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Metabolic profiling of *Malabari does* with subclinical pregnancy toxaemia

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ABSTRACT

Pregnancy toxaemia is a multifactorial disorder of energy metabolism in small ruminants commonly occurs in the last month of gestation characterized by hypoglycemia, hyperketonemia and metabolic acidosis. Does carrying twins and triplets are at higher risk. In the present study 29 does select divided into four groups. Early pregnant, a late pregnant animal with one fetus, late pregnant animal with two fetuses and non-pregnant control group. The objective of this study was the detection of subclinical pregnancy toxaemia, metabolic profiling and assessment of inflammatory cytokines. Among the four groups selected the group of a late pregnant animal with two fetuses had a Beta-hydroxybutyrate level of 0.81 ± 0.10 mmol/l, which shows those animals were in subclinical pregnancy toxaemic stage. Hypoglycemia and hyperketonemia was a striking observation in that group. Serum concentration of Non-esterified fatty acid was increased in the same group with a noticeable increase in the Tumor necrosis factor alpha. This may be due to the inflammatory changes in response to metabolic acidosis as the acetoacetate and beta-hydroxybutyrate are powerful acids. The release of TNF-α implicating the role and importance of stress in the pathophysiology of the condition.

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INTRODUCTION

Goat farming is a venture which has been accomplished by a large sector of Indian population especially in rural areas and their contribution to Indian rural economy is honourable (Kumar and Pant 2003; Singh 2006; Singh and Shalendra Kumar 2007; Kumar *et al.,* 2010). Malabari goats are medium sized, dual-purpose animals with small, slightly twisted horns and medium-sized ears directed outward and downward, which is reputable for its high prolificacy, milk yield, excellent growth rate and adaptability to the hot humid conditions prevalent in the state (Bilaspuri and Singh 1993; Verma *et al.,* 2009). Pregnancy toxaemia is a metabolic disease, commonly occurring in the last six weeks of gestation in does (Marteniuk and Herdt 1988; Rook 2000; Cal-Pereyra *et al.,* 2015). Factors important in the development of the disease are: 1) Presence of two or more fetuses 2) Undernourishment during late pregnancy when the fetuses have the most rapid growth and 3) Addition of stress such as severe weather, sudden changes in feed or other disease or transportation upon the previous factors (East 1983; Marteniuk and Herdt 1988;

Rook 2000; Lima 2012; Cal-Pereyra *et al.,* 2015). Death occurs in 2-10 days in about 80 percent of the cases (Brozos 2011; Abba *et al.,* 2015). The clinical case of disease is diagnosed generally based on the doe's history and the clinical findings which include listlessness, aimless walking, muscle twitching, opisthotonos, grinding of the teeth, and as the disease progresses sternal recumbency, coma, and death (Ermilio and Smith 2011; Abba *et al.,* 2015). Through recognition of early signs and symptoms and avoidance of predisposing factors, pregnancy toxaemia can be reduced to a sporadic condition (Mavrogianni and Brozos 2008; Fthenakis 2012). Hypoglycemia is not a consistent finding, with up to 40% of cases having normal glucose levels and up to 20% having hyperglycemia, increased activities of aspartate aminotransferase, alkaline phosphatase, urea, creatinine, decreased total cholesterol (Schlumbohm and Harmeyer 2008; Smith and Sherman 2009; Menzies 2011; Fthenaki 2012). The most predominant circulating ketone bodies in ruminants are beta-hydroxybutyrate (BHB) and acetoacetate. Ketones are produced from the metabolism of non-esterified fatty acids (NEFAs) or free fatty acids (FFA) and volatile fatty acids (VFA). The primary ketone produced by the liver from NEFA is acetoacetate (Menzies., 2011; Fthenakis., 2012). This is reduced to BHB by hydroxybutyrate dehydrogenase enzyme within the mitochondria. Non-esterified fatty acid (NEFA) reflects the magnitude of mobilization of fat from storage (Smith and Sherman 2009; Menzies 2011; Fthenakis 2012). Excess ketone bodies can generate superoxide radicals and cause oxidative stress and cellular dysfunction. Cytokines are associated with immune responses and are implicated in the pathogenesis of pregnancy toxaemia owing to their roles in endothelial damage. While clinical pregnancy toxaemia in ruminants is relatively well studied, there is a paucity of information regarding metabolic changes in the subclinical form of the disease, especially in does (Menzies 2011; Fthenakis 2012; Lima 2012; Cal-Pereyra *et al.,* 2015). So far very little work has been done on subclinical pregnancy toxaemia in Malabari does. The occurrence of this ailment is worldwide and realising the importance of goat in the agrarian economy of the country this disease drive the researchers explore more on the subclinical stage of this disease because the treatment for pregnancy toxaemia is frequently unsuccessful. Hence the present study is designed with the following objectives: Estimation of Beta-hydroxybutyrate in different groups of Malabari does and metabolic profiling and assessment of Tumor necrosis factor alpha.

MATERIALS AND METHODS

Experimental Animals

The analysis was conducted in the Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India, to detect subclinical pregnancy toxaemia. The study was carried out in clinically healthy Malabari does maintain at the University goat and sheep farm, College of Veterinary and Animal Sciences, Mannuthy, between July and September. Investigations were done in four groups of *Malabari does*. Early pregnant does (40-60 days), Late pregnant does with one fetus (110-150 days), Late pregnant does with two fetuses (110-150 days), Non-pregnant does (sexually matured). The day of mating was considered as the first day of pregnancy. The pregnancy was confirmed by 3.5 MegaHertz B- mode ultrasonography (Minray Pvt. Ltd.). The early pregnant group consists of seven animals, late pregnant does with one fetus consists of six animals and late pregnant does with two fetuses consists of eight animals. Non- pregnant does a group with eight animals is taken as a control group.

Collection of Blood Samples

Blood samples were collected from 29 does. Blood was drawn between 8.30 am and 9.30 am before morning feeding, by jugular venipuncture using a sterile needle. Approximately, 3 ml of blood was dispensed into a vial without anticoagulant and 2ml blood was dispensed into an anticoagulant (Sodium fluoride) coated vacutainer. The tubes for serum collection were allowed to clot at room temperature for 1h and the clear sera were collected by centrifugation at 3000 rpm for 10 min. The blood collected with anticoagulant was centrifuged immediately at 3000 rpm for 10 min and separated plasma used for further investigation.

Estimation of beta-hydroxybutyrate; a golden marker of subclinical pregnancy toxaemia

Beta-hydroxybutyrate concentration was determined kinetically by enzymatic UV method, in RX Monza analyzer using RAN BUT Beta-hydroxybutyrate kit.

Metabolic profiling of does with subclinical pregnancy toxaemia

Glucose concentration was determined immediately after blood collection by GOD/POD method (Endpoint method) using glucose LS kit (Euro Diagnostic Systems Pvt. Ltd). Cholesterol was determined by CHOD/POD method (Endpoint method)

using cholesterol kit, (Euro Diagnostic Systems Pvt. Ltd). Non-esterified fatty acid concentration was determined colourimetrically (Endpoint method) using Randox NEFA kit. Total protein was determined photometrically by Biuret method (End-

point method) using total protein LS kit (Euro Diagnostic Systems Pvt. Ltd). The method of Konturek *et al.,* 2001 assayed the levels of *TNF-α*.

RESULTS

Late pregnant does with two fetuses showed a highly significant increase (p<0.01) when compared to the control group. The mean beta-hydroxybutyrate values were 0.45 ± 0.01, 0.59 ± 0.01, 0.81 ± 0.10 and 0.29 ± 0.02 mmol/L, respectively for early pregnant does, late pregnant does with one fetus, late pregnant does with two fetuses and control group (Figure 1).

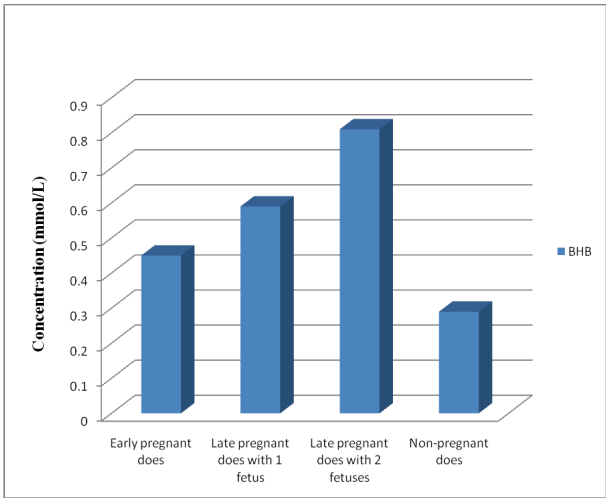

Figure 1: Concentration of BHB (mmol/L) in different groups of Malabari does

A highly significant decrease (p<0.01) in blood glucose concentration was found in early pregnant does, late pregnant does with one fetus and two fetuses when compared to control group as non-pregnant does (Fig.2). The mean values obtained for glucose in early pregnant does, late pregnant does with one fetus and two fetuses were 40.04 ± 4.40, 44.17 ± 4.46, 46.08 ± 3.04 mg/dL, respectively and that of the control group was 62.54 ± 4.36 mg/dL.

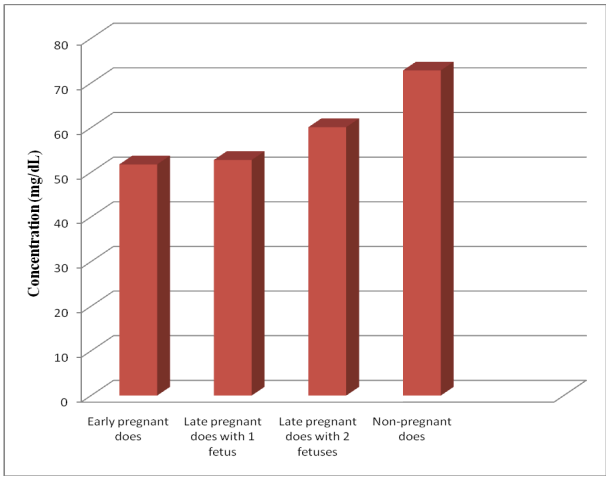

Figure 2: Concentration of Glucose (mg/dL) in different groups of Malabari does

A significant decrease (p<0.01) was observed for serum cholesterol level in early pregnant does, late pregnant does with one fetus, late pregnant does with two fetuses when compared to the control group. The mean values obtained for cholesterol in early pregnant does, late pregnant does with one fetus and late pregnant does with two fetuses were 51.79 ± 2.79, 52.78± 4.61, 60.13± 4.29 mg/dL, respectively and that of the control group was 72.83± 4.46. A highly significant increase (p<0.01) was observed for serum NEFA in late pregnant does with two fetuses and one fetus when compared to the control group, no significant changes (p>0.05) was observed in early pregnancy does when compared with the control group (Fig. 3). The mean values for NEFA in early pregnant does, late pregnant does with one fetus and two fetuses were 0.59 ± 0.02, 0.95 ± 0.05 and 1.27 ± 0.05 g/dL respectively, and that of the control group was 0.48 ± 0.01 g/dL. A highly significant increase (p<0.01) was observed in late pregnant does with two fetuses when compared with the control group. A significant increase (p<0.01) in late pregnant does with one fetus compared to control group. No significant change (p>0.05) was observed in early pregnancy does when compared with the control group. The mean values of TNF-α in early pregnant does, late pregnant does with one fetus, two fetuses and control group were 3.33 ± 0.14, 8.78 ± 0.19, 12.17 ± 0.49 and 2.30 ± 0.16 ng/ml, respectively. (Figure 4).

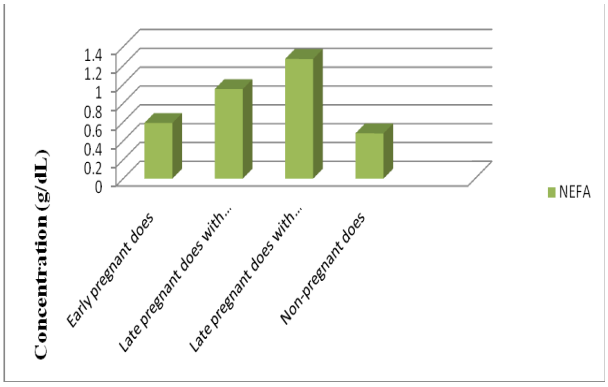

Figure 3: Concentration of NEFA (g/dL) in different groups of Malabari does

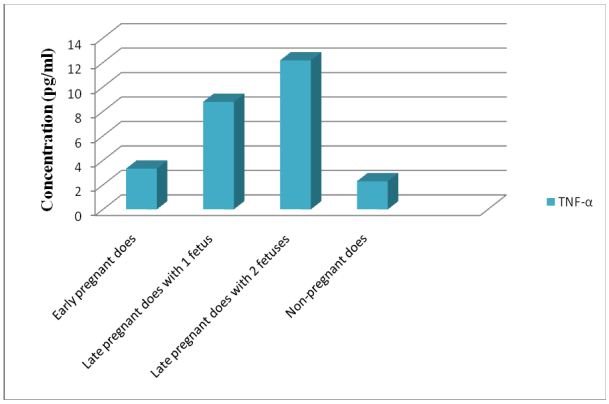

Figure 4: Concentration of TNF-α (pg/ml) in different groups of Malabari does

DISCUSSION

The result from this study showed that group of does with two fetuses, was in subclinical pregnancy toxaemic stage. Pregnancy toxaemia is a multifactorial disorder of energy metabolism hence so many factors other than the plane of nutrition can lead to this condition. The intense genetic breeding for twinning can be an important reason and in addition sudden feed change, transportation and weather change can act as a precipitating cause of pregnancy toxaemia. Since Malabari goat breed is well adapted to the hot and humid weather condition prevailing in the state, the stress due to the climate can be excluded and this may be a rationale behind, a subclinical condition not progressing to a clinically exhibiting condition. From this, it was inferred that the regulatory and metabolic stress in late gestating does bearing twins was disproportionately high. Hence further studies on metabolic parameters were carried out to know more about the aetiopathology of the disease and to find out an easy and cost- effective marker for subclinical pregnancy toxaemia. Ruminants appear to be well adapted to a carbohydrate economy based on their ability of gluconeogenesis (Kaneko *et al.,* 2008). During pregnancy the glucose demand increases, as fetal demands for glucose are high, and the placenta can transport glucose from maternal to fetal plasma (Warnes *et al.,* 1977). When an imbalance occurs between the maternal ability to synthesize or absorb glucose and fetal consumption, hypoglycemia results. Significantly greater hypoglycemic responses in twin-pregnancies than in singleton pregnancies have been reported to occur in sheep (Reid and Hogan, 1959; Reid and Hinks 1962). A highly significant decrease ($p<0.01$) in plasma glucose concentration was found in early pregnant does, late pregnant does with one fetus and two fetuses when compared with the control group. These findings were in accordance with Schlumbohm and Harmeyer (2008) and Anoushepour A *et al.,* (2014). Hefnawy *et al.,* (2011) reported a marked drop in serum total cholesterol in dose affected by clinical pregnancy toxaemia and the finding was in accordance with the present finding. In the present study a significant decrease ($p<0.01$) was observed for serum cholesterol level in early pregnant does, late pregnant does with one fetus, late pregnant does with two fetuses when compared to the control group. Under the hypoglycemic circumstances, there is increased lipolysis in adipose tissue and release of NEFA. According to the study conducted by Anoushepour A *et al.,* (2014) serum concentrations of NEFA were significantly higher in hyperketonemia ewes than normal ewes and the result obtained from our study shows a similar fashion. A significant increase ($p<0.01$) in late pregnant does with one fetus compared to control group. This may be due to the inflammatory changes in response to metabolic acidosis as the acetoacetate and beta-hydroxybutyrate are powerful acids. The release of TNF-α implicating the role and importance of stress in the pathophysiology of the condition. No significant changes ($p>0.05$) were observed in early pregnancy does when compared with the control group. (Fig.5). The mean values of TNF-α in early pregnant does, late pregnant does with one fetus, two fetuses and control group were 3.33 ± 0.14, 8.78 ± 0.19, 12.17 ± 0.49 and 2.30 ± 0.16 ng/ml, respectively and the observation was in agreement with Albay *et al.,* (2014). According to his study, a sharp increase was observed in serum TNF-α activity for the subclinical and clinical pregnancy toxaemic groups. Cytokines are associated with immune responses and are implicated in the pathogenesis of pregnancy toxaemia owing to their roles in endothelial damage (Redman *et al.,* 2005). An intense systemic inflammatory response and augmented production of inflammatory cytokines occur in pregnancy toxaemia, for which trophoblast cell defects and destruction of placental debris are considered to be important inducing factors (Sargent *et al.,* 2003). Hypoxia in the placenta due to reduced blood perfusion, in turn, induces placental production of TNF-α (Benyo *et al.,* 1997). According to this study, the higher levels TNF-α is positively correlated with well-established parameters of pregnancy toxaemia, BHB and NEFA and it suggests that cytokines are also mediators of placental inflammation and subsequent systemic inflammatory reactions in does pregnancy toxaemia and hence TNF-α along with either BHB or NEFA can be used as an additional diagnostic biomarker for subclinical pregnancy toxaemia in does.

CONCLUSION

The fetuses require an increasing amount of carbohydrates in the last trimester and when the metabolic needs of the fetuses are met at the expense of the dam which leads to pregnancy toxaemia. Detection of subclinical condition helps to take necessary prevention measures and that can be readily achieved by nutritional means and is far more rewarding than treatment.

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Conflicts of Interests: None

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