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**EVALUATION OF OXIDATIVE STRESS AND ANTIOXIDANT MARKERS DURING
VARIOUS STAGES OF LACTATION IN SMALL RUMINANTS**

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ABSTRACT

The present study was conducted to evaluate the effect of oxidative stress through various antioxidative/oxidative systems during different lactation stages in sheep and goats. A total of 60 lactating ewes and does between 1.5 to 5 years age group comprised the experimental animals taken from Bahadar Nagar Farm, Okara. Thirty (30) female Lohi sheep and likewise 30 female Beetal goats were grouped into three different milking stages [lac I (n=10), lac II (n=10) and lac III (n=10)]. Blood samples were collected from the animals during three different lactation periods. Serum has been preserved at -20 °C and used for determination of Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), Ceruloplasmin (Cp), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Gamma-glutamyl Transferase (GGT). Statistical analysis was done by two way ANOVA and Duncan Multiple Range Test (DMRT). MDA, SOD, CAT, Cp and GGT were significantly different ($P \leq 0.05$) through various stages of lactation in sheep as well as in goats. Whereas, AST and ALT were not differ significantly during different stages of lactation in both the groups. In Conclusion, lactation may

cause an increase in reactive oxygen species and antioxidant substances predicting that is very stressful for the animals.

Keywords: Oxidants and antioxidants, Lactation, Liver Enzymes, Sheep, Goat

INTRODUCTION

An immense demanding status leads to the extreme production of the radicals and results in oxidative stress, an imbalance between oxidants and antioxidants systems. Oxidative stress means status of oxidative overload on, the cell, organ or organism. Membranes are the principal sites of oxidative stress since ROS can react with unsaturated fatty acids resulting peroxidation of lipids in cell membrane or organelles [1]. Lipid peroxides produced are unstable and decomposed to form a series of compounds like malondialdehyde (MDA). The MDA evaluation is extensively used as marker of lipid peroxidation and oxidative stress [2]. The cells have developed few counterbalancing antioxidant defensive system. Ihejirika *et al.* [3] reported that to improve productivity, emphasis should be on antioxidant system. The innate defense components consists of enzymatic antioxidants like Superoxide dismutase, Catalase and non-enzymatic like Ascorbate, Vitamin E and glutathione [4]. SOD is the

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first line of protection against ROS and is effective for detoxification of superoxide radical. The hydrogen peroxide is changed into water in the presence of CAT [5]. Furthermore, blood metabolic profile including AST, ALT, and GGT are the markers in evaluating animal health [6]. The major factor is the physiological status (pregnancy, lactation) of an animal which influences such metabolic markers [7]. Lactation is a very strenuous phase for the animals which cause enhanced nutritional requirements, thus stimulate oxidative metabolism, which in turn increases the electron stream in mitochondria and accelerates the production of free radicals [8]. The evaluation of free radicals and antioxidant balance predict the actual state of physiological defenses. There is unavailability of data about oxidative stress in Lohi sheep as well as in Beetal goats. Present research is designed for the analysis of oxidative stress during lactation in the ewes and does.

Clinically healthy Lohi sheep (n=30) and Beetal goats (n=30) aged between 1.5-5 years

were selected from Livestock Production Research Institute Bahadur Nagar Farm, Okara from 2013-2014. Both the lactating groups comprised of early, peak and low lactation stages as Lac I (30 days), Lac II (60 days) and Lac III (90 days-onwards). Blood samples were collected during three different lactation periods and serum has been preserved at -20°C . The analyses were done in the laboratory of Institute of Pharmacy, Physiology and Pharmacology at the University of Agriculture, Faisalabad, Pakistan. .

Malondialdehyde (MDA; mmol/mL)

MDA is measured by Lipid Peroxidation (LPO; mmol/L) method. Kits (Abacum, UK) were provided these components along with directions of their use and storage.

Super Oxide Dismutase (SOD; μmL)

Sun *et al.* [9] method was used for SOD measurement. Absorbance of samples was read at 560 nm. Percent inhibition was calibrated using the following formula:

$$\% \text{ inhibition} = (A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} \times 100\%$$

Catalase Activity (CAT; KU/L)

A spectrophotometer assay based on H_2O_2 was used to analyze catalase activity using methodology of Goth [10]. Catalase activity was measured through following formula.

Serum catalase activity (KU/l) =

$$\frac{A(\text{sample}) - A(\text{blank 1})}{A(\text{blank 2}) - A(\text{blank 3})} \times 271$$

Ceruloplasmin Activity (Cp: U/L)

To determine Cp activity Schosinsky *et al.* [11] method was used. Calculation is done as:

$$\text{Ceruloplasmin oxidase activity (U/L)} = \text{Abs}_{15\text{min}} - \text{Abs}_{5\text{min}} \times 6.25 \times 10^2$$

(Abs_{15} & Abs_5 are absorbance at 15 and 5 minute respectively, 6.25×10^2 is dilution factor of the reaction mixture).

Aspartate Aminotransferase (AST; U/L)

By using a calorimetric method kit of Randox Laboratories, the quantitative in vitro in the serum samples of AST was determined.

Alanine Aminotransferase (ALT; U/L)

By using a calorimetric method kit of Randox Laboratories the quantitative in vitro in the serum samples of ALT was determined.

Gamma-glutamyl transferase (GGT; U/L)

Method of Szasz *et al.* [12] was used for calibrating GGT activity. Calculation is done as.

$$\Delta A/\text{min} = [\Delta A/\text{min Sample}] - [\Delta A/\text{min blank}];$$

$$\text{Calculation: Hg } 405 \text{ nm } 1158 \times \Delta A/\text{min}$$

Statistical Analysis:

Data obtained was subjected to two way analysis of variance (ANOVA)

techniques [13]. Duncan Multiple Range (DMR) test was used to explain significant difference [14].

RESULTS

Analysis of variance (ANOVA) in Lohi sheep and Beetal goat

Serum MDA, SOD, CAT, Cp, AST, ALT and GGT concentrations were analyzed to see the difference in both groups by means of two way analysis of variance and the results have been shown in **Table 1**. Results for groups, different stages of lactation and the interaction between groups and stages were statistically significant ($P \leq 0.01$) for Cp and GGT. However, sheep and goats at various stages of lactation and interaction between groups and stages were shown significantly different results for MDA, SOD and CAT. While ALT and AST have shown statistical significant ($P \leq 0.01$) results only for different stages of lactation.

Malondialdehyde (MDA; mmol/mL)

Mean \pm SE of serum malondialdehyde concentration was significantly different ($P \leq 0.05$) through various stages of lactation in Lohi sheep as well as in Beetal goats; the highest value in sheep was seen at Lac-II while in goats at stage-III. In sheep and goats groups interaction, statistical difference was only observed during lactation stage-II.

Overall mean MDA concentrations between sheep and goats groups were significantly the same (**Table 2**).

Superoxide dismutase (SOD; μ /mL)

In both sheep and goats, superoxide dismutase concentration was statistically significant during various stages of lactation; highest value of SOD was observed in lactation stage II and lowest in stage-III. When a comparison was made between the groups, significant different results were shown only during Lac-II. Overall mean between groups was same (**Table 2**).

Catalase (KU/L)

In Lohi sheep catalase concentration was significantly high in lactation stage-I, whereas, in Beetal goats, significantly different results were observed during Lac-II. Interaction between Lohi sheep and Beetal goats groups showed significantly ($P \leq 0.05$) different results in lactation stage-I, II and III Overall mean between sheep and goat groups was significantly same (**Table 2**).

Ceruloplasmin Activity (U/L)

Ceruloplasmin conc. in Lohi sheep were significantly different ($P > 0.01$) during lactation stage-II. However in Beetal goats, a significant increase was observed from stage-I to III does. A comparison between the groups predicted significantly different results during Lac-III. Overall mean

Ceruloplasmin values between groups manifested statistically significant results (Table 2).

Aspartate Aminotransferase (AST; U/L)

In Lohi sheep and Beetal goats, Mean \pm SE of serum Aspartate aminotransferase concentration was significantly same throughout all the stages of lactation. Similarly, Interaction results between sheep and goats among various stages of lactation were significantly the same. However, overall mean AST values were significantly different in both the groups (Table 2).

Alanine Aminotransferase (ALT; U/L)

Mean \pm SE values of serum alanine aminotransferase concentration in both sheep and goats groups did not differ significantly during all the stages of lactation. An interaction between ewes and does exhibited

significantly similar results among all the stages of lactation. Overall mean ALT concentrations were significantly same in both the groups (Table 2).

Gamma-glutamyl transferase (GGT; U/L)

Serum GGT concentration in sheep and goats manifested significantly different results. The highest GGT level was observed in lactation stage-I and lowest during stage III in both ewes and does. Interaction between groups were significantly different throughout all the stages. Overall mean GGT values were also significantly different between sheep and goats (Table 2).

Table 1: Analysis of Variance (ANOVA) of MDA, SOD, CAT, Cp, AST, ALT and GGT of Lohi sheep and Beetal goats during various stages of Lactation

PARAMETERS (F-VALUE)	SOURCE OF VARIATION		
	Groups	Stages	G x S
MDA	0.296 N.S.	152.313**	28.622**
SOD	2.267 N.S.	167.583**	9.338**
CAT	0.248 N.S.	235.384**	87.505**
Cp	65.275**	57.581**	72.725**
AST	693.466**	253.405**	0.714 N.S.
ALT	0.188 N.S.	600.290**	0.179 N.S.
GGT	306.286**	188.496**	8.793**

**Significance = $P \leq 0.01$.

N.S. = Non-significant.

Table 2: Mean \pm SE of MDA, SOD, CAT, Cp, AST, ALT and GGT of Lohi sheep and Beetal goats during various stages of Lactation

PARAMETERS	Lactation Period			
	Lactation (Stage I)	Lactation (Stage II)	Lactation (Stage III)	Overall Mean
MDA Lohi Sheep Beetal Goats	1.02 \pm 0.09d 1.28 \pm 0.04d	2.43 \pm 0.08a 1.81 \pm 0.05c	2.08 \pm 0.09bc 2.35 \pm 0.05ab	1.84 \pm 0.12 1.81 \pm 0.09
SOD Lohi Sheep Beetal Goats	1.58 \pm 0.06d 1.37 \pm 0.05d	2.57 \pm 0.04b 2.97 \pm 0.09a	2.07 \pm 0.08c 2.14 \pm 0.08c	2.07 \pm 0.08 2.16 \pm 0.13
CAT Lohi Sheep Beetal Goats	1.23 \pm 0.05d 1.79 \pm 0.02c	2.48 \pm 0.07b 2.71 \pm 0.05a	2.46 \pm 0.05b 1.74 \pm 0.05c	2.06 \pm 0.11 2.08 \pm 0.09
Cp Lohi Sheep Beetal Goats	132.05 \pm 1.47c 129.60 \pm 1.4c	141.10 \pm 0.81b 142.95 \pm 0.84b	128.40 \pm 1.01c 150.70 \pm 0.84a	133.85 \pm 1.17B 141.08 \pm 1.72A
AST Lohi Sheep Beetal Goats	56.85 \pm 0.84 43.20 \pm 0.80	46.00 \pm 0.56 32.45 \pm 0.59	43.45 \pm 0.30 28.50 \pm 0.69	48.77 \pm 1.13A 34.72 \pm 1.22B
ALT Lohi Sheep Beetal Goats	8.37 \pm 0.14 8.11 \pm 0.18	15.83 \pm 0.38 15.67 \pm 0.49	18.43 \pm 0.25 18.53 \pm 0.25	14.21 \pm 0.81 14.10 \pm 0.84
GGT Lohi Sheep Beetal Goats	24.50 \pm 0.50a 16.30 \pm 0.31bc	16.90 \pm 0.53b 12.10 \pm 0.24d	15.05 \pm 0.37c 9.60 \pm 0.54e	18.82 \pm 0.8A 12.67 \pm 0.56B

a-e; Means in a column followed by the same letter are statistically not different ($P \leq 0.01$; ANOVA, DMRT).

A-B; Means in a row followed by the same letter are statistically not different ($P \leq 0.01$; ANOVA, DMRT).

DISCUSSION

Malondialdehyde (MDA)

The present study revealed that MDA level increased significantly from early to late lactation in goats. Whereas, highest value of MDA was observed during lac-II in sheep. Both of these observations are in line with the findings of Jimoh *et al.* [15] and Ognik *et al.* [16] who had reported an increase in the MDA level at peak of lactation in sheep. The likely cause of this rise in MDA concentration could be due to overproduction of free radicals. However, decrease of MDA level in goats during late lactation might have been due to reduction in milk production at

late lactation or due to the animals having attained balanced energy. This fact has been confirmed by Castillo *et al.* [17] who compared MDA concentration during mid-lactation in various milk producing groups and observed higher level of MDA in high milk producing cows. Likewise, Amer *et al.* [18] reported a significant increase in MDA concentration during lactation in ewes.

Superoxide Dismutase (SOD)

In the present study, SOD increased significantly from lac-I to lac-II but decreased during lac-III in both sheep and goats. Similar findings were reported by Konvicná *et al.* [19] who had observed the

highest activity of SOD during lactation peak. This enhanced SOD might be due to response of the organism to higher superoxide generation just after parturition. Even though the SOD increases, quite a few studies have observed that the antioxidant ability is not enough to counteract the increase in ROS supply [17]. Decreased SOD level during late lactation might be a sign of oxidative stress [4]. However, Ognik *et al.* [16] in ewes had reported decrease in SOD activity after parturition. Jimoh *et al.* [15] observed that antioxidant defense of goats decreases during pregnancy and lactation accompanied with the increase in pro-oxidants; thus indicating oxidative stress.

Catalase (CAT)

The concentration of antioxidant enzyme, catalase in the present study showed a non-significant increase from lactation stage I to II and a decrease in lactation stage III in both the groups. Similar results have been observed by Ognik *et al.* [16] who had reported an increase in catalase activity after lambing. The present study results of decreased catalase concentration during late lactation are in agreement with findings of Jimoh *et al.* [15] in goats who had concluded a decreased CAT level during lactation. Amer *et al.* [18] in ewes observed that there was a negative correlation between chemical

activities of antioxidant and lipid peroxidation or MDA during lactation because oxidative stress indicator (MDA) was enhanced and catalase activity was reduced. One of the probable causes of enhancement of CAT activity during lactation might be due to high value of lipid peroxides from dismutase reaction. It is also involved in breaking of H₂O₂ into water [20]. Moreover, SOD also causes to increase H₂O₂ production and the defense against ROS could only be provided by increase in catalase [21]. Another reason for low antioxidant during early lactation was probably due to utilization of antioxidants for colostrum production [22].

Ceruloplasmin (Cp)

Ceruloplasmin performs a vital function in lipid peroxidation and inhibition of free radical production [23]. In Lohi sheep, there was an increase in Cp from lactation stage I to lac-II but decreasing in lactation-III. Cp concentration in goats increased significantly from early to late lactation and these results were in agreement with those of Hussein and Staufenbiel [24] who had reported an increase in ceruloplasmin with the advancement of lactation in cows. However, Albera and Kankofer [25] observed reduction of Cp in cows in late lactation. Iqbal *et al.* [26] had observed that oxytocin injection

during lactation in buffaloes triggered an oxidative stress by enhancing the Cp oxidase activity and declining activities of antioxidant enzymes; that might cause low reproductive potential of the animals. The reason for increased Cp might be due to the fact that antioxidants enzymes were increased due to quenching of free radicals and lipid peroxidation [27].

Aspartate aminotransferase (AST):

The findings about AST have not shown any significant difference during different stages of lactation in sheep and goats. However, a non-significant decrease in AST concentration was noted from lactation stage I to III. The present study results are in consistent with Antunovic *et al.* [6] had reported that ewe's blood did not show any difference for AST activity considering the reproductive status of the animals. Similar results were observed by Yokus and Cakir [28] in cows affirming that physiological as well as seasonal variations have no effect on AST activity. However, Antunovic *et al.* [29] in sheep had found that AST activity was significantly reduced during late lactation. However, Jimoh *et al.* [15] in sheep and Krsmanovic *et al.* [30] in cows had found increased AST activity during lactation.

Alanine aminotransferase (ALT):

There was no significant difference shown by sheep and goats for serum enzyme ALT concentration in the present study. However, a non-significant increase in AST concentration was observed from lactation stage I to III, respectively. These findings are in agreement with those of Antunovic *et al.* [6] had reported the highest ALT activity in the serum of ewes during lactation, despite the fact that without any significant difference. Similarly, Jimoh *et al.* [15] and Zebari *et al.* [31] had observed that ALT was not significantly affected by different physiological condition of does. Similar results in sheep were also obtained by Yokus and Cakir [28] that physiological as well as seasonal variations did not affect ALT activities in the serum of these animals. However, Antunovic *et al.* [29] reported decreased ALT activities in blood of ewes during lactation period when compared with pregnant and non-pregnant ewes.

Gamma-glutamyl transferase (GGT)

Our results were showing reduction in GGT concentration with advancement of lactation in both sheep and goat groups. These findings were corresponding to the study of Krsmanovic *et al.* [30] whose results suggests a decrease in GGT values from early to late lactation in cattle. GGT is microsomal and membrane-bound enzyme

[32]. The highest GGT activity observed during early lactation might be due to reason that extreme liver function of lactating ewes to be able to meet up the energy and proteins supplies for their maintenance and milk production [7, 35-38]. Additionally, it might be due to liver damage, with the fat infiltration, cell membranes damage causes the destruction of hepatocytes. As a result, GGT levels were increased [33, 39-44]. However, the reason for decrease in GGT with advancement of lactation might be due to level of proteins in the blood. Antunovic *et*

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- al.* [29] and Radavelli *et al.* [34] found an increase in GGT activity in lactating ewes.

CONCLUSION

The results shows that Lohi sheep and Beetal goats does display oxidative stress during lactation period especially during lactation stage II due to peak milking time owing to intense metabolism. Therefore antioxidant should be employed to counteract the oxidative stress.

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