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Review Articles

CLINICAL, BIOCHEMICAL AND HISTOLOGICAL CHANGES DURING DEVELOPEMENT OF PREGNANCY KETOSIS IN GOATS

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SUMMARY

Thin does or very over-conditioned does and does carrying with multiple pregnancies are more likely to develop pregnancy ketosis (pregnancy toxaemia). Pregnancy ketosis is one of the metabolic disorders in ruminant animal such as does and ewes during the last weeks of pregnancy (5 – 6 weeks before gestation) and beside high ketone body levels characterised by free fatty acids levels (FFA). These FFA are derived from the adipose tissue. The ability of adipocytes to respond to changes in the energy balance, by secretion of FFA makes this parameter a main indicator for disorder that coincide with changes in energy balance. It is biochemically and endocrinologically characterized by ketosis, hypoinsulinemia and an elevation of B- hydroxybtyrate concentration in the plasma, free fatty acids (FFA), and cortisol. However, the effect of plasma free fatty acid on pathophysiology, metabolites and histological changes of this disorder remain poorly understood. Clinical signs, often with a slow onset, are characterised by neuorological signs like teeth grinding, stress and dull eyes. Ketosis also affected on pathological part which characterized by fatty liver. In this review, we are focusing various key aspects of the disease with special reference on the clinical, biochemical and histological changes during development of pregnancy ketosis in goat population.

Keywords:Biochemical, B-Hydroxybutyrate, Free fatty acids, Goats, Histological, Ketosis

INTRODUCTION

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 urbanisation, income growth and changing consumer preference (Bisant, 2010). Nevertheless, scientifically based information on goat farms and industry in Malaysia is extremely limited to complement the sudden surge of demand for goat 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 meat and milk production (Bani Ismail *et al*., 2009). Pregnancy ketosis commonly occurs in does during the late stage of gestation. 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 a so called negative energy balance (Schlumbohm and Hameyer, 2004). The disturbance of carbohydrate metabolism leads to high plasma free fatty acids and ketone bodies level. Pregnancy ketosis is also known to further result in low production and economic losses by the goat farms through culling and mortality. 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, information from previous studies has an important role in order to overcome this problem at once to reduce the number of cases mortality rate in farm. Thus, the aim of this review is to summarise and discuss the clinical, biochemical and histological changes occur during development of pregnancy ketosis in goats.

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Overview of pregnancy ketosis in ruminants

Pregnancy is the apart of female life cycle which most anabolic period, and pregnancy ketosis is a diseases resulting of negative energy balance that occur in late gestation which commonly seen in ewes, goats, cows, and many other species (Abd-Elghany *et al*., 2010). According to Mateniuk and Herdt (1988), it has been reported that economic losses because of the pregnancy ketosis have been considerable and it is the most commonly occurring metabolic disease of sheep and goat. This metabolic disease normally occur during last trimester of pregnancy by the sudden extra requirement for energy in the fast-growing lambs or kids (Rook, 2000; González *et al*., 2011). Stress and food deprivation also causes of pregnancy ketosis (Abd-Elghany *et al*., 2010). It is a potentially total metabolic condition of sheep and goats which can cause substantial losses. Pregnancy ketosis normally occur in the last trimester (4 to 6 weeks) of gestation in goat and sheep, mostly in pregnancies with carry more than one foetus, increased energy demands for rapid foetal growth in late gestation and insufficient intake (Smith, 1990). Previous studies also stated about 60% of foetal growth takes place during last stages of pregnancy period and in this time around 33-36% of the circulation glucose during pregnancies is focusing into foetoplacental unit to fulfil its energetic requirements (Twardock *et al*.,

1973; Hay *et al*., 1983). In addition, glucose is the major requirements for energy source increase considerably in the pregnant animal and the uterus and foetus (Lindsay, 1973). Indeed, reported as energy deficit because of ketosis causes of lipid reserves undergo lipolysis and mobilise and results in giving rise a doubling of the plasma free fatty acids and ketone bodies levels in blood and urine (Chaiyabutr *et al.*, 1980). Changes the level of Beta-hydroxybutyrate (BHBA) and free fatty acids (FFA) in the blood will also give reflect on other biochemical and hormonal changes in the plasma. Thus, a study on the role of plasma FFA in pregnancy goat during development of pregnancy ketosis is needed.

Diagnosis

Clinical signs

Does should be in normal health during and after pregnancy so as to produce viable meat and milk. Accurate diagnosis in early stage of subclinical metabolic disorders, like pregnancy ketosis, is important for dairy goat industry in Malaysia. Prognosis of pregnancy ketosis is generally very poor. Symptom of pregnancy ketosis include weakness, depression, dullness, anorexia, staggering gait, reduced appetite, ketonaemia, ketonuria, hypoglycaemia, some affected animals become constipated, and have acetone smell to their breath and suffering from dystocia (Al-Qudah, 2011; Abd-Elghany *et al*., 2010). Moreover, pregnancy ketosis also can affect the neurological signs including circling, in-coordination, stargazing, tremors and convulsion. In a severe stages reported by El- Sebaie (1995), severe ketoacidosis, haemoconcentration, hyperglycaemia and uraemia are often occur during ketosis accompanied by dyspnoea, recumbency and blindness. Excessive salivation and muscle fasciculation were occasionally observed in the head region causing movements of the overlying skin and twitching of the ears. (Scott *et al*., 1995; Sargison *et al*., 1994). Changes in body weight and condition also reflects adequacy of energy. However, in late pregnancy rapid growth of the foetus makes it difficult to interpret body weight and body condition changes accurately (Russel, 1984). Pough (2002) also stated which ketosis animal may lead to death if the case is left untreated or did not giving a proper treatment. Clinically, the disease in cattle (acetonaemia) and in ewes and does (pregnancy toxaemia) are rather different entities and occur in different parts of the pregnancy-lactation cycle but the biochemical disturbance is essentially the same and they occur under similar conditions of management, all of which lead to a state of negative nutritional balance (Barakat *et al*., 2007). Previous studies also reported that developments of clinical ketosis have associated with major changes in blood profile (Anderson, 1988; Foster, 1988).

Subclinical signs

In subclinical signs, the presence of pregnancy ketosis in ruminant can only detected through increasing of ketone body concentrations in the plasma such as BHBA (Duehlmeier *et al*., 2011). To replace for the lack of glucose during ketosis, adipocytes undergo lipolysis process and resulting in increased free fatty acids (FFA) levels in blood plasma. Due to incomplete FFA breakdown in the liver, level of plasma BHBA increase. Plasma BHBA concentrations have been identified to be a specific tool to detect does which under nutrition (normal state), severe under nutrition and pregnancy ketosis in goats. According to Andrews (1997), healthy animals normally have BHBA in the plasma levels below 0.8 mmol/L, while BHBA concentration of 0.8 to 1.6 mmol/L could suggest moderate undernutrition. In addition, ketone bodies in plasma concentration (BHBA) more than 3.0 mmol/L are usually measured in sheep suffering from pregnancy ketosis. (Sargison *et al*., 1994). Ramin *et al*. (2005) stated that most of disorders are characterised by certain changes in the blood parameters of concentration. Plasma and serum components are most common biochemical indicators for diagnosis activity of disease, patterns and concentration changes, although not precise, it could be a diagnostic consequence in metabolic disorder (Moghaddam and Olfati, 2012). Several studies have concentrated on mediators related with the arising metabolic changes in ewe pregnancy ketosis (Harmeyer and Schlumbohm, 2006; Scott *et al*., 1995). Thus, the authors of this review suggested that future studies in animal science focus on the important relationship between diagnosis of pregnancy ketosis and biochemistry profile in different gestational stages of does.

Metabolic changes during pregnancy ketosis in goats

Free fatty acid

Free fatty acids (FFA) provide an essential source of energy for cells. Free Fatty Acids (FFA) is the one of principal precursor ketones in ketotic animals. Ketosis in ruminants is a disease caused by impaired metabolism of volatile fatty acids and carbohydrates. During starvation, the alteration can occur in the amounts and compositions of lipids in liver and plasma lipoproteins (Brumby *et al.,* 1975). As it has been reported that concentration of plasma free fatty acids is directly proportional to the free fatty acids quantity taken up via livers of starved sheep (Katz and Bergman, 1969), fatty acids will accumulate during starvation unless compensatory increases in rates of output of fatty acids in plasma lipoproteins or ketone bodies occur and rated of oxidation to carbon dioxide. It has been suggested that decreasing amount of glycerol precursors for the formation of triglycerides from free fatty acids may account for accumulation of lipid in liver during starvation (Bergman, 1971). It has been shown by Brumby *et al*., (1975) which though the content of free fatty acid increased during ketosis induced reflected in increase lipid observed in liver. The liver of ruminants is a major organ of metabolism of FFA, which is similar to the situation in non-ruminants. Most synthesis of fatty acids in ruminants occurs in adipose tissue (Vernon, 1980). Free fatty acids also can be present in the blood stream through a process adipolysis in which triglycerides broken down into free fatty acids and glycerol. Fatty acids are absorbed by liver in proportion to their concentration in blood

reaching the liver (Bell, 1980). Rate of uptake can be modified further by changes in the FFA to albumin ratio, with higher ratios favouring increased uptake (Bell, 1980). In sheep is about 10% of the fractional FFA from blood will be extracted by liver (Katz and Bergman, 1969; Thompson *et al*., 1975). Major FFA in ruminants are palmitic, stearic, and oleic acids (Bell, 1980), with stearic acid being utilised more poorly by the liver than either palmitic or oleic acids (Thompson *et al*., 1975). All lipid fraction of plasma including the ratio of oleic: stearic acid also has been used as an indicator of increased lipolysis (Wallenius and Witchurch, 1976; Yamdagni and Schultz, 1970). The increased ratios also have been noted during fasting and ketosis condition (Brumby *et al*., 1975; Waterman and Schultz, 1972; Yamdagni and Schultz, 1970). Pothoven *et al*. (1974) has shown that almost 50 % of fatty acids of adipose are oleic. Presumably the increased ratio of oleic:stearic reflects the relative contribution of diet and lipolysis, although differential metabolism of free fatty acids has been proposed (Vernon, 1980). Fatty acids are toxic within cells (Spector and Fletcher, 1978), and are activated quickly to acyl-CoA esters and either oxidised or esterified (Bell, 1980). About 10% of the FFA taken up by ruminant liver is oxidised to carbon dioxide (Jesse *et al*., 1986; Lomax *et al*., 1983). Lomax *et al*. (1983) stated that the FFA proportion was unaffected by starvation in isolated hepatocytes from sheep but according to Jesse et al.,(1986) it was decreased in liver slices from fasted cows. A major fate of FFA within the liver may be conversion to the ketone bodies, acetoacetate and B-hydroxybutyrate (BHBA). The principal of blood metabolites circulating and hormonal changes have been used to evaluate ruminant energy status: FFA, glucose, ketone bodies (BHBA), insulin and cortisol level (Russel, 1984; Russel and Wright, 1983; Bowden, 1971).

Blood profiling changes during ketosis

Serum biochemical profiles comprehensively have been used by veterinarians to analyse the health, metabolic and nutritional status of ruminants such as diagnosis of pregnancy ketosis. Study by Bani Ismail *et al*. (2009) shown the range developed from metabolic constituents in does serum in late stages of pregnancy with and without sub clinical pregnancy ketosis in (Table 1). In addition, an increase in plasma BHBA and decrease in plasma glucose also detected in pregnancy ketosis (Figure 1) (Nonnecke *et al*., 1992). Regulation of blood glucose in ruminants differs from the regulation in most mono-gastric animals. In humans, insulin is releases after glucose consumption but glucagon releases after fodder intake in ruminant (Trenkle, 1981). One of glucose regulator in ruminants is cortisol which acts to increase process of gluconeogenesis from amino acids. During starvation, gluconeogenesis is maintained by raised glucocorticoids levels (Trenkle, 1981). Cortisol levels are used as an indicator of stress and pain and elevated of this hormone in serum has been shown in cows with hypocalcaemia (Waage *et al*., 1984). Pancreas is one of the endocrine system probably is closely involved in the ruminant ketosis development (Brockman, 1979). Ketogenesis process inhibits by insulin when levels of free fatty acids are high (Bieberdorf, 1970), as well as secretions of growth hormone inhibited by free fatty acids and cortisol (Ganong, 2007). Utilisation of ketone bodies as the uptake of B- hydroxybutyrate and acetate by sheep has been regulated by insulin (Jarrett, 1974). Insulin is one of hormone which able to increasing the proportion of elimination of ketone bodies from blood, and during insufficiency of insulin maximum utilisation of ketone bodies was reduced (Keller *et al*., 1988). The significant elevation levels in cortisol and negative correlation presence between glucose and cortisol level and also positive relation with BHBA possibly due to impaired ability of the fatty liver or raising adrenal output, which finding in pregnancy ketosis was a consistent, to excrete and mobilise the hormone (Ford *et al*., 1990). Preview study also reported that there was a positive association significantly between cortisol and B-hydroxybutyrate in subclinical state of pregnancy ketosis in goat (Bani Ismail *et al*., 2009). Alteration of ketogenesis process and fatty acid releases can be affect by insulin hormone. Ketogenesis supressed by insulin appears independent of any consequence of free fatty acid concentration (Brockman and Laarveld, 1985). Thus, from this information collected authors can summarise pregnancy ketosis has effect in *in vivo* as well as it alters concentration of insulin and cortisol that associated with the levels of B-hydroxybutyrate and glucose. Furthermore, insulin could play a role in metabolic adaptive to inhibit ketogenesis and lessening production of ketone bodies in experimentally pregnancy goats with ketosis. Halford and Sanson (1983) indicated ewes with pregnancy toxaemia were decreased in concentration of serum potassium; however, in goats, no differences in these analytes were observed. Study by Bani Ismail *et al*. (2009) shows characterised serum biochemical profile in goat does with subclinical pregnancy ketosis in Jordan. Table 1 shows a ranges of FFA, BHBA, cortisol, glucose and total calcium developed by Bani Ismail *et al*. (2009) and Kristina *et al*. (2010) which may be applied by veterinarians to aid in clinical analysis of herds and help in the understanding of changes in pathophysiologic that occur in goats during late pregnancy.

Histological changes in liver during ketosis

Reports on histological findings in pregnancy ketosis normally are referred to data collected from post mortem examination. Indeed, histological changes due ketosis had reported by few studies which claims the animals faced with early structural maturity of placenta, liver steatosis, and cerebral and cerebellar neuronal necrosis and vacuolation (Snock, 1992; Mitchell and Stratford, 1987; Marteniuk and Herdt, 1988; Jeffrey and Higgins, 1992; Andrews, 1977). According to Herdt (2000) reported that fatty liver occur due to lipolysis interferes with hepatic gluconeogenic capacity. Thus, fatty liver and ketosis disease would play a main role during pregnancy ketosis. During ketosis condition, Snook (1939) stated that nucleus starting being pushed up against the cell wall and many of the liver cells the protoplasm seems to have been almost entirely replaced by fat. In (Figure 2), Snook (1939) and

Constituents	Subclinical Pregnancy Ketosis (n=53) Range	Controls (n=100) Range
3-Hydroxybutyrate (mmol/L)[a]	0.8-19.0	0.1-0.7
Glucose (mg/dL)[a]	17.0-165.0	72.0-170.0
Total Calcium (mg/dL)[a]	5.0-17.0	4.0-19.0
Cortisol (ng/mL)[a]	0.0-10.0	0.0-110.0
Free Fatty Acid (mmol/L)[b]	0.8-1.6	0.0-0.8

Table 1: Serum metabolic constituents in goat does in late pregnancy, with and without subclinical pregnancy ketosis. Retrieved from [a]Bani Ismail *et al.*, (2009); [b]Kristina *et al.*, (2010).

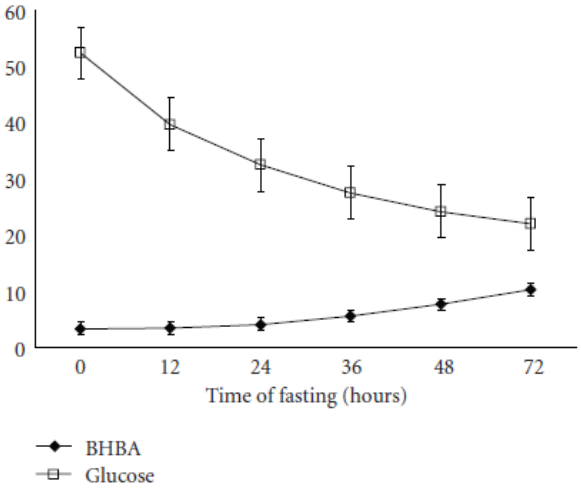

Figure 1: Concentration of BHBA and glucose (mg/dl) in experimentally pregnancy ketosis goats. Differences in the graph indicate beginning of the significant differences. Adapted from Abd-Elghany*et al.*, (2010).

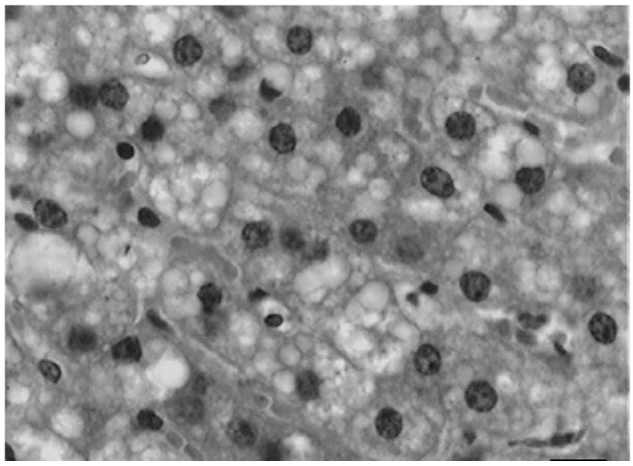

Figure 2: Severe liver microvesicular degeneration, lipid droplets with large amount in almost every hepatocyte cell. Scale bar = 20µm. Adapted by Cal et al., (2009).

Cal *et al*. (2009) was indicated that during pregnancy ketosis, necrosis or degenerative changes of the liver parenchyma were not observed, but during late pregnancy degeneration of hepatic in ketosis ewes was of microvesicular type. During acute starvation, fatty acid started accumulates in the liver which caused microvesicular degeneration (Jaeschke *et al*., 2002). Indeed, sudden onsets of mithochondrial dysfunction caused insufficient time for a progressive coalescene of small lipid droplets into the large fat inclusions seen in macrovesicularsteatosis (Jaeschke *et al*., 2002).

Short period of starvation in ruminant can develop degeneration of liver microvesicular which the whole liver acinus could affect and can be spontaneously reversed during late pregnancy. According to Cal *et al.*(2009), severity of liver damage was found to be related with an elevated activity of Aspartate Aminotransferase (AST) and it has been proved by EL-Dee, (2012) which the healthy ewes has a range of 47.5±0.63 IU/L and for ketosis ewes, the range is 209.5±2.51 IU/L. It was also reported by Barakat *et al*. (2007) that AST levels were increased in shamia goats around parturition. The range of AST level in goat affected by ketosis is (68±0.3 IU) while the range of AST in healthy goats is (6.7±0.3IU). Other study also reported that at day 140 in severe fasting stage, AST level was increased from 84.7±11.9IU/L to 331±152.1 IU/L (Cal, *et al*., 2009). Association between AST and the degree of vacuolation in the liver shows a positive correlation only at day 140, and it suggesting that this enzyme would be a useful and rapid marker of liver damage in ketosis ewes. It is concluded that during late gestation with starvation period in ruminant, liver can produce microvesicular degeneration and could affect the whole liver acinus. The severity of liver damage was found to be related to an increase activity of AST.

CONCLUSION

Pregnancy ketosis is thought to result from disruption of the doe's glucose homeostatic mechanisms in response due to elevated nutritional needed of the rapidly developing fetal placental unit. In addition, extensive research is recommended to assess the potential role of biochemical, diagnosis, hormonal and histological changes during pregnancy ketosis in does at abnormal metabolic states. This information might be applied by veterinarians to aid in clinical examination of herds and individual does in late pregnancy, and help in the understanding of histological changes that occur in these does. To finally interpret the FFA, BHBA, glucose data, and other parameters of the energy metabolism have to be taken into account.

CONFLICT OF INTEREST

Authors state that there is no conflict of interest regarding the publication of this paper.

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