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Sub Clinical Pregnancy Toxaemia Diagnostic Indicators and its Therapeutic Evaluation in Goats

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ABSTRACT

A total of 516 adult non descriptive does brought to Veterinary University Peripheral Hospital, Madhavaram Milk Colony, Chennai – 51, during the period October 2016 to September 2018. were treated for various medical conditions. Of this, 72 does were in their last six weeks of gestation carrying twins/triplets and presented with the history of off feed. They were subjected to determination of blood beta hydroxybutyric acid (BHBA) concentration by means of a portable blood ketone and glucose monitoring system and qualitative urinalysis using urine dip stick. Does with BHBA > 0.8 mmol/L and < 1.6 mmol/L were classified as sub-clinical pregnancy toxaemic group (n = 12) while the remaining does BHBA level were within normal range (< 0.8 mmol/L). The control animals were selected from adult Tellicherry does in the age group of 2 to 4 years maintained at Livestock Farm Complex (LFC), Madhavaram Milk Colony, Chennai – 600 051 and a private goat farm (ECR Goat Farm), Injambakkam, Chennai. The sub- clinical pregnancy toxaemic group were resorted to treatment with intravenous glucose therapy (5 per cent Dextrose) and oral administration of glycerine for 3-4 days @ 25 ml twice daily supported with parenteral Vitamin B_1, B_6 & B_{12} therapy with an overall cure rate of 100 %.. Reliable diagnostic indicators for sub-clinical form of pregnancy toxaemia include presence of ketone body in urine and BHBA > 0.8 mmol/L and < 1.6 mmol/L.

HIGHLIGHTS

- Periparturient mortality in goats have a great economic impact on the livelihood of marginal farmers.
- Reliable diagnostic indicators of negative energy balance in the primary stage of the disease are the need of the hour for better herd health management.

Keywords: Sub-clinical Pregnancy Toxaemic Goats, Diagnostic Indicators, Therapeutic Evaluation

Pregnancy toxaemia also called as gestational ketosis, twin-lamb disease, ketosis of pregnancy, kid disease, lambing sickness, kidding paralysis and lambing or kidding ketosis Sharma *et al.,* 2014) is a metabolic disease affecting pregnant ewes and does after a period of negative energy balance (NEB) and impaired gluconeogenesis (Lima *et al.,* 2012). Pregnancy toxaemia normally occur in the last trimester (last 6 to 4 weeks) of gestation in goat and sheep as a result of negative energy balance consequent

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to enhanced requirement for glucose by the developing foetuses (Schlumbohm and Harmeyer, 2008). Risk factors include multiple fetuses, poor quality of ingested energy, decreased dietary energy level, genetic factors, obesity, lack of good body condition, high parasitic load, stress factors and multiple pregnancies (Hefnawy *et al.,* 2011). The disease is characterized by hypoglycaemia, low concentrations of hepatic glycogen, increased fat catabolism leading to high plasma concentration of non-esterified fatty acids (NEFA), high concentration of ketone bodies (hyperketonaemia) and high mortality rate (Van Saun, 2000). The mortality rate can attain 100 % even with the initiation of treatment due to severe irreversible organ damage. In goat farming reliable diagnostic indicators of negative energy balance in the primary stage of the disease are the need of the hour for better herd health management.

MATERIALS AND METHODS

The study was carried out at Veterinary University Peripheral Hospital (VUPH), Madhavaram Milk Colony, Chennai – 6000 051, Livestock Farm Complex (LFC), Madhavaram Milk Colony, Chennai – 600 051 and a private goat farm (ECR Goat Farm), Injambakkam, Chennai during the period October 2016 to September 2018. The control animals were selected from adult Tellicherry does in the age group of 2 to 4 years maintained at Livestock Farm Complex (LFC), Madhavaram Milk Colony, Chennai – 600 051 and a private goat farm (ECR Goat Farm), Injambakkam, Chennai. Pregnant does (n = 12) carrying twins / triplets, without exhibiting signs of pregnancy toxaemia throughout gestation at Livestock Farm Complex, Madhavaram Milk Colony and Pregnant does (n = 12) carrying twins / triplets, without exhibiting signs of pregnancy toxaemia throughout gestation at ECR Goat Farm, Injambakkam, Chennai served as control. Does in their last six weeks of gestation carrying twins / triplets presented with the history of off feed to Veterinary University Peripheral Hospital, Madhavaram Milk Colony, Chennai (72 does) were subjected to determination of blood BHBA concentration by means of a portable blood ketone and glucose monitoring system and qualitative urinalysis using urine dip stick. The pregnant does were subjected to radiography for conformation of pregnancy and assessment of fetal numbers. Does with BHBA > 0.8 mmol/L and < 1.6 mmol/L were classified as sub-clinical pregnancy toxaemia.

Parameters included in the Study

Clinical Signs

The clinical signs exhibited by the pregnant does were recorded.

Blood BHBA concentration

The blood BHBA concentration was determined using a portable blood ketone and glucose monitoring system (Fig. 1) *(Free Style Optium Neo H – Abbott ®)* (Pichler *et al.,* 2014).

Fig. 1: Portable Blood ketone monitoring system

Urine sample

Urine samples were obtained after a voluntary micturition or induced by covering the nose and mouth of does for a few seconds (Albay *et al.,* 2014). The urine samples were analyzed using Multistix 10 SG reagent strip (Siemens Healthcare Private Limited, India) for qualitative determination of ketone bodies, glucose and protein (Emam and Galhoom, 2008). The test strips were dipped into the collected urine and immediately compared with the colour chart provided on the label of the urine test strip container to determine the presence of ketone, glucose and protein in the urine. (Fig 2).

Fig. 2: Urinalysis using Multistix 10SG reagent strip in sub clinical pregnancy toxaemic doe

Ultrasonography

The pregnant does were subjected to ultrasonography to assess the stage of gestation and the viability of the fetuses. The estimated gestational age of the fetus in weeks was calculated using the formula Y = 4.712 + 0.445 X, where Y = Gestational age (wks) and X = Fetal parameter (cm) in case of crown rump length and Y = 2.675 + 3.229 X where Y = Gestational age (wks) and X = Fetal parameter (cm) in case of bi-parietal diameter (Abdelghafar *et al.,* 2011).

Radiography

To confirm pregnancy and assess the foetal numbers (Fig. 3 & 4).

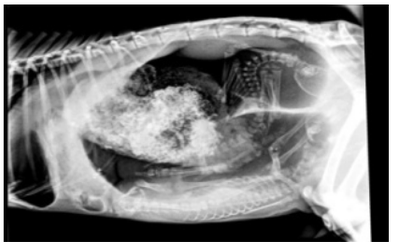

Fig. 3: Radiography in pregnant doe – Twins

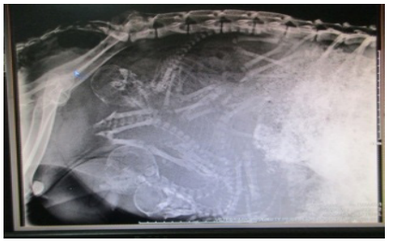

Fig. 4: Radiography in pregnant doe - Triplets

Haematology

Haematological investigation with automated haematology analyzer *(Mindray BC 2800 Vet)*: haemoglobin (g/dL, packed cell volume (%), red blood cell ($X10^6$/cmm), white blood cells (/cmm) and differential count.

Serum Biochemistry

Serum biochemical parameters - blood urea nitrogen (mg/dL), creatinine (mg/dL), aspartate aminotransferase (IU/L), alanine aminotransferase (IU/L), glucose (mg/dL) and total protein (g/dL) were estimated in an automated biochemical analyzer *(A 15 Random Access Analyzer)*.

Serum Electrolytes

The serum electrolytes - sodium (mmol/L), potassium (mmol/L), calcium (mg/dL), magnesium (mg/dL) and chloride (mmol/L) were estimated in an automated electrolyte analyzer. *(Diestro 103 AP)*

Serum Metabolites

The serum was stored at – 20º C until analysis of levels of serum metabolites namely beta hydroxybutyric acid (BHBA) (µmol/L) and non-esterified fatty acid (NEFA) (µmol/L) by Enzyme Linked Immunosorbent Assay (ELISA) method using goat specific BHBA and NEFA ELISA kits (My Bio Source Inc., USA) while the level of serum cortisol (nmol/L) was analyzed by Enzyme Linked Immunosorbent Assay (ELISA) method using goat specific Cortisol ELISA kit (Cusabio Biotech Co. Ltd.,) as per the manufacturer's instruction and the optical density value was read in the ELISA microplate reader at 450 nm.

Therapy

The sub-clinical pregnancy toxaemic does were treated with intravenous glucose therapy (5 per cent Dextrose) and oral administration of glycerine for 3-4 days @ 25 ml twice daily supported with parenteral Vitamin B_1, B_6 & B_{12} therapy. The response to therapy was evaluated 3-5 days post initiation of therapy and the efficacy was assessed based on the clinical signs, haematology, serum biochemistry, metabolic and hormonal parameters.

Cure rate and case fatality rate

The cure rate and case fatality rate were evaluated based on the response to treatment.

Statistical Analysis

The data collected were statistically analyzed by One Way Analysis of Variance (ANOVA) using Statistical Software IBM® SPSS® Version 20.0 for Windows® and critically discussed.

RESULTS AND DISCUSSION

The clinical signs recorded in sub-clinical pregnancy toxaemia anorexia (100 %), dullness in 10 (83 %), bruxism in 7 (58 %). The dung voiding was normal in all the does with a standing posture and normal carriage of head and neck.

The BHBA concentration in blood of non pregnant does and pregnant does without exhibiting signs of pregnancy toxaemia ranged between 0.2 mmol/l to 0.6 mmol/l (Fig. 5) and between 0.9 mmol/l to 1.5 mmol/l in sub-clinical pregnancy toxaemic does (Fig. 6) which were in accordance to Andrews (1997). The values obtained in the portable ketone meter were immediate, reliable and highly useful in screening does for pregnancy toxaemia under field conditions. The human ketone meter can be successfully applied to estimate beta hydroxybutyrate level in goats at field conditions due to the non availability of other reliable spot tests (Yadav *et al.,* 2016).

Fig. 5: Blood BHBA in pregnant doe without signs of pregnancy toxaemia

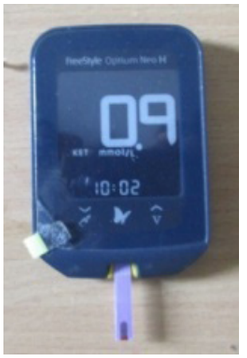

Fig. 6: Blood BHBA in Sub-clinical Pregnancy Toxaemic Doe

Urinalysis in control group indicated absence of ketone bodies, glucose and protein. In the sub-clinical pregnancy toxaemic group, presence of trace quantities of ketone bodies in the urine of 9 does (75%) and small quantities in 3 does (25 %) might be attributed to the increased fat hydrolysis (Cleon, 1988). Protein was completely absent in the urine sample of all the does while trace quantities of glucose was observed in the urine of 6 does (50 %) while the remaining 6 does (50 %) had 1 + grading. The qualitative analysis of urine samples for the presence of ketone bodies, glucose and protein under field conditions can be carried out with accuracy and reliability using Multistix 10 SG reagent strips (Emam and Galhoom, 2008).

The Mean ± S.E. of Haemoglobin, Packed Cell Volume, Red Blood Cells and White Blood Cells in control (LFC and ECR Goat Farm) and pre and post treatment of sub-clinical pregnancy toxaemic group are presented in Table 1. The haemoglobin, packed cell volume and red blood cell values in sub-clinical pregnancy toxaemic group were higher than the control. Highly significant ($P \leq 0.01$) difference was observed between pre and post treatment compared to that of control. The significant increase of the above values in the pregnancy toxaemic does may be due to hemoconcentration and dehydration (Hefnawy *et al.,* 2011).

The Mean ± S.E. of Differential Count in control (LFC and ECR Goat Farm) and pre and post treatment of sub-clinical pregnancy toxaemic group are presented in Table 2. Neutrophilia was observed in sub-clinical pregnancy toxaemic group and showed a decreasing trend during the course of treatment. Highly significant ($P \leq 0.01$) difference was observed between pre and post treatment compared with control. The neutrophilia might be due to the increased cortisol level which created a movement of granulocytes from the bone marrow to the peripheral blood (Alidadi *et al.,* 2012). The Lymphocytes in sub-clinical pregnancy toxaemic group was lower than the control and showed an increasing trend during the course of treatment. Highly significant ($P \leq 0.01$) difference was observed between pre and post treatment compared to that of control. Lymphopenia in sub-clinical pregnancy toxaemic does might be due to the toxic and sub-toxic concentration of beta hydroxybutyrate and acetoacetate in blood which inhibit the lymphocytic proliferation (Franklin and Young, 1991) or may be due to increased cortisol level (Alidadi

Table 1: Mean ± S.E. of Haemoglobin, Packed Cell Volume, Red Blood Cells and White Blood Cells in Control and Pre and Post Treatment of Sub Clinical Pregnancy Toxaemic Group

Parameters	Control : Pregnant Does - Gestation in days				Sub Clinical Pregnancy Toxaemic Group		'F' value
	Livestock Farm Complex (n = 12)		ECR Goat Farm (n = 12)		Pre Treatment (n = 12)	Post Treatment (n = 12)	
	120 days	150 days	120 days	150 days			
Haemoglobin (g/dL)	$8.55^{a} \pm 0.07$	$8.53^{a} \pm 0.05$	$8.45^{a} \pm 0.07$	$8.45^{a} \pm 0.04$	$9.0^{b} \pm 0.15$	$9.01^{b} \pm 0.13$	6.87**
Packed Cell Volume (%)	$24.4^{ab} \pm 0.84$	$26.32^{bc} \pm 0.83$	$22.80^{a} \pm 0.87$	$23.23^{a} \pm 0.83$	$27.75^{c} \pm 0.10$	$27.02^{c} \pm 0.20$	10.33**
Red Blood Cells ($X10^{6}$/cmm)	$15.34^{a} \pm 0.73$	$16.04^{a} \pm 0.73$	$15.19^{a} \pm 0.69$	$15.99^{a} \pm 0.61$	$18.66^{b} \pm 0.08$	$18.57^{b} \pm 0.06$	9.26**
White Blood Cells (/cmm)	19112.5 ± 2046.28	20250 ± 1399.74	20741.66 ± 1773.3	20558.33 ± 1496.93	22091.67 ± 166.27	21683.33 ± 203.69	0.59^{NS}

NS – Not Significant; ** Highly Significant ($P \leq 0.01$); Means bearing the same superscript within the same row do not differ significantly.

Table 2: Mean ± S.E. of Differential Count in Control and Pre and Post Treatment of Sub Clinical Pregnancy Toxaemic Group

Parameters	Control : Pregnant Does - Gestation in days				Sub Clinical Pregnancy Toxaemic Group		'F' value
	Livestock Farm complex (n = 12)		ECR Goat Farm (n = 12)		Pre Treatment (n = 12)	Post Treatment (n = 12)	
	120 days	150 days	120 days	150 days			
Neutrophils (%)	$32.12^{a} \pm 0.63$	$32.0^{a} \pm 0.46$	$32.75^{a} \pm 0.46$	$33.16^{a} \pm 0.62$	$40.91^{b} \pm 1.03$	$39.25^{b} \pm 1.12$	23.79**
Lymphocytes (%)	$63.62^{b} \pm 0.41$	$63.37^{b} \pm 0.26$	$62.33^{b} \pm 0.43$	$62.75^{b} \pm 0.50$	$55.16^{a} \pm 0.96$	$57.0^{a} \pm 1.06$	24.67**
Monocytes (%)	2.5 ± 0.18	2.5 ± 0.26	2.66 ± 0.22	2.75 ± 0.21	2.41 ± 0.14	2.16 ± 0.16	1.13^{NS}
Eosinophils (%)	1.5 ± 0.26	1.75 ± 0.25	1.66 ± 0.25	1.08 ± 0.31	1.5 ± 0.19	1.58 ± 0.14	0.94^{NS}
Basophils (%)	$0.25^{ab} \pm 0.16$	$0.37^{ab} \pm 0.18$	$0.5^{b} \pm 0.15$	$0.25^{ab} \pm 0.13$	$0^{a} \pm 0$	$0^{a} \pm 0$	3.11*

NS – Not Significant * Significant ($P \leq 0.05$) ** Highly Significant ($P \leq 0.01$); Means bearing the same superscript within the same row do not differ significantly.

et al., 2012). With respect to Basophils a significant ($P \leq 0.05$) difference was observed between the sub-clinical pregnancy toxaemic group to that of control.

The Mean ± S.E. of Blood Urea Nitrogen, Creatinine, Aspartate aminotransferase, Alanine aminotransferase, Glucose and Total Protein in control and pre and post treatment of sub-clinical pregnancy toxaemic group are presented in Table 3. A highly significant ($P \leq 0.01$) difference was observed between sub-clinical pregnancy toxaemic group and control in blood urea nitrogen and creatinine levels. Elevated levels observed in sub-clinical pregnancy toxaemic does concurred with Hefnawy *et al.* (2011). The value of blood urea nitrogen started to decrease by 2.64 % and creatinine by 8.27 % during the course of treatment in sub-clinical pregnancy toxaemic does. The reason for increased blood urea nitrogen and creatinine levels observed in the sub-clinical pregnancy toxaemic does may be due to severe kidney dysfunction due to the elevated ketone bodies in general circulation (El-Sayed and Siam, 1994), or due to reduced glomerular filtration due to fatty infiltration in tubular epithelium of kidney (Barakat *et al*., 2007) or due to death and decomposition of fetuses (Radostits *et al*., 2000).

A highly significant ($P \leq 0.01$) difference in aspartate aminotransferase and alanine aminotransferase levels was observed between the pre and post treatment of sub-clinical pregnancy taxaemia groups compared with that of control. Elevated activity of the enzymes correlated with Barakat *et al.* (2007). However the levels of the enzymes started to decrease during the course of treatment in the post treatment group. The reasons for increased aspartate aminotransferase and alanine aminotransferase activities

Table 3: Mean ± S.E. of Serum Biochemical Parameters in Control and Pre and Post Treatment of Sub Clinical Pregnancy Toxaemic Group

Parameters	Control : Pregnant Does - Gestation in days				Sub Clinical Pregnancy Toxaemic Group		'F' value
	Livestock Farm Complex (n = 12)		ECR Goat Farm (n = 12)		Pre Treatment (n = 12)	Post Treatment (n = 12)	
	120 days	150 days	120 days	150 days			
Blood Urea Nitrogen (mg/dL)	$26.02^{a} \pm 1.10$	$26.73^{a} \pm 1.14$	$24.77^{a} \pm 1.13$	$24.90^{a} \pm 0.82$	$39.70^{b} \pm 0.56$	$38.65^{b} \pm 0.52$	69.13**
Creatinine (mg/dL)	$0.62^{a} \pm 0.04$	$0.76^{a} \pm 0.04$	$0.76^{a} \pm 0.04$	$0.73^{a} \pm 0.02$	$1.45^{b} \pm 0.09$	$1.33^{b} \pm 0.09$	26.40**
Aspartate aminotransferase (AST) (IU/L)	$121.5^{c} \pm 3.92$	$122.25^{c} \pm 1.79$	$105.5^{a} \pm 3.04$	$112.91^{b} \pm 0.99$	$131.51^{d} \pm 1.12$	$128.1^{cd} \pm 1.08$	26.38**
Alanine aminotransferase (ALT) (IU/L)	$24.12^{a} \pm 1.24$	$26.12^{a} \pm 0.66$	$44.41^{b} \pm 2.14$	$45.41^{b} \pm 1.99$	$49.23^{b} \pm 2.02$	$47.51^{b} \pm 1.78$	30.14**
Glucose (mg/dL)	$25.25^{a} \pm 2.15$	$29.25^{b} \pm 1.66$	$31.08^{bc} \pm 1.72$	$30.08^{b} \pm 1.15$	$32.0^{bc} \pm 0.69$	$35.0^{c} \pm 0.51$	5.38**
Total Protein (g/dL)	$6.57^{ab} \pm 0.25$	$6.77^{bc} \pm 0.07$	$7.11^{c} \pm 0.13$	$7.12^{c} \pm 0.12$	$6.36^{a} \pm 0.07$	$6.46^{ab} \pm 0.06$	7.44**

** Highly Significant ($P \leq 0.01$); Means bearing the same superscript within the same row do not differ significantly.

in the sub-clinical pregnancy toxaemic group might be due to the damage of the hepatic cells and release of cellular enzymes into circulation as a result of fatty infiltration of the liver due of adipolysis and hepatic ketogenesis following energy deficit (Nassif *et al*., 2005).

A highly significant ($P \leq 0.01$) difference in glucose level was observed between the post treatment sub-clinical pregnancy toxaemic group and control group. The value of glucose started to increase during the course of treatment in the post treatment group. The hypoglycemia observed in sub-clinical pregnancy toxaemic group might be due to long periods of starvation (Andrews, 1997) or due to the increased demand for glucose by the developing twins or triplets or due to decreased hepatic gluconeogenesis and hypoglycemic effect by the increased level of BHBA in blood which can suppress endogenous glucose production and reduction in food intake (Marteniuk and Herdt, 1988; Schlumbohm and Harmeyer, 2004).

A highly significant ($P \leq 0.01$) difference was observed in protein levels between the pregnant does of ECR Goat Farm, pregnant does of LFC at 120 days of pregnancy and sub- clinical pregnancy toxaemic group. Decreased protein levels were observed in sub-clinical pregnancy toxaemic group compared to that of control concurred with Barakat *et al.* (2007) and Hefnawy *et al.* (2011). The total protein levels started to increase during the course of treatment in the post treatment group. The reason for decreased total protein levels observed in the sub-clinical pregnancy toxaemic group might be due to the anorexia and reduction in albumin synthesis due to hepatic insufficiency and albuminuria (Yarim and Ciftci, 2009) or it might be due to malnutrition resulting in inadequate provision of amino acid substrate for general protein production (Nasr *et al.,* 1997).

The Mean ± S.E. of Sodium, Potassium, Calcium, Magnesium and Chloride in control and sub-clinical pregnancy toxaemic group are presented in Table 4.

A highly significant ($P \leq 0.01$) difference in sodium levels was observed between pre and post treatment groups of sub-clinical pregnancy toxaemic group, pregnant does of ECR Goat Farm and pregnant does of LFC at 150 days of pregnancy. However hyponatremia was observed in sub-clinical pregnancy toxaemic group correlated with Hefnawy *et al.* (2011). The value of sodium started to increase during the course of treatment in the sub-clinical pregnancy toxaemic group. The hyponatremia observed might be attributed to the decrease in feed intake, dehydration or large quantity of sodium loss in the renal excretion of acetoacetate and beta hydroxybutyrate (Judith and Thomas, 1988).

A highly significant ($P \leq 0.01$) difference in potassium levels was observed between pre and post treatment of sub-clinical pregnancy toxaemic group compared with that of pregnant does of LFC. Hypokalemia observed in sub-clinical pregnancy toxaemic group compared to control

Table 4: Mean ± S.E. of Serum Electrolytes in Control and Pre and Post Treatment of Sub Clinical Pregnancy Toxaemic Group

Parameters	Control : Pregnant Does - Gestation in days				Sub Clinical Pregnancy Toxaemic Group		'F' value
	Livestock Farm Complex (n = 12)		ECR Goat Farm (n = 12)		Pre Treatment (n = 12)	Post Treatment (n = 12)	
	120 days	150 days	120 days	150 days			
Sodium (mmol/L)	$142.2^{ab} \pm 0.45$	$154.45^{d} \pm 1.04$	$146.35^{c} \pm 0.75$	$145.97^{c} \pm 0.48$	$141.33^{a} \pm 0.35$	$143.18^{b} \pm 0.29$	56.22**
Potassium (mmol/L)	$5.37^{b} \pm 0.15$	$5.43^{b} \pm 0.10$	$4.94^{a} \pm 0.09$	$5.08^{a} \pm 0.08$	$4.89^{a} \pm 0.04$	$5.05^{a} \pm 0.03$	6.09**
Chloride (mmol/L)	$108.38^{a} \pm 0.56$	$109.61^{b} \pm 0.76$	$108.75^{ab} \pm 0.38$	$108.72^{ab} \pm 0.30$	$111.50^{c} \pm 0.17$	$111.09^{c} \pm 0.22$	12.39**
Calcium (mg/dL)	$12.71^{c} \pm 0.61$	$12.17^{c} \pm 0.17$	$11.35^{b} \pm 0.10$	$11.32^{b} \pm 0.15$	$9.75^{a} \pm 0.11$	$9.95^{a} \pm 0.09$	26.21**
Magnesium (mg/dL)	$3.03^{c} \pm 0.05$	$3.03^{c} \pm 0.04$	$3.05^{c} \pm 0.05$	$3.09^{c} \pm 0.06$	$2.57^{a} \pm 0.04$	$2.8^{b} \pm 0.06$	14.55**

** Highly Significant ($P \leq 0.01$); Means bearing the same superscript within the same row do not differ significantly.

correlated with Albay *et al.* (2014). The value of potassium started to increase during the course of treatment in sub-clinical pregnancy toxaemic group. The hypokalemia observed in pregnancy toxaemic does may be attributed to the decrease in feed intake and dehydration (Judith and Thomas, 1988) or may be due to inadequate feed intake and incomplete renotubular absorption of potassium (Henze *et al.,* 1998), or may be due to lowered feed intake and due to los of potassium ions in the urine as observed in human patients with ketonuria and ketoacidosis (Lima *et al.,* 2016).

A highly significant ($P \leq 0.01$) difference was observed in calcium levels between pre and post treatment groups of sub-clinical pregnancy toxaemic group and control. The hypocalcemia observed in sub-clinical pregnancy toxaemic group correlated with Hefnawy *et al.* (2011). The level of calcium in sub-clinical pregnancy toxaemic group started to increase during the course of treatment. The hypocalcemia observed in sub-clinical pregnancy toxaemic goats may be due to the disturbance in the electrolytes and minerals which might be due to stress of starvation, dehydration, electrolyte imbalance or due to enhanced lipolysis (Judith and Thomas, 1988). Alternate reasons might be due to the high demand of calcium by the developing offspring at the late stage of gestation, due to enhanced lipolysis as a result of high cortisol level in circulation, or fatty liver interfering with hydroxylation of Vitamin D and decreased intestinal absorption of calcium (Andrews, 1997) or anorexia and disturbance of acid base balance (acidosis) with the excretion of calcium ions in urine or might be the sequelae to renal insufficiency (Rook, 2000).

A highly significant ($P \leq 0.01$) difference was observed in magnesium levels between pre and post treatment of sub-clinical pregnancy toxaemic group compared with that of control. The hypomagnesemia observed in sub-clinical pregnancy toxaemic group correlated with Hefnawy *et al.* (2011). However the magnesium levels started to increase during the course of treatment. Hypomagnesemia in pregnancy toxaemic goats may be due to the disturbance in the electrolytes and some minerals related to stress of starvation, dehydration, involvement of the kidney or due to enhanced lipolysis (Judith and Thomas, 1988).

A highly significant ($P \leq 0.01$) difference in chloride levels was observed between sub- clinical pregnancy toxaemic group and control. The hyperchloridemia observed in sub-clinical pregnancy toxaemic group correlated with Abdallah *et al.* (2015). However the value of chloride started to decline in the post treatment group. The reasons for hyperchloridemia in sub-clinical pregnancy toxaemia might be attributed to the metabolic acidosis as a result of proportionally smaller loss of chloride than bicarbonate and improved renal reabsorption of chloride in response to decreased bicarbonate (Kaneko *et al.,* 1997).

The Mean ± S.E. of serum beta hydroxybutyric acid (µmol/L), non esterified fatty acid (µmol/L) and cortisol (nmol/L) concentration in control and sub-clinical pregnancy toxaemic group assessed by ELISA method are presented in Table 5.

A highly significant ($P \leq 0.01$) difference in serum beta hydroxybutyric acid concentration was observed between sub clinical pregnancy toxaemic group and control which correlated with the findings of Ismail *et al.* (2008). Elevated levels of beta hydroxybutyric acid in the blood

Table 5: Mean ± S.E. of Serum Beta Hydroxybutyric acid (BHBA), Non Esterified Fatty Acid (NEFA) and Cortisol Concentration by ELISA method in Control and Sub Clinical Pregnancy Toxaemic Group

Parameters	Control : Livestock Farm Complex (LFC)		Sub Clinical Pregnancy Toxaemic Group (n = 12)	'F' value
	Pregnant Does 120 days (n = 12)	Pregnant Does 150 days (n = 12)		
Beta hydroxybutyric acid (BHBA) (µmol/L)	$275.0^{c} \pm 31.34$	$312.5^{c} \pm 29.51$	$1308.33^{a} \pm 58.34$	8.86**
Non Esterified Fatty Acid (NEFA) (µmol/L)	406.56 ± 49.23	434.42 ± 77.14	534.52 ± 89.17	2.03 NS
Cortisol (nmol/L)	$295.61^{a} \pm 54.53$	$348.32^{a} \pm 33.98$	$600.76^{b} \pm 111.55$	6.13**

NS – Not Significant; ** Highly Significant ($P \leq 0.01$); Means bearing the same superscript within the same row do not differ significantly.

might be attributed to the oxidation of long chain fatty acids into ketone bodies namely acetoacetate and beta hydroxy butyrate in the liver following lipolysis during periods of negative energy balance (Nassif *et al.,* 2005) or to the reduction of acetoacetate produced by the liver to beta hydroxybutyrate by hydroxybutyrate dehydrogenase enzyme amounting to higher blood concentration of beta hydroxybutyrate (Hefnawy *et al.,* 2011). Elevated level of serum non esterified fatty acid in sub-clinical pregnancy toxaemic does correlated with Ismail *et al.* (2008). Elevated levels of non esterified fatty acid might be the result of adipolysis during periods of negative energy balance (Vasava *et al*., 2016). A highly significant ($P \leq 0.01$) difference in serum cortisol concentration was observed between sub-clinical pregnancy toxaemic group and control. Increasing trend of cortisol concentration in pregnant and sub-clinical pregnancy toxaemic does correlated with Abdallah *et al.* (2015). Increase in cortisol concentration might be due to hyperactivity of the adrenal glands as a result of hypoglycemia (Adel *et al.,* 2005) or due to reduced hepatic metabolism of cortisol (Radostits *et al.,* 2000) or due to increasing stress in the pregnant animals (Aly and Elshahawy, 2016).

CONCLUSION

The present study showed a cure rate of 100 % in sub-clinical pregnancy toxaemic does. The early indicators of sub-clinical form of pregnancy toxaemia include presence of ketone body in the urine and blood BHBA concentration ≥ 0.8 mmol/L. Hence, the determination of blood BHBA concentration using a portable blood ketone meter and qualitative urinalysis using urine dip stick for the presence of ketone bodies are reliable indicators in the diagnosis of sub-clinical form of pregnancy toxaemia under field conditions.

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