Serum Biochemical, Hormonal and Fatty Acid Profiles During the Late Gestation of Pregnancy Ketosis in Boer Cross Goats

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ABSTRAK

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Ketosis pada kehamilan merupakan salah satu penyakit metabolisme umum yang mempengaruhi produksi daging dan susu kambing. Penelitian ini menggunakan 16 ekor kambing betina bunting umur 80 hari. Sebanyak total 8 kambing betina dikategorikan sebagai kelompok kontrol (kambing betina bunting sehat) diberikan pakan rumput *Napier* dan konsentrat untuk kambing serta air secara *ad libitum*. Delapan kambing betina bunting lainnya dikategorikan ke dalam kelompok yang diberikan perlakuan yang menderita ketosis berdasarkan tanda-tanda klinis dan keberadaan badan keton pada urin. Sampel darah diperoleh dari semua kambing untuk keperluan analisis profil biokimia yaitu: glukosa, *Beta-hydroxybutyrate* (BHBA), asam lemak bebas (FFA), kalsium, elektrolit (sodium, potasium, klorida), enzim hati dan tingkat hormon (kortisol dan insulin). Setelah dilakukan penyembelihan 3 ekor kambing dari masing-masing kelompok, hatinya dikumpulkan dan kemudian dipelajari profil asam lemaknya. Hasil penelitian menunjukkan BHBA, FFA, kalsium, *transaminase aspartate* (AST), *transmirase glutamil gama* (GGT) dan hormon kortisol secara signifikan lebih tinggi pada kambing yang menderita ketosis dibandingkan dengan kontrol. Sementara itu, konsentrasi glukosa, sodium, potassium, klorida dan hormon insulin lebih rendah pada kambing yang menderita ketosis dibandingkan dengan kontrol. Selanjutnya, komposisi asam lemak dalam plasma darah kambing betina bunting dengan riwayat ketosis menunjukkan level yang lebih tinggi pada palmitat, asam stearik dan oleik, sementara itu pada hati menunjukkan nilai yang lebih tinggi untuk palmitat, asam *oleic* dan linoleik.

Kata Kunci: *Beta-Hydroxybutyrate*, Sampel Darah, Asam Lemak, Glukosa, Hati

ABSTRACT

Affan AA, Amirul FMA, Ghani AAA, Annas S, Zamri-Saad M, Hassim HA. 2018. Serum biochemical, hormonal and fatty acid profiles during the late gestation of pregnancy ketosis in Boer cross goats. JITV 23(4): 180-188. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1922

Pregnancy ketosis has been recognized as one of the common metabolic disease affecting goat's meat and milk production. For the present study, sixteen (n=16) individuals of pregnant does at day 80 of pregnancy had been used. A total of 8 does were categorized as control group (healthy pregnant goats), were fed on Napier grass and goat concentrate with water ad libitum, and another 8 does were considered as treatment group which categorized as ketosis based on the clinical signs and presence of ketone body in urine. Blood sample were collected from all goats for biochemical profiles analysis which were glucose, Beta-hydroxybutyrate (BHBA), free fatty acid (FFA), calcium, electrolytes (sodium, potassium, chloride), liver enzyme and hormonal levels (cortisol and insulin). Three does from each group were slaughtered and liver samples were collected for fatty acid profiles study. In this study, the BHBA, FFA, calcium, amino aspartate transferase (AST), gamma glutamyl transferase (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. Furthermore, the fatty acid composition in blood plasma of pregnant goat with ketosis showed higher level of palmitic, stearic and oleic acid, while in liver, palmitic, oleic and linoleic acid was found higher.

Key Words: Beta-Hydroxybutyrate, Blood Sample, Fatty Acid, Glucose, Liver

INTRODUCTION

There has been a sharp increase in the demand for goat's 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 preference (Bisant 2010). Nevertheless, scientifically based information on goat farm and industry in Malaysia is extremely limited to complement the sudden surge of

demand for goat's milk and meat. Among the urgent issues faced by goat farmers include the improper rearing management, feed and feeding, diseases and marketing (Jamaludin et al. 2012).

Pregnancy ketosis has been recognized as one of the common metabolic disease affecting goat's meat and milk production (Bani Ismail et al. 2008). The condition has been observed in Malaysia but not being reported. In Faculty of Veterinary Medicine, UPM, most of the farmer of goat's farm under the "Program Ladang Angkat Fakulti' has reported that pregnancy ketosis is the common metabolic disease in the farm with the morbidity and mortality rate of 5-10% and 80%, respectively (Syahirah et al. 2015). Indeed, this has resulted in poor performance of the goat which further caused high morbidity and mortality.

Pregnancy ketosis commonly occurs in does during the late stage of gestation. The main cause of pregnancy ketosis in goat is a disturbance of carbohydrate metabolism due to the high demands for glucose by the developing fetuses in the last trimester of pregnancy, resulting in negative energy balance (Schlumbohm & Harmeyer 2004). Clinical signs, often with a slow onset, are characterized by separating of the flock, anorexia, locomotion disorders and neurological signs like bilateral blindness, teeth grinding and muscle tremors. While morbidity is low, the reported mortality in absence of therapy for pregnancy ketosis is 90% (Schlumbohm & Harmeyer 2004). It is biochemically characterized by hypoinsulinemia, hypokalemia and high levels of ketone bodies found in the plasma of does diagnosed with pregnancy ketosis (Van Saun 2000). The serum glucose level decreases which causes the breakdown of triglycerides into fatty acids and glycerine mobilizes from the fat reserves. Due to the excessive degradation of fat reserves, β-hydroxybutyrate acid (BHBA), acetoacetate and acetone are produced. A variety of metabolic disorders may occur during ketosis from the examination of energy metabolism, oxidative stress clinical pathology and immune function.

There are a lot of studies related to pregnancy ketosis in goats. However, there are still lacks of information available on the diagnosis of clinical and serobiochemical study as well as hormonal status conducted on goats in Malaysia. Therefore, this study was carried out to assess the effect of pregnancy ketosis in Boer cross goats during late trimester on clinical and serobiochemical parameters, endocrine response (changes in the insulin and cortisol hormones) as well as changes in electrolytes concentration.

Thus, the objectives of this research were to determine the serum biochemical and hormonal profiles in does with pregnancy ketosis, and to assess the fatty acids composition in the blood and liver of does with pregnancy ketosis.

MATERIALS AND METHODS

Animal induction and selection of pregnancy ketosis

Sixteen pregnant goats that are in the last trimester were used in this study. All goats were divided randomly into control (n=8) and treatment (n=8) groups. The animals in control and treatment group were placed into different pen. The pregnant goats in control group were fed based on the standard feeding requirement which enough to meet nutrient requirement energy for pregnant goats (602.5 kJ ME/body weight kg/day) (NRC 2007). The amount of feed given to control group were 70% of Napier grass (dry matter: 10.57%, crude protein: 14.62%, crude fibre: 30.39%, energy: 8.30 MJ/kg) and 30% of goat concentrate (moisture: 13%, crude protein: 14.5%, crude fiber: 20%, dry matter: 87%, ash: 10%, crude fat: 6.65%, energy: 10.41%). Water was given *ad libitum* during experiment. For treatment group, they are induced with ketosis by restricted the energy intake for up to 50% of the daily requirement.

All procedures and techniques related to these does for research, and the experimental design were undertaken following the guidelines of the Research Policy of the Universiti Putra Malaysia. The animal utilization protocol and sampling procedure was approved by Ethical Committee for Animal Experiments, Universiti Putra Malaysia (UPM/IACUC/AUP-R071/2016).

Blood collection

All does were bled in the morning prior to feeding at 8.00 am and the blood sample were transported to the Physiology Laboratory by using ice boxes to avoid blood haemolysed. Blood sample were collected from jugular vein of both control and treatment goats with a proper restrain after clinical examination. The blood was collected by using needle size 21G and the sample was collected in Vacutainer and EDTA tubes. Plasma were harvested from EDTA tube by separating the blood using centrifuge (SH120-II) under 3000x rpm for 15 minutes. All plasma was stored at -80°C until further used. Furthermore, 1 ml of plasma was collected and kept in -20°C for fatty acid analysis.

Serobiochemical assay

Blood samples were used for serobiochemical analysis which was glucose, calcium, BHBA, sodium, chloride and potassium by using chemistry analyzer (Siemens Dimension Xpand Plus, USA). Commercial ELISA kits were used to determine BHBA and free fatty acids. All parameters were determined from

diluted plasma samples with buffer recommended in the commercially available ELISA kits.

Hormonal assays

Cortisol and insulin levels were determined by using plasma sample which represent in duplicate. Cortisol antiserum was obtained from Cortisol EIA Kit. The inter and intra assay coefficients was 6.7% and 1.1% respectively. All hormones levels were determined from diluted plasma samples with buffer recommended in the commercially available ELISA kits.

Liver collection

Three goats from each treatment and control group were slaughtered according to the Islamic traditions (Halal Slaughter Method) by severing the jugular veins, carotid arteries, trachea and esophagus (Ahmed et al. 2015). The liver organ was collected and cut into dice at randomly part on the liver after the slaughtering process. The samples were wrapped in aluminum foil, placed in polyvinyl chloride (PVC) plastic bags and stored at -80°C until further analysis for total FFA extraction.

Fatty acid profile determination

Chemical and glassware

All apparatus such as methylation tubes, screw caps and extraction tube stoppers were soaked for two hours in Decon 90 (Decon Laboratories Ltd., Sussex UK), before and after the fatty acid extractions. Then, all apparatus was soaked in distilled water overnight and rinsed again before oven dried at 60°C. Other glassware such as separating flasks, funnels, extraction tubes and round bottom flasks were washed in an automated laboratory glassware washer using acid and alkaline washes for about three hours. All chemicals, solvents and laboratory supplies used for total lipid extraction and preparation of fatty acid methyl esters (FAME) were of analytical grade. All chemicals and solvents were free from contamination with rubber or fat derivatives.

Total lipid extraction

Based on the method by Folch et al. (1957), the total fatty acids were extracted from plasma and liver tissues using chloroform: methanol 2 : 1. The blood plasma samples were extracted differently, where 1.0 to 2.0 ml of the sample was aspirated into 50 ml stoppered ground-glass extraction flask containing 40 ml of chloroform-methanol (2 : 1, v/v). The tube was gases with nitrogen, stoppered and then vigorously shakes before allowed to stand for 12 hours. The mixture containing the extracted fatty acids was filtered through a No. 1 Whatman paper (Whatman International Ltd., Maidstone, England) into a 250 ml separating flask using a filter funnel. The paper was washed with 10 ml of fresh chloroform-methanol (2 : 1, v/v). Ten ml of normal saline solution were added to facilitate phase separation. The mixture was then shaken vigorously for one minute and was left to stand for four hours. After this washing phase, the lower phase contained 86 parts chloroform: 14 parts methanol: 1part water (Shahidi & Wanasundara 1998). The upper phase would contain 3:48:47 parts of chloroform, methanol and water respectively. After complete separation at the end of fourth hour, the upper phase was discarded and lower phase was collected in a round bottom flask and evaporated by rotary evaporation (Heidolph GmBH, Germany) at 700°C. The total lipid extract was then immediately transferred to a capped methylation tube by re-diluting it with five ml fresh chloroform-methanol (2 : 1, v/v).

Furthermore, for liver sample, about 1 g of tissues were cut and homogenized in 40 ml of chloroform:methanol (2:1, v/v) using an Ultra-Turrax T5 FU homogenizer (IKA Analysentechnik GmBH, Germany) without thawing (Shahidi & Wanasundara 1998) within a 50 ml stoppered ground-glass extraction tube.

Preparation of fatty acid methyl esters

Transmethylation of the extracted fatty acids to fatty acid methyl esters (FAME) were carried out using 14% methanolic boron trifluoride (BF3) according to methods in AOAC (2007). The internal standard, heneicosanoic acid (21 : 0) (Sigma Chemical Co., St. Louis, Missouri, USA) was added to each sample prior to transmethylation to determine the individual fatty acid concentrations within the samples.

The sample extract was then dried on a heating block (40°C) under a constant and mild flow of pure nitrogen gas. After drying the chloroform: methanol, two ml of 0.66 N methanolic potassium hydroxide (R & M Chemicals, Essex, U.K.) was added to saponify the lipid sample. The methylation tube was flushed with nitrogen, stoppered and heated in a boiling water bath for 10 min with occasional shaking. After the mixture had cooled down, two ml of 14 % boron trifluoride (BF3) (Sigma Chemical Co., St. Louis, Missouri, USA) were added to initiate trans-esterification and the mixture was reheated for 20 min in a boiling water bath (Rajion 1985).

After cooling, four ml of distilled water and four ml of petroleum ether (boiling point 40-60°C) were added and the mixture was vortexed for 60 sec. The mixture was then centrifuged at 1500G for 10 minutes to

increase phase separation. The upper petroleum phase was transferred to another test tube using pasture capillary pipettes and washed with one ml of distilled water to remove residual BF3. The upper phase from this test tube was then transferred accurately again to a second test tube and 0.5 g anhydrous sodium sulphate (R & M Chemicals, Essex, U.K.) was added to dry the sample and remove any residual water. Finally, the petroleum ether containing the FAME was transferred to a four ml screw-capped vial (Kimble Glass Inc., USA), flushed with nitrogen, closed tightly and stored at 4°C until analysis by gas-liquid chromatography.

Gas liquid chromatography

The methyl esters were quantified by GC using a 30m x 0.25mm ID (0.20 µm film thickness). One microlitre was injected by an auto sampler into the chromatograph, equipped with a split/splitless injector and a FID detector. High purity hydrogen (Dominick Hunter, Parker Hannifin ltd, UK) and compressed air (Malaysian Oxygen Bhd., Malaysia) were used for the flame ionization detector in the gas-liquid chromatograph. The injector temperature was programmed at 250°C and the detector temperature was 300°C. The column temperature program initiated runs at 100°C, for 2 min, warmed to 170°C at 10°C /min, held for 2 min, warmed to 220°C at 7.5°C /min, and then held for 20 min to facilitate optimal separation.

Identification of fatty acids was carried out by comparing relative FAME peak retention times of samples to standards obtained from Sigma (St. Louis, MO, USA). Both gravimetric calculations and normalised percentage (%) of total Fatty acid (FA) were used to determine the differences in FA composition. Peak areas were determined and calibrated using a personal computer integrator (Hewlett-Packard, Avondale, PA). Automatic expression of the peak areas as absolute and percentage amount of a detected fatty acid was obtained with a programmed PC under Microsoft Excel 2000 (Microsoft Corp., Redmond, USA).

The amount of fatty acid is determined by their relative proportions (normalized percentages to total fatty acids) (Huerta-Leidenz et al. 1991; Alfaia et al. 2006). The normalised percentages describe the interactive and comparable relationship among fatty acids regarding lipid quality, while the gravimetric concentration can show the actual amount of fatty acids in tissues, which relates to nutritional intake.

Data analysis

All the analysis was done with SPSS version 22.0 software. The data was analyzed using independent T-test to compare between means of different groups. Data represent as a mean were considered significantly different when $P<0.05$.

RESULTS AND DISCUSSION

All goats in treatment group showed more than three clinical signs of pregnancy ketosis which appear at 3 to 6 days of induction with pregnancy ketosis in the form of anorexia, dullness, teeth grinding and weakness. However, for control group which were healthy pregnant goats, there were no clinical signs shown throughout the experiment.

Pregnancy ketoses in goats are due to the animals failed to meet the energy demand for fetal unit. The diagnosis of pregnancy ketosis is based on the physical signs, hematological and biochemical measures. Based on the observation in treatment group, depression and teeth grinding were the first clinical signs. A study by Barakat et al. (2007) reported that anorexia and sternal recumbence were observed during ketosis state of pregnancy goat. Indeed, the clinical signs that showed by the pregnancy ketosis goats in this study are similar with the previous study. These clinical signs are caused by negative energy balance due to the lack of energy which is low energy intake. As a result, it will lead the animals become recumbence and lack of energy to support daily activities.

In regard to biochemical analysis, the value of glucose, BHBA, FFA, calcium, AST, GGT and cortisol hormone were significantly higher in treatment group ($P<0.05$) than those of control as shown in Table 1 and Figure 1. However, insulin hormone was shown significantly lower in concentration in treatment group ($P<0.05$) when compared to control (Figure 2). The values of sodium, potassium, and chloride were not significantly different between treatment groups than those of control (Table 1).

For serobiochemical profiles, it was characterized as subclinical pregnancy ketosis based on the FFA result. According to Brozos et al. (2011). The level of FFA in the blood can be categorized in three part which are; (healthy pregnant goats = 0.00 – 0.79 mmol/L; subclinical pregnancy ketosis = 0.80 – 0.99 mmol/L; clinical pregnancy ketosis = 1.00 – 3.00 mmol/L). In present study, there were elevation of FFA and BHBA during decrement of glucose value in ketosis goats. It is similar with the study by Schlumbohm & Harmeyer (2003), which stated that increment of BHBA values resulted in a significant drop of glucose turnover. According to Barakat et al. (2007), the increasing of FFA concentration in plasma of affected goats could be attributed to the increasing mobilization of fatty acids from the adipose tissue in response to a requirement for endogenous substrate for energy production during pregnancy. In other words, the elevation level of FFA

due to ketosis inside plasma reflected the formation of fatty liver in pregnant does.

Furthermore, elevation of BHBA level also able to inhibit the hepatic gluconeogenesis, which could increase the maternal hypoglycaemia (Schlumbohm & Harmeyer 2004). Besides, the study showed that the glucose level for ketotic goats was lower than healthy goats. This is because glucose is the main source of energy supply in the body. Indeed, glucose level shows relation to the animal status which values related falling with a negative energy balance.

In this study, there was declining of calcium level in the blood (hypocalcemia) with slightly locomotion disturbance in the pregnancy ketosis goats. A study by Henze et al. (1998) showed that elevation of ketone bodies and free fatty acid could lead to significant decreased in plasma calcium concentration. This is because elevation concentration of calcium in the circulation of pregnancy does is required for skeletal development in growing fetus especially during the last trimester (Schlumbohm & Harmeyer 2003).

Table 1. Serobiochemical profiles in control vs treatment groups

Parameters	Control Group Healthy Pregnant Goat	Treatment Group Pregnancy Ketosis Goat	Standard Normal Range
Glucose (mmol/L)	2.88±0.11[a]	1.13±0.33[b]	2.70-4.20
Beta- Hydroxybutyrate (mmol/L)	0.13±0.01[a]	1.37±0.11[b]	0.10-0.70
Free Fatty Acid (mmol/L)	0.18±0.02[a]	0.98±0.05[b]	0.00-0.79
Calcium (mmol/L)	2.24±0.07[a]	1.83±0.06[b]	2.20-3.20
Sodium (mmol/L)	143.60±0.82[a]	143.80±1.15[a]	142.00-155.00
Chloride (mmol/L)	104.80±0.74[a]	103.60±2.31[a]	99.00-100.00
Potassium (mmol/L)	4.54±0.09[a]	4.18±0.05[a]	3.50-6.70
Amino aspartate transferase (U/L)	87.29±2.46[a]	104.00±0.09[b]	50.00-100.00
Gamma glutamyl transferase (U/L)	39.00±1.67[a]	48.06±1.87[b]	30.00-50.00

Note: (Means±SEM) in goats with pregnancy ketosis (n=8) and control goat (n=8); [a,b]different letters between columns denote significance (P<0.05)

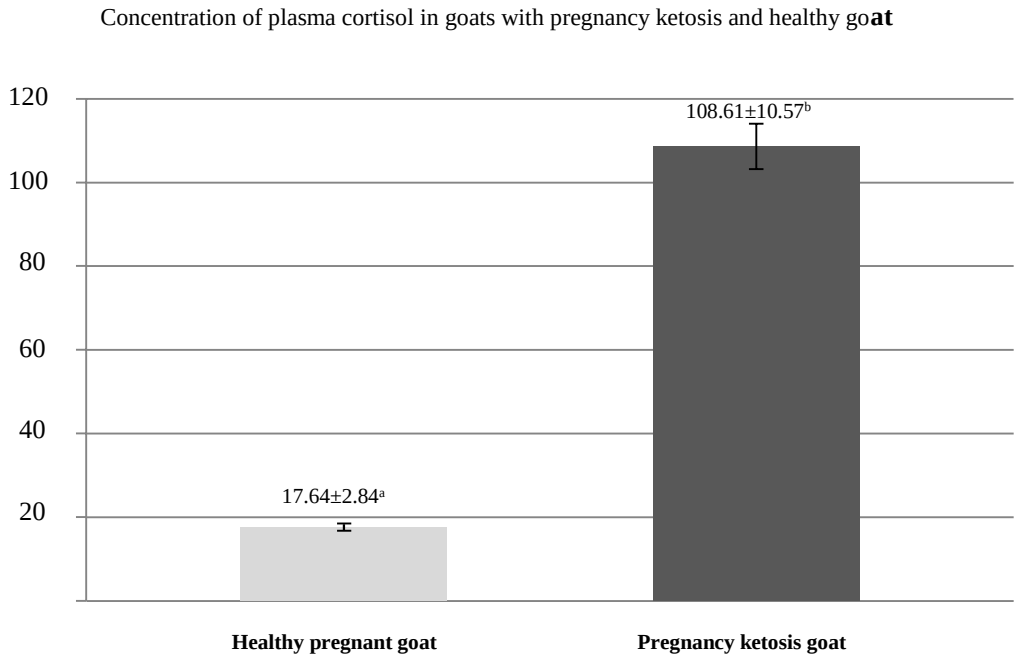

Figure 1. Concentration of plasma cortisol hormone in goats with pregnancy ketosis and healthy goat.

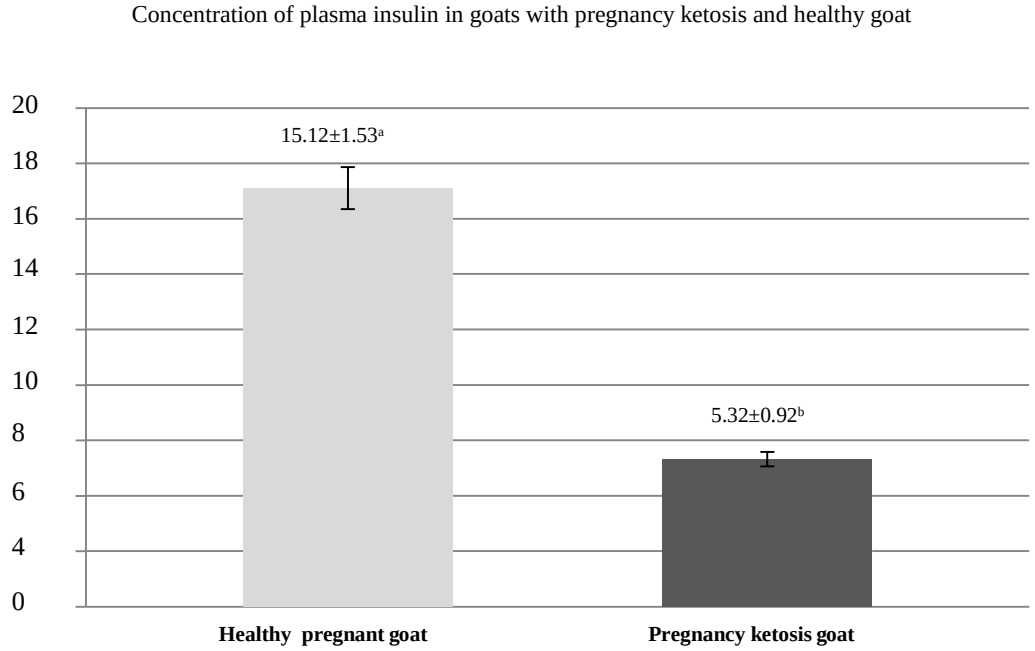

Figure 2. Concentration of plasma insulin hormone in goats with pregnancy ketosis and healthy goat.

In treatment group, there were elevation of AST and GGT concentration recorded, which indicated that there was liver damage in ruminant (Barakat et al. 2007). According to study by Abd-Elghany et al. (2007), it was reported that significant elevation of AST and GGT levels could throw some diagnosis on the hepatic influence of caprine pregnancy ketosis which attributed to fat mobilization and associated with an increasing of FFA in plasma.

According to Halford & Sanson (1983), electrolytes in the blood of pregnancy ketosis in ewes were decreased in concentration; however, no differences of electrolyte concentration were observed between treatment and control group in this study. It is indicated that there could be a tremor in the electrolytes and some minerals which may be essence to dehydration, stress of starvation, and kidney involvement in the pathogenesis of goat pregnancy ketosis (Judith & Thomas 1988). Based on the result, there is high level of cortisol in treatment group compared to control group. These findings are supported by Moyes et al. (2009) which reported that higher serum cortisol concentration present in negative energy balance ruminant animal as compared to animal with an *ad libitum* diet. As mentioned by Kristina et al. (2010), cortisol is one of the hormones used as an indicator of stress and pain during ketosis state in ruminant.

Apart from that, insulin is one of important hormones in regulation of energy homeostasis which can alter fatty acid release and ketogenic process (Abd-Elghany et al. 2010). Insulin secretion plays roles in regulating the utilization of ketone bodies, and uptake of BHBA as well as acetate. In this study, the lower insulin value was observed in treatment group (5.32±0.92 μU/ml) as compared to the control (15.12±1.53 μU/ml). The result indicated that significant decrement of insulin values in treatment group may refer to the fact that insulin may have potential in inhibitory role of ketogenesis (Abd-Elghany et al. 2010). As mentioned by Ganong (2007), rising rate of lipolysis and production of ketone bodies are caused by deficiency of insulin. This theory is in line with the finding of this study which FFA and BHBA concentration was high while the insulin level was low during ketosis state. Apart from that, insulin is one of important hormones in regulation of energy homeostasis which can alter fatty acid release and ketogenic process (Abd-Elghany et al. 2010). Insulin secretion plays roles in regulating the utilization of ketone bodies, and uptake of BHBA as well as acetate.

Fatty acid composition in blood

The composition of fatty acids in blood was tabulated in Table 2. In general, most of the fatty acids composition in treatment group showed a significant higher concentration as compared to control group except for alpha linoleic acid (C18:3) and docosapentanoic acid (C22:5) (Table 2). Among these fatty acids, the palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and docosahexanoic acid (C22:6) in treatment group showed 3 to 4 fold high concentration as compared to the control group (palmitic acid: 14.62±0.98mg/dL vs 5.33±0.87 mg/dL, stearic acid: 16.18±1.17mg/dL vs 5.83±1.12 mg/dL, oleic acid: 27.95±1.88 mg/dL vs 6.90±1.18 mg/dL, linoleic acid: 13.58±1.19 mg/dL vs 6.26±1.47 mg/dL and docosahexanoic acid: 13.97±1.86 vs 5.99±1.23 mg/dL) (Table 2).

Fatty acid composition in liver

Most of the fatty acids composition in the liver of treatment group showed a significant high concentration as compared to the control group, except for eicosapentanoic acid (C20:5) (Table 3). The higher composition of fatty acids in the liver for both groups was palmitic acid (C16:0), linoleic acid (C18:2) and oleic acid (C18:1) while the lower composition of fatty acids were Alpha Linoleic acid (C18:3) and Docosapentanoic acid (C22:5).

In the current study, results showed that fatty acids compositions in treatment group were high as compared to the control. This could be due to different adaption of physiological state and body needs particularly of energy demand in both groups. Indeed, high concentration of fatty acids in blood observed during ketosis condition may cause by lipolysis occurred in adipocyte cell. A study by Nogalski et al. (2012), reported that most of fatty acids present in blood during negative energy balance state caused by lipolysis originated from subcutaneous adipose tissue.

Based on the results, there are three types of fatty acids that showed higher concentration in treatment group which are palmitic acid (C`16:0), stearic acid (C18:0), and oleic acid (C18:1). These three fatty acids showed an increment up to 3 to 4 folds as compared to the control, which indicating that the lipolysis process has been occurred extensively during inadequate of energy condition in pregnancy ketosis. Based on the previous study by Mansbridge & Blake (1997), those fatty acids were also found increased in the blood stream which arise from a source other than *de novo* synthesis in the liver of ruminant.

In this current study, fatty acids composition in the liver of treatment group was also found higher as compared to control group. Indeed, some of the fatty acids such as palmitic acid (C 16:0), oleic acid (C 18:1), and linoleic acid (C 18:2) showed an increment up to 5 – 7 folds in comparison to control animals. The increment of these fatty acids concentration in liver could also be observed in the blood as discussed earlier. The sudden increase in the concentration of fatty acids content in the blood as well as in the liver in pregnancy ketosis goat could be attributed to the increased mobilization of fatty acids from adipocyte and undergo oxidization process in liver, responses to an increased requirement for endogenous substrate for energy

Table 2. Fatty acid composition in the blood of control and treatment group

Fatty acid composition		Control Group (mg/dL) (n=8)	Treatment Group (mg/dL) (n=3)	Changes
Palmitic acid	C16:0	5.33±0.87[a]	14.62±0.98[b]	↑
Palmitoleic acid	C16:1	0.45±0.08[a]	0.83±0.10[b]	↑
Stearic acid	C18:0	5.83±1.12[a]	16.18±1.17[b]	↑
Oleic acid	C18:1	6.90±1.18[a]	27.95±1.88[b]	↑
Linoleic acid	C18:2	6.26±1.47[a]	13.58±1.19[b]	↑
Alpha Linoleic acid	C18:3	0.42±0.08[a]	0.51±0.07[a]	▬
Arachidonic acid	C20:4	1.62±0.28[a]	3.49±0.29[b]	↑
Eicosapentanoic acid	C20:5	0.25±0.05[a]	0.82±0.17[b]	↑
Docosapentanoic acid	C22:5	0.41±0.06[a]	0.42±0.08[a]	▬
Docosahexanoic acid	C22:6	5.99±1.23[a]	13.97±1.86[b]	↑

Notes: All value express in (means±SE) mg/dL
Control group = healthy pregnant goat
Treatment group = pregnancy ketosis goat
a,b different letters between columns denote significance (P<0.05)
↑ = increasing of fatty acid composition in the blood
▬ = no changes of fatty acid composition in the blood

Table 3. Fatty acid composition in the liver of control and treatment group

Fatty acid composition		Control Group (mg/dL) (n=8)	Treatment Group (mg/dL) (n=3)	Changes
Palmitic Acid	C16:0	39.20±3.58[a]	207.00±8.91[b]	↑
Palmitoleic Acid	C16:1	4.49±1.30[a]	11.02±0.60[b]	↑
Stearic Acid	C18:0	50.66±2.15[a]	92.10±14.26[b]	↑
Oleic Acid	C18:1	62.65±4.57[a]	454.20±19.32[b]	↑
Linoleic Acid	C18:2	28.49±1.88[a]	130.60±6.21[b]	↑
Alpha Linoleic Acid	C18:3	0.85±0.16[a]	2.89±0.25[b]	↑
Arachidonic Acid	C20:4	26.43±1.65[a]	46.86±1.82[b]	↑
Eicosapentanoic Acid	C20:5	2.26±0.12[a]	6.63±2.05[b]	↑
Docosapentanoic Acid	C22:5	1.82±0.12[a]	3.42±0.28[b]	↑
Docosahexanoic Acid	C22:6	12.31±1.78[a]	75.67±5.06[b]	↑

Notes: All value express in (means±SE) mg/dL
Control group = healthy pregnant goat
Treatment group = pregnancy ketosis goat
a,b different letters between columns denote significance ($P<0.05$)
↑= increasing of fatty acid composition in the liver

production during last trimester of pregnancy (Noble et al. 1971). In addition, during ketosis state, high amount of fatty acid delivered into mitochondria in the liver and fatty acid oxidation-derived acetyl –CoA is diverted from Kreb cycle to ketogenesis which generating acetoacetate (AcAc) and BHBA and releasing back to blood stream (Schugar & Crawford 2012). Therefore, it concluded that lipolysis is the main cause that triggers gluconeogenesis and ketogenesis occurred during pregnancy ketosis.

CONCLUSION

In conclusion, the severity of pregnancy ketosis in goat may cause changes in serobiochemical and hormonal profiles. Pregnancy ketosis affected the liver organ when enzyme AST and GGT level were increased in the blood analysis. Moreover, muscular imbalance and weakness in pregnancy ketosis goat appeared concurrently with declining of calcium level in blood. Meanwhile, no changes in electrolytes levels and mineral balance recorded during ketosis state in pregnancy goat. Furthermore, plasma free fatty acids levels were observed to be higher in concentration of blood as well as in liver of pregnancy ketosis does. The elevation of free fatty acid was found during high level of BHBA and low glucose level in blood. Stress hormone, cortisol was found to be significantly elevated in pregnancy ketosis does which proved that pregnancy ketosis is a stressful condition that may affect the productivity of meat and milk production. Meanwhile, insulin hormone was found lower in concentration due to low of glucose levels during ketosis condition.

REFERENCES

Abd-Elghany H, Saad S, Seham Y. 2011. Hematobiochemical profile of pregnant and experimentally pregnancy toxemic goats. J Basic Appl Chem. 1:65-69.

Abd-Elghany H, Seham Y, Saad S. 2010. Some Immunohormonal changes in experimentally pregnant toxemic goats. Vet Med Int. 2010:5.

Ahmed HO, Zaiton H, Mohd NAM. 2015. Effect of slaughtering methods on meat quality indicators,chemical changes and microbiological quality of broiler chicken meat during refrigerated storage. J Agric Vet Sci. 8:12-17.

Alfaia CM, Ribeiro SS, Lourenco MRA, Quaresma MAG, Martins SIV, Portugal AP, Fontes CMGA, Bessa RJB, Castro MLF, Prates, JAM. 2006. Fatty acid composition, conjugated linoleic acid isomers and cholesterol in beef from crossbreed bullocks intensively produced and from Alentejana purebred bullocks reared according to Carnalentejana PDO specifications. Meat Sci. 72:425-436.

[AOAC] Association of Official Analytical Chemists. 2007. Official Methods of Analysis of AOAC International. 18th ed. Gaithersburg (UK): AOAC International.

Bani Ismail ZA, Al-Majali AM, Amireh F, Al-Rawashdeh OF. 2009. Metabolic profiles in goat do in late pregnancy with and without subclinical pregnancy toxemia. Vet Clin Pathol. 37:434-437.

Barakat SEM, Al-Bhanasawi NM, Elazhari GAO, Bakhiet. 2007. Clinical and Serobiochemical Studies on Naturally Occuring Pregnancy Toxemia in Shamia Goats. J Anim Vet Adv. 6:768-772.

Bisant K. 2010. Consumer preference for goat meat in Malaysia: Market opportunities and potential. J Agribusiness Marketing. 3:40-55.

Brozos C, Mavrogianni VS, Fthenakis GC. 2011. Treatment and Control of Peri-Parturient Metabolic Diseases: Pregnancy Toxemia, Hypocalcemia, Hypomagnesemia. Vet Clin North Am: Food Anim Pract. 27:105-113.

Folch J, Lees M, Sloane-Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 226:497-509.

Ganong WF. 2007. Review of medical physiology. 22nd ed. New York (USA): McGraw Hill.

Halford DM, Sanson DW. 1983. Serum profiles determined during ovine pregnancy toxemia. Agric Pract. 4:27-33.

Henze P, Bickhardt K, Fuhrmann H, Sallmann HP. 1998. Spontaneous pregnancy toxaemia (ketosis) in sheep and the role of insulin. J Vet Medic Series A. 45:255-266.

Huerta-Leidenz NO, Cross HR, Luntt DK, Pelton LS, Savell JW, Smith SB. 1991. Growth, carcass traits, and fatty acid profiles of adipose tissues from steers fed whole cottonseed. J Anim Sci. 69:3665-3672.

Jamaludin AA, Idris K, Roslaini R. 2012. Challenges facing dairy goat farmers in Malaysia. Abdullah R, Omar MA, Makkar H, Otte J, Rajion MA, Alimon AR, Boo LJ, Kam HA, Li CW, editors. Proceeding of 1st Asia Goat Conference. Kuala Lumpur (Malays): Universiti Putra Malaysia and The Food and Agricultural Organization of the United Nations. p. 11-13.

Judith VM, Thomas HH. 1988. Pregnancy toxemia and ketosis in ewes and does. Vet Clin North Am**:** Food Anim Pract. 4:307-315.

Kristina BF, Orjan AL, Bernt VJ. 2010. Low cortisol levels in blood from dairy cows with ketosis: a field study. Acta Veterinaria Scandinavica. 52:31.

Mansbridge RJ, Blake JS. 1997. Nutritional factors affecting the fatty acid composition of bovine milk. Br J Nutr. 78:37-47.

Moyes KM, Drackley JK, Salak-Johnson JL, Morin DE, Hope JC, Loor JJ. 2009. Dietary induced negative energy balance has minimal effects on innate immunity during a *Streptococcus uberis* mastitis challenge in dairy cows during midlactation**.** J Dairy Sci. 92:4301-4316.

[NRC] National Research Council. 2007. Nutrient Requirements of Small Ruminants: sheep, goats, cervids, and New World camelids. Washington DC (USA): National Research Council, National Academies Press.

Noble RC, Steel W, Moore JH. 1971. The plasma lipids of the ewe during pregnancy and lactation. Res Vet Sci. 12:47-53.

Nogalski Z, Wroński M, Sobczuk-Szul M, Mochol M, Pogorzelska P. 2012. The Effect of Body Energy Reserve Mobilization on the Fatty Acid Profile of milk in High-yielding Cows. Asian-Austr J Anim Sci. 25:1712-1720.

Rajion MA. 1985. Essential fatty acid metabolism in the fetal and newborn lamb (Thesis). [Melbourne (Australia)]: The University of Melbourne. 315p.

Schlumbohm C, Harmeyer J. 2003. Hypocalcaemia reduces endogenous glucose production in hyperketonemic sheep. J Dairy Sci. 68:1953-1962.

Schlumbohm C, Harmeyer J. 2004. Hyperketonemia impairs glucose metabolism in pregnant ewes. J Dairy Sci. 87:350-358.

Shahidi F, Wanasundara JPD. 1998. Extraction and Analysis of Lipids. In: Akoh CC, Min DB, editors. Food lipids: Chemistry, nutrition, and biotechnology. New York (USA): Marcel Dekker Inc. 914p.

Van Saun RJ. 2000. Pregnancy toxaweemia in a flock of sheep. J Am Vet Medic Assoc. 217:1536-1539.