

## SERUM PARAOXONASE-1 ACTIVITY AND METABOLIC PROFILE IN EWES WITH PREGNANCY TOXAEMIA

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### ABSTRACT

This research intended to assess the energy metabolism indices (BHBA, NEFA and glucose) and evaluate the diagnostic relevance of oxidative stress indicators oxidant (MDA) and antioxidant (TAC and PON-1 enzyme), lipid profile and liver enzymes in pregnancy toxemia in ewes. The study comprised a total number of 72 late pregnant ewes (14-20 weeks of gestation). Ewes were divided based on the result of clinical and biochemical findings (glucose and BHBA) into three groups; a clinical pregnant toxemic group (CPTG) (n=36), a subclinical PTG (n=20) and a control group (CG) (n=16). Blood samples were collected for each group and a panel of biochemical parameters was measured. Most of the biochemical markers showed notable variations in comparison with the control group ( $P < 0.05$ ). BHBA, NEFA, MDA, Liver enzymes (ALT, AST and  $\gamma$ GT), Triglycerides and VLDL in clinical and subclinical pregnant toxemic groups showed a significant increase above normal values of control group ( $P < 0.05$ ). Glucose, TAC, PON-1 enzyme, Cholesterol, HDL, LDL and serum proteins in clinical and subclinical pregnant toxemic groups showed a significant decrease below normal values of control group ( $P < 0.05$ ). Based on these findings, we suggest that monitoring changes in the presented energy metabolism indices, oxidant-antioxidant status, lipid profile, liver enzymes and protein assays in late pregnant ewes could be useful in early diagnosis of pregnancy toxemia. The present study also showed that paraoxonase-1 enzyme (PON1) appears to hold potential as a biomarker for diagnosis of pregnancy toxemia in ewes.

**Key words:** Pregnancy toxemia, metabolism indices, paraoxonase-1 enzyme (PON1), lipid profile, oxidative stress.

### INTRODUCTION

Pregnancy toxemia (PT) is regarded as a serious illness that poses a significant threat to the productivity of sheep and goats (Kulcsar *et al.*, 2006). It affects ewes and does throughout the periparturient phase, ranking

among the most prevalent metabolic diseases (Santos *et al.*, 2011).

Metabolic profile of sheep is defined as a series of specific analytical tests or combination of blood constituents carried out on groups of animals at several critical stages of the periparturient period and used as a diagnostic aid to detect abnormalities on the herd basis (Hernández *et al.*, 2020).

Negative energy balance (NEB) typically affects sheep during their last stages of

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pregnancy (Cal-Pereyra *et al.*, 2015). According to Changzheng Guo and co-workers (2020), sheep possess a system for adaptability, that has the capability to mobilize lipids and free the adipose tissue's stored energy to prevent NEB. The insufficiency of oxaloacetate results in the accumulation of acetyl-CoA produced from the oxidation of fatty acids, which prevents it entering the tricarboxylic acid cycle (Changzheng Guo *et al.*, 2020). As a result, the creation of ketone bodies rises as a compensatory mechanism, but does not generally resolve negative energy balance (NEB). Furthermore, a fatty liver can develop as a result of an excessive mobilisation of free fatty acids (FFA), which overwhelms the liver's ability to oxidize them (Changzheng Guo *et al.*, 2020). As a consequence, the metabolic function of the liver is compromised.

Beta hydroxybutyrate (BHB) and cortisol, which are essential for initiating oxidative stress and oxidative degradation of lipids in ewes with excessive ketone body concentrations, are significantly elevated in the serum during PT (Al-Qudah, 2011). BHB concentrations in healthy ewes are typically less than 0.8 mmol/L (Olfati and Moghaddam, 2013). However, BHB levels between (0.8-1.6 mmol/L) may indicate moderate malnutrition (subclinical pregnancy toxemia) and concentrations in ewes with clinical pregnant toxemia are greater than 3 mmol/L (Olfati and Moghaddam, 2013).

Oxidative stress can occur during the periparturient period, resulting in certain periparturient problems, that could have significant impacts on both animal welfare and production (Celi, 2011). Evaluating the intensity and severity of oxidative stress are both possible through the detection of lipid peroxidation metabolites (Ghada and Hayam, 2019).

Paraoxonase-1 enzyme is a glycoprotein, produced mainly by the liver and connected to high density lipoprotein. It plays a critical role in protecting lipoproteins from oxidative

stress damage (Fukumori *et al.*, 2020). Furthermore, it has been classified as negative acute phase protein (Pradeep, 2014). Negative acute phase proteins are thought to be an effective biomarker for assessing the impact of inflammation throughout the periparturient phase (Hanan *et al.*, 2019).

Paraoxonase-1 is considered as an essential part of the mammalian normal anti-oxidative mechanism, that's breakdown lipid hydroperoxides and oxidized phospholipids formed during oxidative stress, so it is specified as an anti-oxidative and anti-inflammatory enzyme (Fukumori *et al.*, 2020). PON1 plays multiple roles in a variety of metabolic processes, including preventing lipid peroxidation, supporting innate immunity and detoxifying reactive metabolites (Ceron *et al.*, 2014).

NEB stimulates homeostatic pathways including several metabolic processes and how the body distributes its energy. So, liver glucose synthesis and fat mobilization rates have significantly increased, this lead to more variations in serum concentrations of lipid parameters (Van Knegsel *et al.*, 2005). Fat mobilization also associated with increased levels of NEFA and BHBA during NEB, both of which are considered as markers of lipid mobilisation (Van Knegsel *et al.*, 2005).

Given that using metabolism indices and oxidative stress biomarkers are the suggested method for assessing health of late pregnant ewes during this critical period, we hypothesize that activity of paraoxonase-1 (PoN-1), BHBA, NEFA and changes in lipid profile may be used to predict the risk of pregnancy toxemia in ewes.

Therefore, this research aimed to investigate the energy metabolism indices, including BHBA, NEFA and glucose, assessing the diagnostic potential of oxidative stress markers, including oxidant (MDA) and antioxidants (TAC and PON1 enzyme), lipid profile and liver enzymes in ewes with pregnancy toxemia during late pregnancy. Additionally, the purpose of this research was

to evaluate the impact of pregnancy toxemia on the mobilisation of lipids and paraoxonase-1 activities in late-pregnant ewes.

## MATERIALS AND METHODS

### Animals:

For this research, 72 late-pregnant ewes, aged 2-5 years, with a gestational period of 14-20 weeks were examined, from October 2021 to July 2022. Ten ewes were selected from the Veterinary Teaching Hospital, Assiut University, while the remaining 62 ewes were selected from two private farms at El-Minia Governorate, Egypt. According to the results of clinical and biochemical findings, including glucose and BHBA levels, ewes were classified into three groups (Gurdogan *et al.*, 2014).

### Clinical examination:

Complete clinical investigation was conducted to all ewes in the study as reported elsewhere with the techniques of (Diffay *et al.*, 2004), including complete case history, temperature of the animal, heart rate and rhythm, respiratory rate and rhythm and rumen contraction were monitored.

### Blood samples:

Blood samples were obtained from each case under study. Serum and plasma samples were collected from the jugular vein. Serum samples were obtained using plain tubes without an anticoagulant for the estimation of metabolic profile indices, oxidative stress markers, lipid profile, liver enzymes, and serum proteins. To measure the amount of glucose in plasma, sodium fluoride tubes were used. Both blood samples were centrifuged for 15 minutes at 3000 rpm, and then preserved at -20°C for analysis.

### Biochemical investigation:

Serum biochemical variables including BHBA, NEFA and PON1 enzyme were measured using commercial ELISA test kits (SinoGeneClon Biotech Co., Ltd.). Glucose, serum MDA, TAC, total cholesterol, triglyceride, high density lipoprotein (HDL-C),  $\gamma$ -Glutamyltransferase ( $\gamma$ GT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) albumin and total proteins were measured using commercially available reagent test kits (Bio-Diagnostic, Giza, Egypt for MDA and TAC and by Spectrum Diagnostics, Egyptian Company for Biotechnology). Spectrophotometric assays were performed using (Optizen 3220 UV, Mecasys, Korea). Low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were estimated according to Basoglu *et al.* (2002):

- VLDL = triglyceride / 5.
- LDL = total cholesterol - (HDL cholesterol + triglyceride / 5).

### Statistical Analysis:

The obtained data were statistically analyzed using the statistical software program (IBM SPSS version 21). The data are presented as mean  $\pm$  standard deviation (SD) at a significant level of  $P < 0.05$ . The results of control group (n=16), subclinical group (n=20) and clinical group (n=36) analysis were compared by one-way ANOVA, followed by LSD test to detect the differences between the groups at each point of examination.

## RESULTS

Based on the results of clinical and biochemical findings, including glucose and BHBA levels, animals were classified into three groups (table 1).

**Table 1:** Classification of animals groups according to the results of clinical and biochemical findings.

Group parameter	Control group (CG) (n=16)	Subclinical group (SCPTG) (n=20)	Clinical group (CPTG) (n=36)
Temperature	39.3±0.4°C	39.1±0.5°C	Early stages (39.1±0.2°C), terminal stage ( 38±0.4°C )
Heart rate (beats/min)	77±8	72±8	102±6
Respiratory rate (breaths/ min)	18±5	22±3	36±8
Appetite	Normal	Decreased	Decreased and anorexia
Ruminal cycle	3-4/2 minutes	1-2/2 minutes	1/2 minutes or completely absent
BHBA (mmol/l)	0.81 ± 0.15	1.45 ± 0.2	4.09 ± 1.25
Glucose (mg/dl)	50.75±13.63	45.81 ± 4.21	31.64 ± 7.3

**Clinical examination:**

Subclinical pregnant toxæmic ewes did not show any apparent signs of disease, but they exhibited a decreased appetite, while their body temperatures, heart rates and respiration rates were all within normal limits.

Clinical pregnancy toxæmic ewes showed, in the first stage (early stage), inappetence (75%), scanty faeces (97.2%), dullness, depression (72.2%) and unwilling to move (69.4%) while in a standing position (photo 1). They also exhibited a decrease in ruminal cycles (72.2%), grinding of teeth (77.8%) and normal values for heart rate, respiratory rate and temperature. In the second stage (advanced stage), ewes showed sternal recumbency (33.3%), weakness, loss of condition, anorexia (25%) and a pronounced acetone odor from the mouth

and breath (47.2%). They also displayed nervous manifestations (41.7%) such as apparent blindness, head pressing, incoordination to movement (47.2%), neck muscular spasms, stiffness in the whole body and lateral deviation of the head and neck (photo 2). Heart and respiratory rates were decreased, and as the disease developed rapidly, medical intervention was required to induce parturition. The ewe was pregnant with triplets, all of which were stillborn (photo 3). In the third stage (terminal stage), ewes displayed lateral recumbency (13.9%), anorexia, severe dehydration (25%) and were comatose (photo 4). They also showed subnormal body temperature, tachycardia and labored breathing. The average number and percentage of animals that showed each set of symptoms was presented in table 2.

**Table 2:** Number and percentage of clinical symptoms recorded from clinical pregnant toxemic ewes.

Clinical symptoms	Number of affected animals Total number (36)	Percentage (%)
Inappetence	n=27	75 %
Dullness and depression	n=26	72.2 %
Anorexia	n=9	25 %
Sternal recumbency	n=12	33.3 %
Lateral recumbency	n=5	13.9 %
Acetone odor from mouth	n=17	47.2 %
unwilling to move	n=25	69.4 %
Incoordination	n=17	47.2 %
Scanty feces	n=35	97.2 %
Decrease ruminant cycles	n=26	72.2 %
Grinding of teeth	n=28	77.8 %
Nervous manifestation	n=15	41.7 %
Dehydration	n=9	25 %

**Photo 1:** A pregnancy toxaeic ewe shows dullness and depression**Photo 2:** A depressed pregnancy toxaeic ewe with sternal recumbency, exhibiting weakness, and lateral deviation of the head towards the flank region.**Photo 3:** A recumbent, dehydrated pregnancy toxaeic ewe with a deviated head, due to the rapid development of the disease, medical intervention was necessary to induce parturition, which resulted in the delivery of triplets that were stillborn.**Photo 4:** A pregnancy toxaeic ewe exhibiting lateral recumbence and a comatose state.

**Biochemical investigations:**

BHBA and NEFA levels in subclinical and clinical PTG exhibited significantly increased levels ( $P < 0.05$ ) than the healthy CG. Additionally, the levels of BHBA and NEFA was significantly higher ( $P < 0.01$ ) in between the subclinical and clinical PTG (fig. 1 and 2). In comparison with CG, glucose levels showed no significant changes in subclinical PTG, whereas its level exhibited a highly significant decrease in clinically PTG ( $P < 0.01$ ) (fig. 3). The results are summarized in table 3.

Both of the subclinical and clinical PTG had increased levels ( $P < 0.01$ ) of malondialdehyde (MDA) (fig. 4) and lower levels ( $P < 0.01$ ) of paroxonase-1 enzyme (PON1) and TAC compared to CG (fig. 5 and 6). Additionally, malondialdehyde (MDA) and paroxonase-1 enzyme (PON1) levels between the groups with subclinical and clinical pregnancy toxemia did not differ significantly (fig. 4 and 6). All results were presented in table 3.

In the subclinical pregnancy toxemia group, AST and  $\gamma$ GT levels increased in a highly significant way ( $P < 0.01$ ) in comparison to

CG. (fig. 7), while no changes were observed in ALT levels (fig. 7). In contrast, comparing the clinical pregnant toxemic group with CG, both AST and ALT levels showed a very significant rise ( $P < 0.01$ ), while  $\gamma$ GT levels revealed a substantial increase ( $P < 0.05$ ) (fig. 7). All results were presented in table 3.

As compared to CG, triglyceride and VLDL levels in both subclinical and clinical PTG exhibited a very significant rise ( $P < 0.01$ ) (fig. 8), while cholesterol, HDL, and LDL levels in both groups revealed a highly significant reduction ( $P < 0.01$ ) (fig. 8). Even so, no discernible variations in HDL and LDL values between the groups with subclinical and clinical pregnancy toxemia were seen (fig. 8). All results were presented in table 4.

Serum total proteins and albumin levels revealed a very significant reduction ( $P < 0.01$ ) in both subclinical and clinical PTG compared to CG (fig. 9), while globulin levels and A/G ratio had significantly decreased ( $P < 0.05$ ) in both groups in comparison with CG (fig. 9). All results were presented in table 4.

**Table 3:** Mean  $\pm$  SD values of metabolic profile, oxidative stress markers and liver enzymes in control group, subclinical and clinical PT groups.

Parameter	Control group	Subclinical group	Clinical group
BHBA (mmol/l)	0.81 $\pm$ 0.15 <sup>a</sup>	1.45 $\pm$ 0.2 <sup>b</sup>	4.09 $\pm$ 1.25 <sup>c</sup>
NEFA (umol/l)	183.5 $\pm$ 12.84 <sup>a</sup>	260 $\pm$ 14.73 <sup>b</sup>	314.56 $\pm$ 12.03 <sup>c</sup>
Glucose (mg/dl)	50.75 $\pm$ 13.63 <sup>a</sup>	45.81 $\pm$ 4.21 <sup>a</sup>	31.64 $\pm$ 7.3 <sup>b</sup>
MDA (nmol/ml)	5.18 $\pm$ 1.88 <sup>a</sup>	8.27 $\pm$ 2.507 <sup>b</sup>	9.17 $\pm$ 3.05 <sup>b</sup>
TAC (mmol/L)	0.67 $\pm$ 0.12 <sup>a</sup>	0.50 $\pm$ 0.09 <sup>b</sup>	0.38 $\pm$ 0.10 <sup>c</sup>
PON-1 (ng/ml)	98.25 $\pm$ 10.6 <sup>a</sup>	47.38 $\pm$ 13.39 <sup>b</sup>	42.67 $\pm$ 7.97 <sup>b</sup>
ALT (U/L)	25.4 $\pm$ 3.57 <sup>a</sup>	32.46 $\pm$ 4.35 <sup>a</sup>	49.24 $\pm$ 3.65 <sup>b</sup>
AST (U/L)	70.71 $\pm$ 13.55 <sup>a</sup>	156.32 $\pm$ 20.97 <sup>b</sup>	173.06 $\pm$ 29.95 <sup>c</sup>
GGT (U/L)	40.75 $\pm$ 10.37 <sup>a</sup>	60.71 $\pm$ 18.06 <sup>b</sup>	51.52 $\pm$ 15.83 <sup>c</sup>

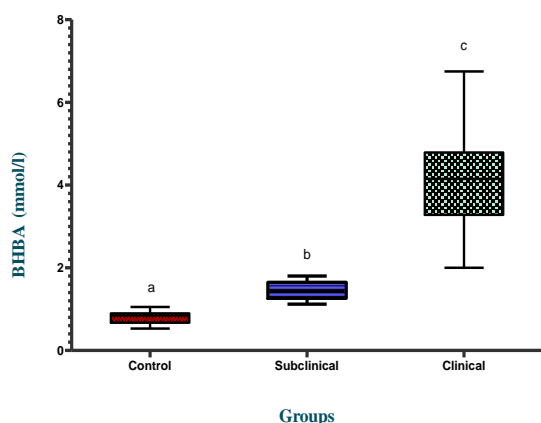
In each raw value followed by different superscript letters (a, b, c) within the same row indicate significant variations among groups ( $P < 0.05$ ).



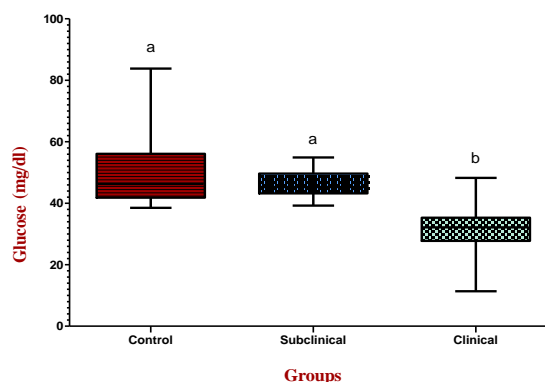
**Table 4:** Mean ± SD values of lipid profile and serum proteins in control group, subclinical and clinical PT groups.

Parameter	Control group	Subclinical group	Clinical group
Cholesterol (mg/dl)	92.88 ± 15.10 <sup>a</sup>	58.28 ± 8.22 <sup>b</sup>	50.59 ± 7.99 <sup>c</sup>
Triglycerides (g/dl)	46.96 ± 11.64 <sup>a</sup>	71.49 ± 15.63 <sup>b</sup>	79.34 ± 12.20 <sup>c</sup>
HDL (mg/dl)	48.50 ± 5.96 <sup>a</sup>	30.87 ± 8.23 <sup>b</sup>	30.53 ± 10.18 <sup>b</sup>
LDL (mg/dl)	31.24 ± 16.92 <sup>a</sup>	15.53 ± 10.01 <sup>b</sup>	12.06 ± 5.85 <sup>b</sup>
VLDL (mg/dl)	9.22 ± 2.21 <sup>a</sup>	14.24 ± 2.53 <sup>b</sup>	13.91 ± 3.42 <sup>c</sup>
Total proteins (g/dl)	5.50 ± 0.88 <sup>a</sup>	4.10 ± 1.09 <sup>b</sup>	3.49 ± 0.74 <sup>c</sup>
Albumin (g/dl)	3.14 ± 0.36 <sup>a</sup>	2.70 ± 0.54 <sup>b</sup>	2.42 ± 0.44 <sup>c</sup>
Globulins (g/dl)	2.37 ± 0.89 <sup>a</sup>	1.43 ± 1.16 <sup>b</sup>	1.10 ± 0.86 <sup>b</sup>
A/G ratio	1.34 ± 0.4 <sup>a</sup>	1.91 ± 0.22 <sup>b</sup>	2.30 ± 0.89 <sup>c</sup>

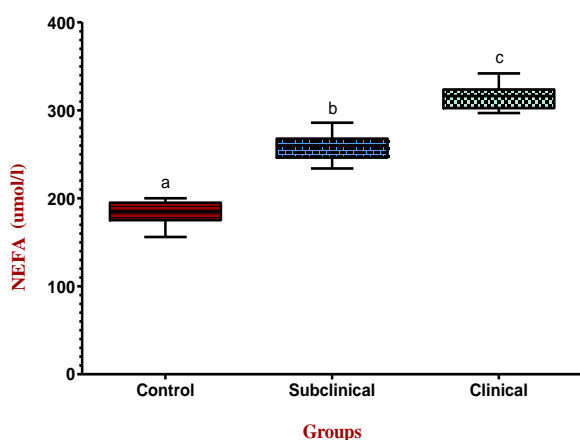
In each row value followed by different superscript letters (a, b, c) within the same row indicate significant variations among groups (P<0.05).



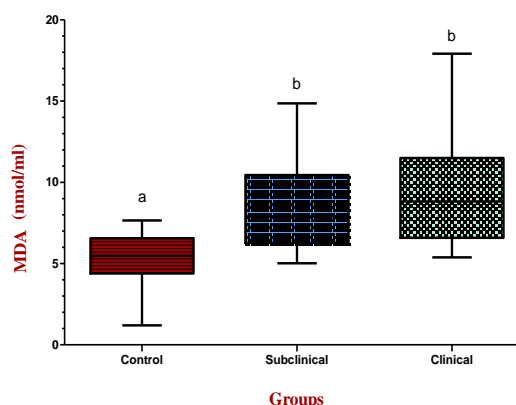
**Figure 1:** Mean ± standard deviation of serum levels of β-hydroxybutyric acid (BHBA)(mmol/l) in clinically healthy and diseased ewes. Values with different superscripts are statistically different (P<0.05).



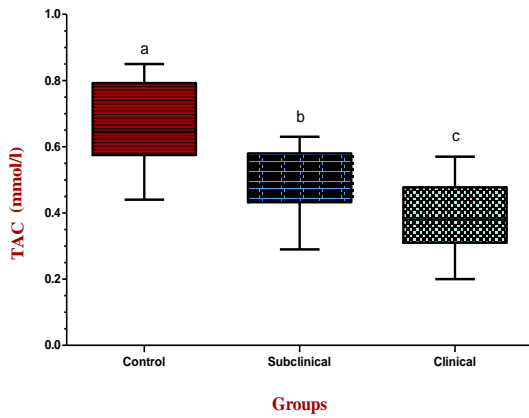
**Figure 3:** Mean ± standard deviation of glucose level (mg/dl) in clinically healthy and diseased ewes. Values with different superscripts are statistically different (P<0.05).



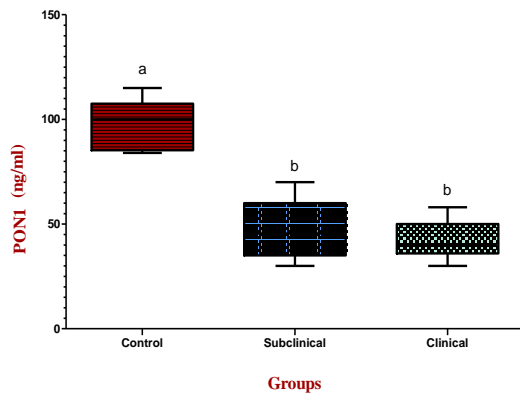
**Figure 2:** Mean ± standard deviation of serum NEFA (umol/l) in clinically healthy and diseased ewes. Values with different superscripts are statistically different (P<0.05).



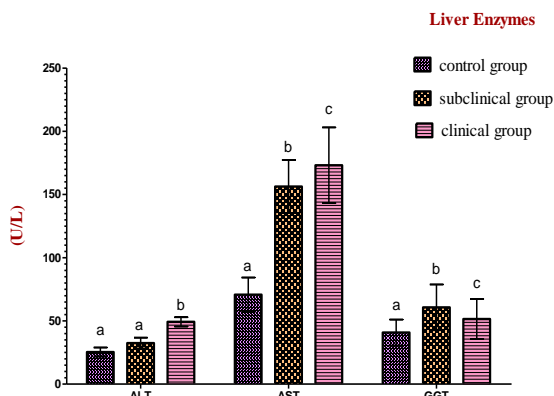
**Figure 4:** Mean ± standard deviation of serum levels of malondialdehyde (MDA) (nmol/ml) in clinically healthy and diseased ewes. Values with different superscripts are statistically different (P<0.05).



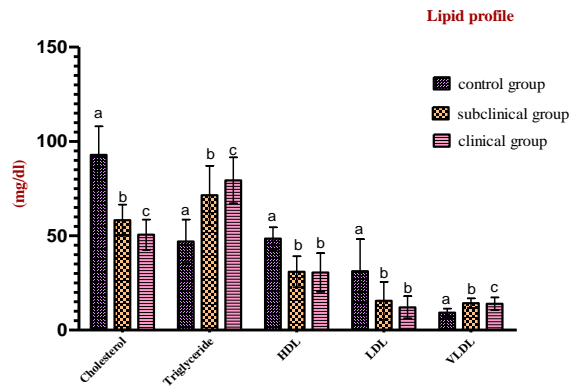
**Figure 5:** Mean ± standard deviation of serum levels of TAC (mmol/l) in clinically healthy and diseased ewes. Values with different superscripts are statistically different (P<0.05).



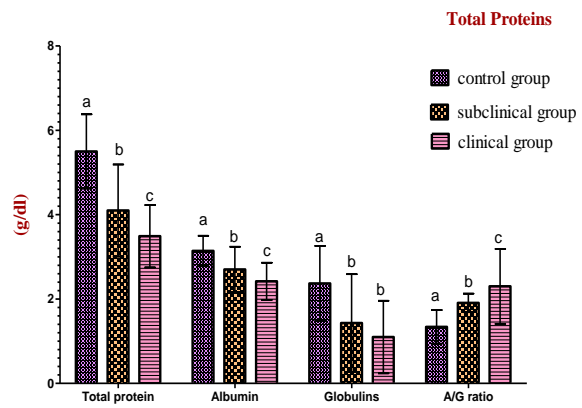
**Figure 6:** Mean ± standard deviation of serum levels of paroxonase-1 enzyme (PON1) (ng/ml) in clinically healthy and diseased ewes. Values with different superscripts are statistically different (P<0.05).



**Figure 7:** Mean ± standard deviation of serum levels of liver enzymes (U/L) in clinically healthy and diseased ewes. Values with different superscripts are statistically different (P<0.05).



**Figure 8:** Mean ± standard deviation of serum levels of lipid profile (mg/dl) in clinically healthy and diseased ewes. Values with different superscripts are statistically different (P<0.05).



**Figure 9:** Mean ± standard deviation of serum total proteins (g/dl) in clinically healthy and diseased ewes. Values with different superscripts are statistically different (P<0.05).

## DISCUSSION

The present study aimed to elucidate the alterations in metabolic profile indices of ewes in late pregnancy, as well as assessing the consequences of PT on paraoxonase-1 enzyme (PON1) and lipid profile.

Pregnancy toxæmia may have an impact on the productivity and health of lambs and dams, as well as increasing economic losses, Consequently, an early diagnosis is necessary for management of PT (Vasava *et al.*, 2016).



According to Macedo *et al.* (2015), the primary clinical changes found in sheep with PT include neurological disorders and behavioral issues, recumbency, and mortality, these changes vary depending on the severity of the condition and may indicate a poor prognosis. Therefore, the amount of ketone bodies produced in blood and the lipolytic process both affect how the severity of the disease is (Cal-Pereyra *et al.*, 2015).

Clinical assessment of the subclinical pregnant toxemic group in the current investigation revealed decreased appetite, normal body temperature, respiration rate, and heart rate, these findings were consistent with the results mentioned in a prior research (Gurdogan *et al.*, 2014). Alteration in appetite could result from, Ewes don't consume enough nutrition to fulfil their energy demands, since they don't have adequate rumen space as a results of excessive accumulation of fats in abdominal cavity and as pregnancy progresses, the uterus occupies more space (Uma Rani *et al.*, 2015).

Clinical findings showed in the clinical PTG in the current study were in agreement with results of (Gaadee and Gehan, 2021; Affan *et al.*, 2022), neurological signs as apparent blindness, head pressing, incoordination to movement, neck muscular spasms and stiffness throughout the body with lateral deviation of the head and neck may be attributed to impairment of glucose metabolism and inability of the nerve cell to utilize glucose due to high levels of cortisol which producing hypoglycemic encephalopathy, or due to the production of isopropyle alcohol, which resulted from the acetoacetic acid breakdown in the rumen (Al-Qudah 2011).

In the terminal stage of the disease, decrease heart and respiration rates may be attributed to the toxemic states which is accompanied by peripheral circulatory failure and death (Kabakci *et al.*, 2003), during this period, incomplete breakdown of NEFA was responsible for the production of ketone bodies, as a result, lack of energy, hypoglycemia and hypocalcemia may be the causes of recumbency in the pregnancy toxemic ewes (Al-Qudah 2011).

Starvation, accelerated lipolysis in adipose tissue as a result of hypoglycemia, and excessive production of FFA which converted by liver into ketone bodies could be the causes of hyperketonemia and changes in the lipid profile. These outcomes matched those that had been reported for sheep and goats (Kacar *et al.*, 2010).

In the current study, serum levels of BHBA showed significant increase in clinical and subclinical PTG, which explained by (Olfati and Moghaddam, 2013; Gurdogan *et al.*, 2014) in ewes, and (Vasava *et al.*, 2016) in goats, this may suggest that it might be used as a biomarker for diagnosis of pregnancy toxemia in ewes as in goats (Vasava *et al.*, 2016). As time goes on, lower glucose levels and increasing levels of BHBA produce more ketone bodies, which cause more severe clinical symptoms. According to Roberts *et al.* (2012), pregnant ewes with BHBA concentrations more than 3 mmol/L may experience severe pregnancy toxemia.

Elevated blood levels of BHBA show that the tri-carboxylic acid cycle did not fully oxidise NEFA during NEB (Doepel *et al.*, 2002). The disturbance of the metabolism of fats and carbohydrates may also be responsible for these elevated levels of

BHBA, this increase breakdown of body fats and excessive production of free fatty acids, which the hepatocytes then converted it to ketone bodies, a process known as "hepatic ketogenesis" (Kacar *et al.*, 2010).

The rate of fat mobilization from fat storage as a result of NEB is accompanied by changes in NEFA concentrations. The steady decrease in DMI seen during NEB can be used to explain the gradual rise in plasma NEFA during late pregnancy (Sadjadian *et al.*, 2013).

The mean value of NEFA in the clinical PTG was nearly two times more than the mean in CG, this explained the metabolic disorder brought on by lipolysis and shows that fat was severely mobilised to satisfy the increased energy needs for dam and fetus (Vasava *et al.*, 2016).

Clinically pregnant toxemic ewes in the present study demonstrated a highly significant reduction in glucose levels when compared to CG. These findings concur with the earlier findings of (Gurdogan *et al.*, 2014; Uma Rani *et al.*, 2015).

Decrease glucose level in this study may be caused by dietary deficiencies in net energy as well as an increase in energy requirements in the final weeks of pregnancy due to twin or triplet pregnancies (Gurdogan *et al.*, 2014), a decrease in hepatic gluconeogenesis (Darwish, 2019), as well as a decrease in endogenous glucose production that results from low calcium levels that occur during pregnancy (Gonzalez *et al.*, 2011).

Increased levels of MDA with decreased values of TAC in this research could be explained to oxidative stress and these

results were consistent with those published by (Gurdogan *et al.*, 2014).

Several studies on animal pregnancies have observed that oxidative stress can develop in late pregnancy (Castillo *et al.*, 2005). This is attributed to increased metabolic activity during pregnancy, NEB and the synthesis of ketone bodies, decreased antioxidant reserves throughout pregnancy and physiological adaptations to lactation, this is brought on by increased free radical production (Sahoo *et al.*, 2009). The elevated NEFA and BHB in PT trigger the creation of pro-inflammatory cytokines, which in turn increases the release of free radicals and ROS, which raises the level of MDA and reduced TAC due to much of TAC being depleted to eliminate increased ROS, could be another cause for the development of oxidative stress (Gurdogan *et al.*, 2014).

The antioxidant defense of ewes declines from late pregnancy to lactation and this drop is followed by an increase in the production of pro-oxidant, which predisposes ewes to oxidative stress (lipid peroxidation), this may explain the substantial rise in MDA levels seen in our investigation (Gurdogan *et al.*, 2014; Darwish, 2019). This may reflect lipid peroxidation process in ewes with pregnancy toxemia (Pilarczyk *et al.*, 2012).

High-density lipoprotein (HDL) is crucial as an anti-oxidative/anti-inflammatory particle. This action of HDL is mediated by several structural proteins and enzymes carried on the particle, of these, PON1 enzyme, synthesized and stored mostly in the liver (Turk, 2009). PON1 inhibits the accumulation of lipid oxidation products in low-density

lipoproteins (LDL) and HDL (Link *et al.*, 2007).

Lower serum PON1 activity showed in this study agreed with results of (Fukumori *et al.*, 2020; Tashla *et al.*, 2021), and was associated with the changes in lipid metabolic profiles (Turk, 2009). Also the lower HDL concentration could be one of the causes of decreased paraoxonase activity considering the role of HDL as a carrier of most paraoxonase molecules in the blood (Turk, 2009).

According to Fukumori *et al.* (2020) liver injury brought on by inflammation or excessive NEFA oxidation may cause the liver to produce less PON1.

Lower blood cholesterol levels in this study may be caused by NEB and hepatic insufficiency during pregnancy as well as mild liver steatosis, which causes the liver of pregnant toxemic sheep to produce less cholesterol (Salar *et al.*, 2018). Waziri *et al.* (2010), claim that three causes; reduced food intake, liver dysfunction, and alterations in endocrine function are responsible for the drop in concentration of cholesterol.

Reduction in levels of HDL was ascribed by Farid *et al.* (2013) to reduced hepatic production of protein responsible for HDL formation (apolipoprotein A), or due to decrease concentration of cholesterol, assuming that HDL contains roughly 60% cholesterol. Reduced lipoprotein lipase enzyme, which has a positive correlation with HDL, could be another contributing factor (Cheung *et al.*, 2003).

Serum triglycerides levels increased in the current study were agreed with results of (Salar *et al.*, 2018), and could be attributed to excessive mobilisation and

breakdown of fats very late in pregnancy to balance off the marked reduction in glucose during this period (Aly and Elshahawy, 2016).

Elevated levels of liver enzymes are indicative of hepatic dysfunction, which is typically seen due to liver steatosis (Vasava *et al.*, 2016), or due to increased fat mobilisation brought on by an energy deficit, which raises the amount of circulating FFA in the liver and frequently causes steatohepatitis and hepatic damage (Aly and Elshahawy, 2016; Vasava *et al.*, 2016).

Serum  $\gamma$ GT appears to be a more accurate diagnostic tool than serum AST for hepatic injury and elevated levels of this enzyme are indicative of liver cirrhosis, damage to hepatocyte cell membranes or liver and bile duct dysfunction (Lubojacka *et al.*, 2005).

Increases the mother's basal metabolic rate, the maximum nutritional requirements for dam and growing fetus, and the passage of serum proteins and necessary amino acids from blood to udder for milk production, may be a contributing factors to decreases in serum albumin and globulin levels (Batavani *et al.*, 2006). The emergence of fatty liver infiltration causes a reduction in the liver's ability to produce albumin (Lubojacka *et al.*, 2005).

Finally, the elevation in liver enzymes (AST and ALT), along with the reduction in serum proteins and cholesterol, may provide some insight into the role of liver in PT, this origin could be related to fatty acids mobilisation, which has been linked to insufficient food intake, hepatic injury, or fatty liver disease (Smith and Sheramn, 2009).

## CONCLUSIONS

In conclusion, serum Paraoxonase-1 activity is reduced in ewes with pregnancy toxemia, so, it is considered a helpful indicator for diagnosis of such conditions. Further research on PON1 could lead to improvement in diagnosis and management of PT in ewes. Additionally, lipid profiles and oxidant-antioxidant status may be helpful in assessing oxidative stress during the periparturient stage of sheep pregnancy.

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## نشاط إنزيم الباروكسوناز - ١ ومؤشرات التمثيل الغذائي في مصل الدم في النعاج المصابة بتسمم الحمل

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يهدف هذا البحث إلى تقييم مؤشرات استقلاب الطاقة البيتاهايدروكسي بوتيرات والأحماض الدهنية الحرة الغير مؤسرة وتقييم الأهمية التشخيصية لعلامات الإجهاد التأكسدي مثل المألونديالديهيد المؤكسد ومضادات الأكسدة مثل مضادات الأكسدة الكلية وإنزيم الباروكسوناز - ١ و مؤشرات الدهون وإنزيمات الكبد في تسمم الحمل في النعاج. اشتملت الدراسة علي عدد كلي اثنان وسبعون نعجة في المراحل الأخيرة من الحمل (أربعة عشر إلي عشرين أسبوعاً من الحمل). تم تقسيم النعاج بناءً علي النتائج السريرية والكيميائية الحيوية (الجلوكوز والبيتاهايدروكسي بوتيرات) إلي ثلاث مجموعات (مجموعة كلينيكية مصابة بتسمم الحمل ومجموعة تحت الإكلينيكية مصابة بتسمم الحمل والمجموعة الضابطة). تم تجميع عينات الدم لكل مجموعة وتم قياس مجموعة من المتغيرات البيوكيميائية. أظهرت معظم نتائج المتغيرات البيوكيميائية اختلافات ملحوظة بالمقارنة مع المجموعة الضابطة. أظهرت نتائج البيتاهايدروكسي بوتيرات والأحماض الدهنية الحرة الغير مؤسرة والمألونديالديهيد وإنزيمات الكبد مثل الألبومين أمينوترانسفيريز والأسبرتيت أمينوترانسفيريز والجاماجلوتاميل ترانسفيريز والدهون الثلاثية والبروتينات الدهنية المنخفضة الكثافة جدا في مجموعتي السريرية وتحت الإكلينيكية للنعاج المصابة بتسمم الحمل زيادة معنوية فوق القيم الطبيعية للمجموعة الضابطة. أظهرت نتائج الجلوكوز وإنزيم الباروكسوناز - ١ والكوليستيرول والدهون عالية الكثافة والدهون منخفضة الكثافة وبروتينات مصل الدم في مجموعتي السريرية وتحت الإكلينيكية للنعاج المصابة بتسمم الحمل انخفاضا معنويا أقل من القيم الطبيعية للمجموعة الضابطة. بناءً علي هذه النتائج فاننا نقترح أن مراقبة التغيرات في مؤشرات استقلاب الطاقة وحالة الأكسدة ومضادات الأكسدة ومؤشرات الدهون وإنزيمات الكبد وفحوصات البروتين للنعاج في مراحل الحمل المتأخرة يمكن أن تفيد في تشخيص تسمم الحمل في النعاج. تظهر نتائجنا ايضا ان إنزيم الباروكسوناز - ١ يحمل إمكانات كمؤشر بيولوجي لتشخيص تسمم الحمل في النعاج.