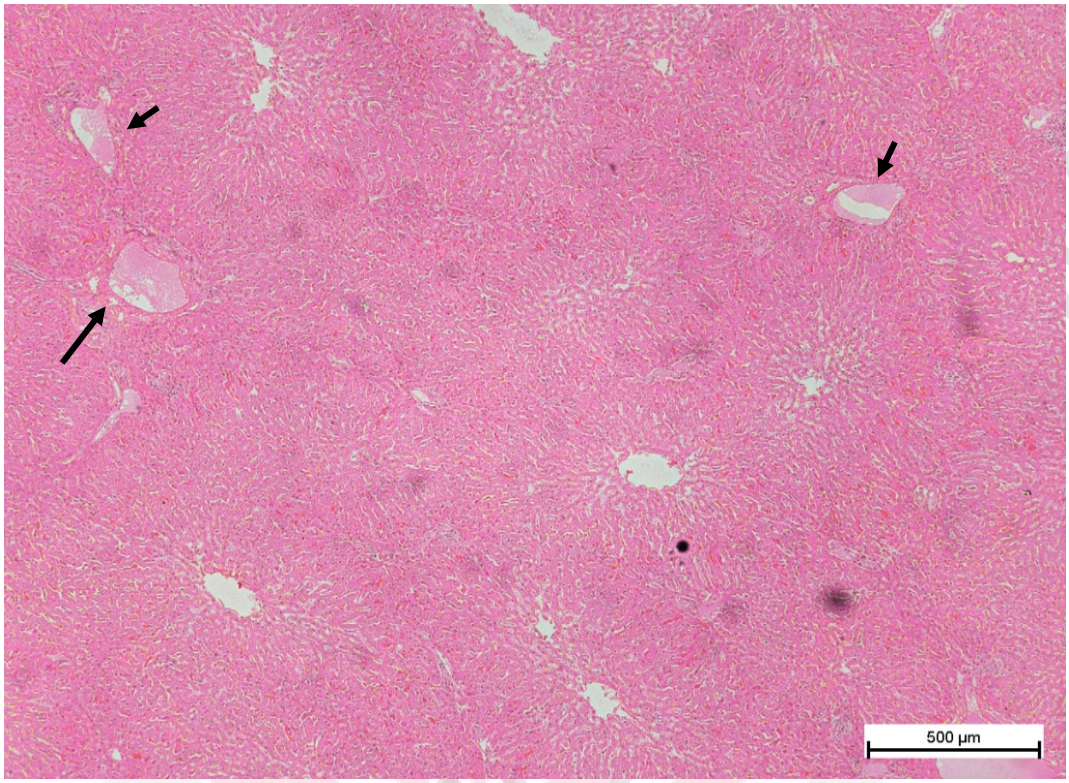

Figure 4 : Thrombosis is observed as blood clot in the blood vessel as indicated by the arrow

Table 1 : Histological lesions scoring of different cases

Sample	Liver				
	Congestion	**Inflammation**	**Necrosis**	**Fatty Liver**	**Thrombosis**
L1	1	0	0	3	2
L2	2	0	0	3	2
L3	1	0	0	3	1
L1a	1	0	0	3	1
L2a	1	0	0	3	1
L3a	1	0	0	3	1
L4	0	0	0	0	1
L5	1	0	0	0	1
L6	1	0	0	0	1

The results are scored based on lesion distribution

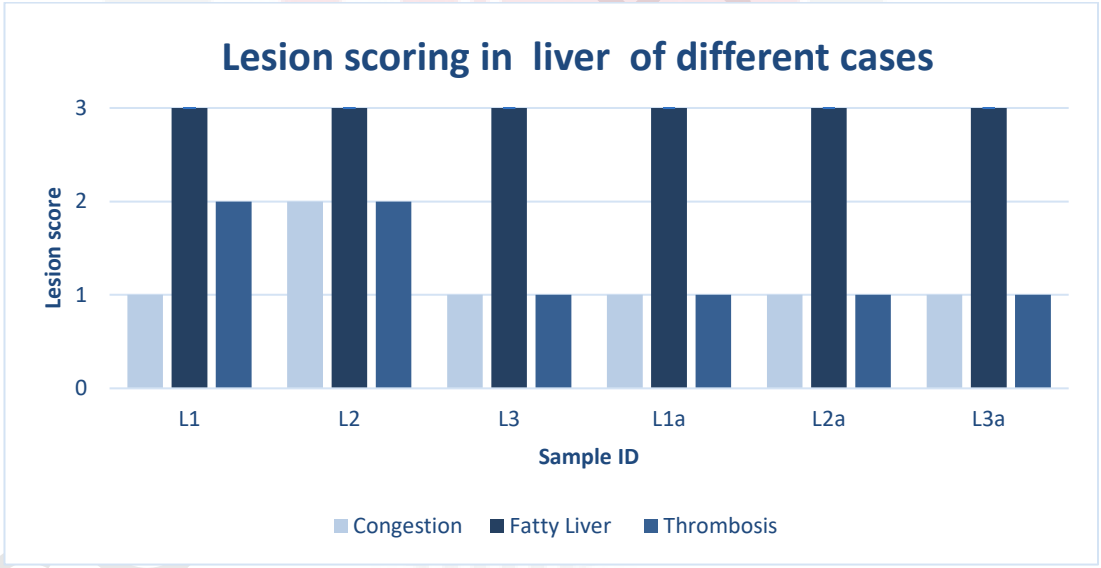

Figure 5 : Histological lesions scoring of different cases

4.2 Clinical signs and Blood parameter

Table 2 shows frequency of clinical signs between ketosis and control groups. All goats with ketosis in this study showed clinical features. The observed clinical signs included dullness, depression, teeth grinding and recumbency. The clinical signs were observed only among the ketosis group and the control group showed no clinical sign.

Table 2.0 The frequency of clinical signs with ketosis and control group

	All c/s	C/s except Recumbency	No clinical signs	Total
Ketosis	33.3	66.7	0	100
Control	0	0	100	100
Total	33.3	66.7	100	

Table 3 shows the mean value of glucose and BHBA for ketosis and control groups. Blood glucose level in ketosis group was significantly ($p<0.05$) lower than control group while BHBA of ketosis group was significantly ($p<0.05$) higher than control group. Correlation analysis revealed that BHBA showed negative correlation with glucose but positive correlation with histological score. Similarly, histological score showed a negative correlation with the glucose level.

Table 3.0: The Glucose and BHBA (mean ± SD) for ketosis and control group

	Glucose (mmol/L)	BHBA (mmol/L)
Ketosis	0.9 ± 0.1414 [a]	1.475 ± 0.224 [a]
Control	2.8 ± 0.4583 [b]	0.21 ± 0.0264 [b]

[ab]Value on the same column with different superscript indicate significant difference (P<0.05)

5.0 DISCUSSION

The lesion of fatty liver was highly associated with ketosis and was graded severe. Since the affected goats were undernourished at late pregnancy, the does' glucose homeostatic mechanisms were disrupted and therefore, triggerred the mobilization of body fat, which resulted in the release of non-esterified fatty acids (NEFAs) from adipose tissues. The NEFAs would then be taken up by liver and esterified. The product of esterification, which was triglyceride would then accumulate in the liver causing fatty liver (Cal et al., 2009). The lesions of congestion and thrombosis were not statistically significant. Studies have shown that pathophysiology of ketosis did not interfere with circulatory system. According to Albay et al. (2014), these lesions could also be a result of post-mortem change or artefacts during histological procedure.

Herdt (2000) also proved that fatty liver and ketosis are strongly associated in ketosis and interfere with gluconeogenic capacity. Thus, clinical signs were observed only in ketosis group. This finding shows the relationship between fatty liver and the clinical signs in a distinct comparison. Fatty liver associated with ketosis was strongly associated with the presence of clinical signs and therefore the clinical ketosis. Studies have shown that ketosis could be divided into clinical and subclinical kitoses. Clinical ketosis is the type that expressed clinical signs while the subclinical does not expressed the clinical signs (Oetzel, 2007).

The finding of clinical signs in clinical ketosis was similar with Hassim et al. (2016) whio indicate that common clinical signs are weakness, dull, depression, reduced appetite and in some severe cases neurological syndrome. In this study, the animal was slaughtered without allowing it to die from the course of the disease.

Barakat (2007) reveals that nervous signs are often manifested in chronic severe ketosis cases. Therefore, since this study involved a short duration during late pregnancy, nervous symdromme was not observed.

The biochemistry parameters such as blood glucose and BHBA were chosen due to the fact that they are commonly used parameters in clinically diagnosis of ketosis. The mean value of blood glucose level of ketosis goat was significantly ($p<0.05$) lower than control group with 0.9 ± 0.1414 compared to 2.8 ± 0.458. On the other hand, BHBA of ketosis group with 1.475 ± 0.224 and was statistically higher than control group 0.21 ± 0.0264. Thus, there was negative correlation between BHBA and glucose but positive correlation between BHBA and histological score. The histological score showed a negative correlation with the glucose level. The reduced blood glucose level during late gestation following lack of feed, particularly energy supply leads to lack of gluconeogenic capacity. Serum β-hydroxybutyric acid reading showed higher mean value due to the fact that pathophysiological pathway of ketosis includes ketone bodies released into plasma resulting in higher ketone bodies to compensate the energy supply. Besides that, serum HBA concentration has been identified to be a specific tool to detect does that are undernuourish, suffering severe undernutrition and pregnancy ketosis (Bousquet et al., 2005).

Similar study should be repeated with larger sample size and stratified groups for subclinical and clinical ketosis. This could be done to further understand the pathophysiology of subclinical ketosis and clinical ketosis to establish a gold standard for diagnosis of ketosis.

6.0 CONCLUSIONS

In conclusion, this study showed a strong association between ketosis cases with fatty liver infiltration lesion. However, no significant differences ($P>0.05$) were observed in congestion and thrombosis for ketosis group. These were attributed to various factors mainly due to presence of low lesion distribution in the liver. Fatty liver lesion is significantly associated with ketosis with various severity. This is because fatty liver occurs due to lipolysis that interferes with hepatic gluconeogenic capacity. Blood parameters such as BHBA and glucose could aid in clinical analysis of herds or individual goats during late pregnancy.

7.0 REFERENCES

Albay, M. K., Karakurum, M. C., Sahinduran, S., Sezer, K., Yildiz, R., & Buyukoglu, T. (2014). Selected serum biochemical parameters and acute phase protein levels in a herd of Saanen goats showing signs of pregnancy toxaemia. *Veterinarni Medicina*, *59*(7), 336–342.

Andrews, A. H., Holland-Howes, V. E., & Wilkinson, J. I. D. (1997). Naturally occurring pregnancy toxaemia in the ewe and treatment with recombinant bovine somatotropin. *Small Ruminant Research*, *23*(96), 191–197. https://doi.org/10.1016/S0921-4488(96)00912-1

Barakat, S. M. ., AL-Bhanasawi, N. ., Elazhari, G. ., & Bakhiet, A. . (2007). Clinical and Serobiochemical Studies on Naturally-Occurring Pregnancy Toxaemia in Shamia Goats. *Journal of Animal and Veterinary Advances*.

Bousquet, A., Brozos, C., Mavrogianni, V. S., Fthenakis, G. C., Doré, V., Dubuc, J., … Universities. (2005). Metabolic profiles in goat does in late pregnancy with and without subclinical pregnancy toxemia. *Journal of Dairy Science*, *22*(3), 404–405. https://doi.org/10.3906/vet-1412-25

Brown, S. (2001). The Science and Application of Hematoxylin and Eosin Staining. *Robert H. Lurie Comprehensive Cancer Center Northwestern University*, 1–5. Retrieved from http://www.feinberg.northwestern.edu/research/docs/cores/mhpl/HandE_troubleshooting.pdf

Cal, L., Borteiro, C., Benech, a., Rodas, E., Abreu, M. N., Cruz, J. C., & González Montaña, J. R. (2009). Histological changes of the liver and metabolic correlates in ewes with pregnancy toxemia. *Arquivo Brasileiro de Medicina Veterinária E Zootecnia*, *61*(2), 306–312. https://doi.org/10.1590/S0102-09352009000200004

Fasidi, I. O. (1994). Carbohydrate metabolism in, *51*, 211–213. https://doi.org/10.1136/bmj.2.3524.127

Fatet, A., Pellicer-Rubio, M. T., & Leboeuf, B. (2011). Reproductive cycle of goats. *Animal Reproduction Science*, *124*(3–4), 211–219. https://doi.org/10.1016/j.anireprosci.2010.08.029

Hefnawy, A. E., Shousha, S., & Youssef, S. (2011). Hematobiochemical Profile of Pregnant and Experimentally Pregnancy Toxemic Goats. *Journal of Basic and Applied Chemestry*, *1*(8), 65–69.

Gibson-Corley, K. N., Olivier, a K., & Meyerholz, D. K. (2013). Principles for valid histopathologic scoring in research. *Veterinary Pathology*, *50*(6), 1007–15. https://doi.org/10.1177/0300985813485099

Lima, M. S., Pascoal, R. A., & Stilwell, G. T. (2012). Glycaemia as a sign of the viability of the foetuses in the last days of gestation in dairy goats with pregnancy toxaemia. *Veterinary Ireland Journal*, *2*(5), 1–6. https://doi.org/10.1186/2046-0481-65-1

Mahmoud, S., & Azab, M. (2014). Regulation of glucose level during late pregnancy and onset of lactation in Egyptian female Baladi goats. *Small Ruminant Research*, *121*(2–3), 320–324. https://doi.org/10.1016/j.smallrumres.2014.06.009

Marutsova, V. (2015). Changes in blood enzyme activities in ewes with ketosis, *3*(6), 462–473.

Amirul, Mokrish, Lai, K. S., Zuki, A. B., & Hassim, H. A. (2016). Clinical, biochemical and histological changes during developement of pregnancy ketosis in goats, *28*, 1–6.

Oetzel, G. R. (2007). Herd-level ketosis: diagnosis and risk factors. *Preconference Seminar 7C :Dairy Herd Problem Investigation Strategies: Transition Cow Troubleshooting*, 67–91.

Poobathy, R., Sinniah, U. R., Rathinam, X., & Subramaniam, S. (2013). Histology and scanning electron microscopy observations of cryopreserved protocorm-like bodies of Dendrobium sonia-28. *Turkish Journal of Biology*, *37*(2), 191–198. https://doi.org/10.3906/biy-1206-22

Rawashdeh, O. (n.d.). B- ENERGY RELATED PROBLEMS 1 . KETOSIS OF RUMINANTS ACETONEMIA OF CATTLE :

Saun, R. Van. (n.d.). Common Nutritional and Metabolic Diseases of Goats, (1).

Us, C., & Offices, C. (2015). Pregnancy Toxemia (Ketosis) in Ewes and Does, (1), 1–3.

Vasava, P. R., Jani, R. G., Goswami, H. V., Rathwa, S. D., & Tandel, F. B. (2016). Studies on clinical signs and biochemical alteration in pregnancy toxemic goats. *Veterinary World*, *9*(8), 869–874. https://doi.org/10.14202/vetworld.2016.869-874