

## Investigation on Some Biochemical and Clinical Parameters for Pregnancy Toxemia in Akkaraman Ewes

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**Abstract:** The aim of this study was to investigate some clinical and biochemical parameters for subclinical and clinical pregnancy toxemia and to determine the effect of early diagnosis on the success of curing. According to the results of clinical and biochemical parameters, 16 ewes were healthy (control group), 11 ewes had subclinical pregnancy toxemia (subclinic group) and 15 ewes had clinical pregnancy toxemia (clinic group). There was an increase at the levels of glucose, albumin, globulin in clinic group and Ca both in clinic and subclinic groups and a decrease at the levels of urea, AST, ALT in clinic group and BHBA both in clinic and subclinic groups after the treatment when compared with the period before treatment. Cure rates for subclinic and clinic groups were determined as 100% (11/11 ewes) and 73% (11/15 ewes), respectively. The parameters such as BHBA, glucose, ALT and AST should be taken in care especially in the period of last 6 weeks of pregnancy in ewes for early diagnosis of subclinical pregnancy toxemia.

**Key words:** Pregnancy toxemia,  $\beta$ -hydroxybutyrate, glucose, ewe, biochemical, parameters, diagnosis

### INTRODUCTION

Pregnancy toxemia is a metabolic disease commonly occurring in the last 6 weeks of gestation which causes significant economic losses and with a high mortality rate in pregnant ewes (Caldeira *et al.*, 2007). The disease is usually seen when the ewe is carrying 2 or more lambs (Schlumbohm and Harmeyer, 2008). It is rarely observed in ewes carrying singles. The disease is associated with low plasma concentrations of glucose and markedly increased plasma concentrations of ketone bodies (Scott *et al.*, 1995; Van Saun, 2000). It is believed that the disorder is caused by an inability of the twin-pregnant ewe to meet the increased glucose demand of the uteroplacental unit (Rook, 2000). Although, intense research for the underlying mediators of this disease remains unclear, a key role for high carbohydrate demand of multiple fetuses and negative energy balance in late pregnancy has been hypothesized (Hay and Baird, 1991).

The first symptoms for pregnancy toxemia are lie down, become sluggish and show a loss of appetite. Keto-acidosis is also common during toxemia and needs to be treated daily. Encephalopathy results from depressed glucose metabolism in the brain

(Andrews, 1997). As the disease progresses, the neurological systems become compromised due to lack of glucose. Blindness, stargazing, tremors, aimless walking, ataxia, are seen and eventually the ewes becomes comatose (Sargison, 2007).

The determination of blood glucose and  $\beta$ -Hydroxybutyrate (BHBA) concentrations is very important for early diagnosis (Bickhardt and Konig, 1985; Lacetera *et al.*, 2001). Laboratory findings in individual ewes may include hypoglycemia (often  $<2 \text{ mmol L}^{-1}$ ), elevated urine ketone levels, elevated BHBA levels (normal  $<0.8 \text{ mmol L}^{-1}$ , subclinical ketosis  $>0.8 \text{ mmol L}^{-1}$  and clinical disease  $>3.0 \text{ mmol L}^{-1}$ ) and frequently hypocalcemia and hyperkalemia due to severe ketoacidosis (Ramin *et al.*, 2007).

The treatment of advanced pregnancy toxemia is usually unsuccessful (Marteniuk and Herdt, 1988). If pregnancy toxemia is diagnosed in the early stages, medical treatment can be successful (Andrews, 1997; Sargison, 2007).

The aim of this study is to investigate some clinical and biochemical parameters for subclinical and clinical pregnancy toxemia and to determine the effect of early diagnosis on the success of curing.

## MATERIALS AND METHODS

**Study area and animals:** The disease was occurred in the flock existing 54 Akkaraman sheep. All animals in the flock were checked for the periods of pregnancy and the number of fetus by ultrasonography. In addition, the ewes were examined by systemic and clinical inspection.

According to the results of ultrasound inspection, 12 of these ewes were not pregnant and 42 ewes were pregnant (27 bearing single and 15 bearing twins or multiple). Blood samples were taken from the sheep that were pregnant only. The biochemical parameters were analysed. According to the results of clinical and biochemical parameters, it has been determined for 42 ewes that 16 of them were healthy ewes (control group), 11 ewes had subclinical pregnancy toxemia (subclinic group) and 15 ewes had clinical pregnancy toxemia (clinic group). The ewes in the study were at 17th week of gestation and aged between 3 and 6 years and weighing 40-60 kg. The mean BCS was  $3.7 \pm 1.4$  measured on a 0-5 point scale (Russel, 1991).

Clinical pregnancy toxemia in 15 ewes was diagnosed according to declared symptoms by Scott *et al.* (1995) and demonstration of plasma BHBA concentrations  $>3.0 \text{ mmol L}^{-1}$  (Marteniuk and Herdt, 1988). Subclinical pregnancy toxemia developed in 11 ewes (plasma BHBA concentration  $>0.86 \text{ mmol L}^{-1}$  without any clinical signs of disease) (Lacetera *et al.*, 2001).

The ewes were fed a diet of hay (1 kg/day/ewe, metabolizable energy  $8 \text{ MJ kg}^{-1}$  dry matter) and concentrate (0.5 kg/day/ewe, metabolizable energy  $9.5 \text{ MJ kg}^{-1}$  dry matter) by the animal keeper up to 17 weeks of the gestation. After the diagnosis of the disease at the 17th week of the gestation, the ewes were begun to feed a diet of hay (1 kg/day/ewe, metabolizable energy  $8 \text{ MJ kg}^{-1}$  dry matter), concentrate (0.9 kg/day/ewe, metabolizable energy  $11.9 \text{ MJ kg}^{-1}$  dry matter) and a commercial mineral mixture until the parturition (Table 1). The animals had free access to water throughout the experiment.

**Blood and urine analysis:** Blood samples (5.0 mL) were collected into heparinized tubes from the jugular vein. Blood was centrifuged at  $3000 \times \text{rpm}$  for 10 min plasma was decanted. The levels of glucose, total protein, albumin, triglyceride, cholesterol, creatinin, urea, Ca, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were analysed within 6 h of collection on a Technicon RA-XT autoanalyser using commercial kits (Sigma Chemical Co. Ltd., Poole, Dorset, UK). Globulin concentration was calculated as total protein minus albumin values. Plasma BHBA

Table 1: Average daily intake of metabolizable energy (MJ) and digestible crude protien (nitrogen  $\times 6.25 \text{ g}$ ) by the ewes

	Pregnant ewes (days pregnant)	
	64-119	119-147
Diet	64-119	119-147
Metabolizable energy	12.75	18.71
Digestible crude protien	95.00	115.0

concentration was determined using the ultraviolet enzymatic method (Williamson *et al.*, 1962). Urine analyses including leukocyte counts, nitrate, urobilinogen, protein, pH, erythrocyte, specific gravity, ketone, glucose were performed with Bayer Multistix 10 SG®. One week after the initial therapy, physical examination blood and urine analysis of the animals were repeated.

**Treatment of pregnancy toxemia:** An i.v. injection of 100 mL of 30% dextrose was given to each ewe that had pregnancy toxemia at 1st day. Then, it was continued to a cure with 100 mL of 10% dextrose. These ewes also received 50 mL propylene glycol orally twice daily. In addition, i.v. administration of 250 mL electrolyte solutions with sodium bicarbonate and multiple B vitamins were done. The calcium borogluconate was given 50 mL by i.v. to ewes with a low plasma Ca levels. Treatment continued until the ewe had recovered, with normal locomotion, alert appearance and a good appetite. Avarage period of cure has been continued for 5 days.

**Post-mortem examination:** Four sheep with a heavy pregnancy toxemia which died in clinics group were examined post mortem and major macroscopic problems noted.

**Statistical analysis:** All the results were expressed as the mean  $\pm$  SD. A one-way repeated measures Analysis of Variance (ANOVA) was used to determine statistical differences between mean values of the studied parameters of the observation period. The data were evaluated to be within the groups and among the groups. All results were statically compared between before and after treatment ewes using an independent-sample t-test.

## RESULTS

**Clinical and post-mortem findings:** Of ewes in subclinic group, 6 have got single fetus (54%) and 5 have got twin fetus (46%). Of ewes in clinic group, 5 (33%) have got singe fetus and 10 have got twin fetus (66%). The mean BCS for 2, 4 and 5 of ewes in subclinic group and for 2, 5 and 8 of ewes in clinic group were 2-3, 3-4 and 4-5, respectively.

The predominant clinical findings observed in clinic group were depression, anorexia, weakness, slightly dehydration. Few of them displayed signs of incoordination, lipping, reluctance to walk, teeth gnashing, amaurosis and nervous symptoms such as circling movements, wandering, chewing movements with salivation. Temperature, respiration and pulse usually were within normal limits in all groups. Only, at 6 ewes in clinic group, the respiratory rate was decreased and the type of respiration was abdominal, at 6 ewes tachycardia was found. A smell of ketones was detected on the breath, which strongly suggested pregnancy toxemia. However, it was clearer in the clinic group.

Urine analysis was unremarkable, except for ketonuria (urine ketone bodies in group subclinic ++, in group clinic +++ with Bayer Multistix 10 SG®).

There was extensive fatty infiltration of the liver in necropsia of dead ewes. The color ranged from pale pink to brilliant orange-yellow. There was no gross lesion in the other organs on macroscopic examination.

**Biochemical findings:** Changes of the biochemical parameters in the plasma and significant differences of data between control, subclinic and clinic groups are presented in Table 2.

Before treatment, glucose, cholesterol, total protein, albumin, globulin and Ca levels were found statistically lower ( $p < 0.05$ ) than the other 2 groups in clinic group; as for BHBA, triglyceride, urea, AST and ALT were found higher ( $p < 0.05$ ) than the other groups.

After treatment, cholesterol, total protein levels were statistically found lower ( $p < 0.05$ ) than the other 2 groups; as for BHBA, triglyceride, urea, AST and ALT were found higher ( $p < 0.05$ ) than the other groups in clinic group.

Before treatment, glucose, Ca levels were found statistically lower ( $p < 0.05$ ) than the control groups in subclinic group; as for BHBA and urea were found higher ( $p < 0.05$ ) than the control group. There were no significant difference ( $p > 0.05$ ) for amongs of measured parameters in subclinic group and clinic group after treatment.

There was an increase at the levels of glucose, albumin, globulin in clinic group and Ca both in clinic and subclinic groups and a decrease at the levels of urea, AST, ALT in clinic group and BHBA both in clinic and subclinic groups after the treatment when compared with the period before treatment.

**Cure rates:** Cure rates for subclinic and clinic groups were determined as 100% (11/11 ewes) and 73% (11/15 ewes), respectively.

Table 2: Some biochemical parameters in control (n = 16), subclinic (n = 11) and clinic (before treatment n = 15, after treatment n = 11) groups

Parameters	Control	Subclinic	Clinic
<b>BHBA (mmol L<sup>-1</sup>)</b>			
Before treatment	0.45±0.02 <sup>a</sup>	1.28±0.19 <sup>ab</sup>	5.57±0.64 <sup>ac</sup>
After treatment	0.52±0.04 <sup>a</sup>	0.64±0.08 <sup>ba</sup>	1.05±0.09 <sup>bb</sup>
<b>Glucose (mmol L<sup>-1</sup>)</b>			
Before treatment	2.84±0.13 <sup>a</sup>	1.90±0.07 <sup>ab</sup>	1.07±0.11 <sup>ac</sup>
After treatment	2.74±0.09	2.78±0.05 <sup>b</sup>	2.75±0.12 <sup>b</sup>
<b>Triglyceride (mmol L<sup>-1</sup>)</b>			
Before treatment	0.61±0.02 <sup>a</sup>	0.68±0.03 <sup>a</sup>	1.12±0.05 <sup>b</sup>
After treatment	0.64±0.03 <sup>a</sup>	0.66±0.02 <sup>a</sup>	1.01±0.06 <sup>b</sup>
<b>Cholesterol (mmol L<sup>-1</sup>)</b>			
Before treatment	2.06±0.11 <sup>a</sup>	1.77±0.06 <sup>a</sup>	1.17±0.05 <sup>b</sup>
After treatment	2.00±0.09 <sup>a</sup>	1.88±0.05 <sup>a</sup>	1.30±0.05 <sup>b</sup>
<b>Urea (mmol L<sup>-1</sup>)</b>			
Before treatment	5.39±0.46 <sup>a</sup>	6.85±0.54 <sup>ab</sup>	9.13±0.82 <sup>ac</sup>
After treatment	5.85±0.58 <sup>a</sup>	5.78±0.48 <sup>ba</sup>	7.03±0.94 <sup>bb</sup>
<b>Total protein (g L<sup>-1</sup>)</b>			
Before treatment	71.7±4.43 <sup>a</sup>	65.4±5.84 <sup>ab</sup>	53.4±5.42 <sup>b</sup>
After treatment	69.4±2.05 <sup>a</sup>	68.3±3.45 <sup>a</sup>	63.1±5.61 <sup>b</sup>
<b>Albumin (g L<sup>-1</sup>)</b>			
Before treatment	34.2±2.21 <sup>a</sup>	32.4±3.32 <sup>a</sup>	26.4±2.34 <sup>ab</sup>
After treatment	33.0±3.14	33.4±2.27	30.5±2.29 <sup>b</sup>
<b>Globulin (g L<sup>-1</sup>)</b>			
Before treatment	37.4±2.30 <sup>a</sup>	33.1±4.40 <sup>a</sup>	26.9±2.37 <sup>ab</sup>
After treatment	35.9±2.34	35.2±3.36	32.5±3.43 <sup>b</sup>
<b>AST (U L<sup>-1</sup>)</b>			
Before treatment	38.1±3.32 <sup>a</sup>	48.2±5.63 <sup>ab</sup>	114.3±8.77 <sup>ab</sup>
After treatment	40.3±2.82 <sup>a</sup>	45.4±3.45 <sup>a</sup>	79.1±5.42 <sup>bb</sup>
<b>ALT (U L<sup>-1</sup>)</b>			
Before treatment	18.3±2.10 <sup>a</sup>	20.5±3.04 <sup>a</sup>	53.4±6.36 <sup>ab</sup>
After treatment	20.1±1.83 <sup>a</sup>	21.3±2.41 <sup>a</sup>	39.1±4.79 <sup>bb</sup>
<b>Ca (mmol L<sup>-1</sup>)</b>			
Before treatment	2.23±0.18 <sup>a</sup>	2.05±0.15 <sup>ab</sup>	1.95±0.12 <sup>ab</sup>
After treatment	2.21±0.19	2.23±0.16 <sup>b</sup>	2.17±0.14 <sup>b</sup>

Different superscript letters (A, B, C) within same column indicate significant ( $p < 0.05$ ) differences among groups. Different superscript letters (a, b, c) within same row indicate significant ( $p < 0.05$ ) differences among groups

## DISCUSSION

Reported predispose factors (Van Saun, 2000; Schlumbohm and Harmeyer, 2008) such as obesity, inadequate ration with regard to carbohydrate in the late pregnancy and lack of feeding according to the number of fetus had been established in the flock. It was also reported that (Hay and Baird, 1991) the disease was determined in single pregnant and weak ewes which is similar to our findings. In clinic group, ewes with twin fetus were more than single ones and in subclinic group in opposite the single ewes were in generality. In this study, glucose levels of ewes in clinic group were lower than ewes in subclinic group. This may be because of inability of the twin-pregnant ewe to meet the increased glucose demand of the uteroplacental unit (Rook, 2000).

Although, the temperature, heart and respiration rates of animals both in subclinic and clinic groups were usually normal which was similar to the literature (Kabakci *et al.*, 2003; Sargison, 2007; Barakat *et al.*, 2007).

But, the respiratory rate decreased at 6 ewes and tachycardia was found at 4 ewes in clinic group. However, it was met to severe neural symptoms at four ewes in clinic group. These ewes dead in 1-3 days period. There was smell of ketones on breath and ketonuria in both groups. These had been pointed out to be the characteristic findings for pregnancy toxemia (Barakat *et al.*, 2007).

The measured values in the control group were similar to the values in our previous studies that we established for healthy ewes in the same race (Yildiz *et al.*, 2005; Balıkcı *et al.*, 2007).

Blood glucose levels between 20 and 40 mg dL<sup>-1</sup> or lower (Henze *et al.*, 1994; Kabakcı *et al.*, 2003) are common in pregnancy toxemia. The value below 20 mg dL<sup>-1</sup> was considered as subclinical pregnancy toxemia (Robinson, 1980; Ramin *et al.*, 2007). In this study, blood glucose levels were found as 1.90 mmol L<sup>-1</sup> (34.2 mg dL<sup>-1</sup>) in subclinic group and 1.07 mmol L<sup>-1</sup> (19.3 mg dL<sup>-1</sup>) in clinic group. Furthermore, the relationship between the severity of neurological symptoms and hypoglycemia (Moghaddam and Hassanpour, 2008) provides additional clinical evidence of hypoglycemic encephalopathy. Hypoglycemic encephalopathy may become irreversible in the later stages of pregnancy toxemia (Burswell *et al.*, 1986). In this study, the fixed neurological symptoms in some patients of clinic group were thought to result from severe hypoglycemia. In later stages of the disease they may enter renal failure and have an altered acidbase status. Comatose animals may show terminal hyperglycemia, especially associated with fetal death (Marteniuk and Herdt, 1988; Moghaddam and Hassanpour, 2008). In the study, normoglycemia was determined in 2 ewes and hyperglycemia was also determined in other 2 ewes in clinic group and then these 4 ewes dead in 1-3 days period. The extensive fatty infiltration of the liver determined in necropsy of this dead animals has been reported by other researchers (Tontis and Zwahlen, 1987; Kabakcı *et al.*, 2003).

BHBA is the predominant circulating ketone body (Radostits *et al.*, 2007). Bickhardt and König (1985) found that the increase in plasma BHBA level is a sensitive indicator for the complex alteration of glucose and lipid metabolism in pregnancy toxemia. A BHBA level >0.85 mmol L<sup>-1</sup> in ewes is a sign of energy deficiency (Dawson *et al.*, 1999). Scott and Woodman (1993) found that BHBA levels increased to 7.2 mmol L<sup>-1</sup> in pregnant ewes with energy deficiency. This observation was consistent with the results of some previous reports (Scott and Woodman, 1993; Everts, 1990; Durak and Altiner, 2006). If the concentration of BHBA is >0.7 mmol L<sup>-1</sup>, it is considered as sub clinical pregnancy

toxemia (Rook, 2000; Ramin *et al.*, 2007). Plasma BHBA concentration around 5-7 mmol L<sup>-1</sup> was regarded to be similar to that usually present in ewes with clinical symptoms of pregnancy toxemia (Ford *et al.*, 1990; Henze *et al.*, 1998). But according to Robinson (1980) and Lacetera *et al.* (2001), the increase of BHBA to 0.86 and 1.6 mmol L<sup>-1</sup> leads subclinical and clinical pregnancy toxemia in ewes, respectively. In this study, BHBA concentrations were found to be 1.28 and 5.57 mmol L<sup>-1</sup> in the subclinic and the clinic groups, respectively.

It has been reported that serum triglycerid levels increase and serum cholesterol levels decrease in pregnancy toxemia ewes (Marteniuk and Herdt, 1988; Van Saun, 2000; Kabakcı *et al.*, 2003). In this study, the triglycerid levels increased and cholesterol levels decreased statistically in both clinic and subclinic groups in comparison with control group. This may be due to anorexia and potential hepatic dysfunction associated with fatty infiltration.

Hypoalbuminemia and slight increase in serum globulin were reported in pregnancy toxemia (Barakat *et al.*, 2007). But in another study (Yarim and Ciftci, 2008), serum levels of albumin, globulin and total protein were found to be significantly lower in pregnancy toxemia than in uncomplicated pregnant ewes. In present study, plasma albumin, globulin and total protein levels decreased nonstatistically in subclinic group when compared with control group and a statistical decrease was determined in clinic group when compared with the other 2 groups. Decreased serum albumin levels in ewes with pregnancy toxemia may also be explained by hepatic and renal failure associated with pregnancy toxemia.

High plasma urea levels occur generally in ewes suffering from poor nutrition in the last period of pregnancy (Andrews, 1997). In this study, urea levels increased statistically more in the clinic group than subclinic and control groups. But, the increase of urea levels in subclinic group was higher than control group. Our observation was similar to the results reported by other researchers (Durak and Altiner, 2006; Ramin *et al.*, 2007; Yarim and Ciftci, 2008; Moghaddam and Hassanpour, 2008). This increase is related to the severity of the disease. The reason of this increase could be because of increased cortisol levels in the catabolism of proteins in the body (Silanikove, 2000). And also, this increase is thought to be a result of hepatic and renal failures in clinic group. Thus, affected proximal tubular epithelium and adrenal cortex of the kidney in this disease were reported by some researchers (Tontis and Zwahlen, 1987; Marteniuk and Herdt, 1988).

Plasma AST and ALT levels increase in kidney infection, mononucleosis and liver damage (Kaneko *et al.*, 1997). In the study, plasma AST and ALT levels increased in clinic group when compared with the other two groups. This observation was consistent with the results of some previous reports (Kabakci *et al.*, 2003; Sargison, 2007; Yarim and Ciftci, 2008). The increases in this study may be due to the liver damage. Severe hepatic changes were observed in necropsy of 4 dead ewes in the study.

Occasionally pregnancy toxemia is with hypocalcemia (Scott *et al.*, 1995; Rook, 2000). Reduction in feed intake by the late gestating multiparous ewe will enforce negative energy balance, mobilization of body stores and hepatic ketogenesis. Hypocalcaemia augments the depressive action of hyperketonaemia on hepatic glucose production (Schlumbohm and Harmeyer, 1999). In fact the contributing role of hypocalcaemia on development of pregnancy ketosis has been indicated in earlier studies (Simensen, 1971). In the present study, the decrease in plasma Ca levels was statistically more important than the control group. The observed decrease in calcium level at late pregnancy could be attributed to increased demand for calcium for mineralization of foetal skeleton (Azab and Abdel-Maksoud, 1999). Furthermore, starvation also reduces intestinal calcium absorption and lowers blood calcium concentration (Rook, 2000).

In this study, 4 ewes that had severe neurological symptom didn't answer to cure and then they dead in a period of 1-3 days. The treatments of ewes with severe pregnancy toxemia and the encephalopathy development were usually unsuccessful (Burswell *et al.*, 1986; Marteniuk and Herdt, 1988). Cure rates for subclinical and clinic groups in this study were determined as 100% (11/11 ewes) and 73% (11/15 ewes), respectively. Early diagnosis effects the cure positively and by the way the chance of healing increases (Kabakci *et al.*, 2003; Sargison, 2007).

### CONCLUSION

All the ewes in subclinical group with no clinical symptoms and 11 ewes in clinic group healed as a result of therapy. The parameters such as BHBA, glucose, ALT and AST should be taken in care especially in the period of last 6 weeks of pregnancy in ewes for early diagnosis of subclinical pregnancy toxemia.

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