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OXIDANT AND ANTIOXIDANT STATUS DURING DIFFERENT STAGES OF LACTATION IN NILLI-RAVI BUFFALOES AND SAHIWAL COWS

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ABSTRACT

Lactation is an important period in dairy animals during which many physiological changes takes place and produced a measureable changes in diagnostic parameters in blood. Stages of lactation also affect the oxidative status of animals. Stress expressed by dairy cattle during lactation is a challenged situation. The present study was aimed to assess the status of oxidants and antioxidants status in Nili Ravi buffaloes and Sahiwal cows during different stages of lactation. Lactating buffaloes (n=30) and cows (n=30) were randomly selected from two different farms and categorized into three stages, stage I (n=10), stage II (n=10) and stage III (n=10). From each stage blood sample were drawn. Melanodialdehyde (MDA) was determined by measuring serum lipid peroxidation (LPO), while serum enzymes were measured by applying standard methodologies and using spectrophotometer (Biosystem BTS-330). Non significantly lower concentration of TAS was found in stages I and stage III of lactation. TOS, SOD and catalase were significantly higher at lactation stage II. MDA and Ceruloplasmin were significantly higher at lactation stage II in buffaloes and lactation stage III in cows. These parameters assist to describe the changes in physiology of animals which is associated with oxidative stress during lactation.

Keywords: Oxidative stress, antioxidants, lactation, buffaloes and cows

INTRODUCTION

During lactation dairy animals need high energy mainly in the early period when the milk production is increased. Cattles used lipids as energy substrate and increased energy requirement for milk production **[1, 2]**. In physiological conditions, the antioxidants and certain enzymes acts as defense system and controlled the oxidative damage battling with reactive oxygen species (ROS) **[3]**. Oxidative stress occurs when free a radicals increased in production and decreased antioxidants thus caused the physiological disorders in lactating animals **[4, 5]**. Oxidative stress weakened the dairy animals and animals can be susceptible to many metabolic disorders particularly during lactation which in turn affected the health of dairy animals **[6, 7]**. Evaluation of peroxidative stress and antioxidant enzymes during this period would be critical in deciding the health of animals. The present study was conducted to determine the oxidative stress by evaluating the lipid peroxidation levels (malondialdehyde) and antioxidant status in Nili Ravi buffaloes and Sahiwal Cows during different stages of lactation. The activities of main antioxidant enzymes were also determined.

MATERIALS AND METHODS

The study was carried out on 30 lactating Nili-Ravi buffaloes and 30 Sahiwal cows of age group of 3-7 years will be selected from Buffalo Research Station, Pattoki District Kasur, Pakistan and from Livestock Production Research Institute Bahadar Nagar, Okara, Pakistan during the years 2012-2013. Ear tags were used for identification. Lactation period will be divided in to three stages early, mid and late and considered as stage I, II and stage III. 10 animals were concluded in each stage. Twenty ml of blood from each buffalo and cow was collected at different stages. After centrifugation at 196x g for 15 minutes, serum will be harvested and stored at -20 ^{0}C till analysis. In the laboratory of Institute of pharmacy, physiology and pharmacology (University of Agriculture, Faisalabad) analysis of blood has been done. Before the analyzing the sample was kept in refrigerator for thawing and then kept at room temperature.

Total Measurement of total oxidant status (TOS)

The TOS of the body was estimated using the method, developed by Erel **[8]**. The TOS concentrations were calculated in terms of micromolar per liter of hydrogen peroxide.

Measurement of Total Antioxidant Capacity (TAC)

The total antioxidant capacity of the body was determined by using a novel automated ABTS radical cation method Erel **[9]**.

Ceruloplasmin oxidase activity

The enzymatic activity of ceruloplasmin oxidase was measured by using the slightly modified colorimetric method of Schosinsky *et al.* **[10]**. The following formula was used to calculate the enzymatic activity.

Ceruloplasminoxidase activity(U/L)=Abs15min–Abs5min×6.25×102

Ceruloplasmin oxidase activity (U/L) =

$$\mathbf{Abs_{15min} - Abs_{5min} \times 6.25 \times 10^2}$$

Whereas, Abs_{15min} and Abs_{5min} were the absorbance of samples after 15 and 5minutes respectively. 6.25×10^2 was the dilution factor of the reaction mixture

Super Oxide Dismutase (SOD)

Sun *et al.* **[11]** method was used for measurement of SOD and the results were measured in Unit/L.

Catalase Activity (KU/L)

By using the method of Goth [12], in this method using a spectrophotometer based on hydrogen peroxide. Chemical reagents used in this method was H_2O_2 sixty five µmol per ml and Na K buffer was sixty mmol

Serum catalase activity (KU/l) =

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

Malondialdehyde (MDA; mmol/mL):

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

Statistical Analysis

Data was investigated by two way ANOVA **[13]** and SPSS statistical software. Duncan Multiple Range Test was employed for analysis of significance difference between different lactation stages **[14]**.

RESULTS AND DISCUSSION

A two way analysis of variance was conducted to determine the significance of the differences between group, stages, and groups × stage interaction. Among groups, stages levels, and their interaction, total oxidant status (TOS), ceruloplasmin and superoxide dismutase (SOD) differ significantly while total antioxidant status (TAS) differed significantly among groups and stages and their interaction was not significant. Catalase and Melanodialdehyde (MDA) differed significantly among stages and their interaction but not significant among their groups. Analysis of variance was shown in **Table 1**.

Table 2 reveals that serum TAS concentration was not significant during

different lactation stages and TOS was lower at lactation stage I and significantly high at lactation stage II and then decreased in lactation stage III in both buffaloes and cows. According to Adela *et al*. **[15]** in the first lactation period antioxidants level were low and lipid peroxidation was high as compared to remaining lactation period in dairy cows. Similar studies in lactating cows were reported by Castillo *et al*. **[16, 17]**. From the previous reports it showed that just after calving the TAS reduced may be due to the increased production of reactive oxygen metabolites (ROM). Increased these metabolites induce imbalance between TAS and assembly of free radicals leading to lipid per oxidation **[18]**. In lactating buffaloes ceruloplasmin concentration was significantly high at stage II of lactation and in lactating cows, cows did show a significantly high concentration of serum ceruloplasmin concentration at stage III of lactation revealed in **Table 2**. Hussein *et al*. **[19]** reported that cerulolplasmin concentration was increased gradually throughout lactation in Holstein dairy cows. In the serum of goat has low Cu, the cerulolplasmin activity also low [20]. Ceruloplasmin is a ferric oxidase enzyme scavenges free radicals that acts as an antioxidative defence of the organism **[21]**. In the present study increased activity of ceruloplasmin in buffaloes at stage II of lactation and in cow in third stage of lactation might be due to tissue damaging factor, any infections which stimulate the liver to secrete ceruloplasmin **[22]** or might be due o the diet that consists of rich in cu supplements thus its concentration rised. So, ceruloplasmin oxidase activity is the important health parameter that could be used to evaluate the health status and welfare of the animals **[23]**.

Table 1: Analysis of variance data concerning of Total antioxidant status (TAS;), Total oxidant status (TOS), Ceruloplasmin (Cp), Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) concentrations in Nili-Ravi buffaloes and Sahiwal cows during different stages of lactation

Source of variation	TAS	TOS	CP	SOD	CAT	MDA
Groups (G)	36.384**	263.098**	61.117**	7.107**	0.009NS	0.431NS
Stages (S)	123.887**	264.789**	50.296**	151.263**	244.853**	134.823**
G x S	0.401NS	17.526**	69.498**	6.406**	84.027**	23.470**

****Significant at P≤0.01; NS=Non-significant**

Table 2: Mean ± SE of Total Antioxidant Status (TAS;), Total Oxidant Status (TOS), Ceruloplasmin (Cp), Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) in Nilli Ravi buffaloes and Sahiwal cows during different stages of lactation

Parameters	Lactation Stage-I	Lactation Stage-II	Lactation Stage-III	OVER ALL MEAN
TAS Buffalo Sahiwal cow	1.99±0.08 2.43±0.14	3.03±0.08 3.55±0.08	1.79±0.04 2.15±0.09	2.27±0.11B 2.71±0.13A
TOS Buffalo Sahiwal cow	1.93±0.07d 2.39±0.06bc	2.57±0.04b 3.65±0.04a	1.62±0.05e 2.24±0.06c	2.04±0.08B 2.76±0.12A
Ceruloplasmin Buffalo Sahiwal cow	131.90±1.43c 129.40±1.40c	140.80±1.10b 142.50±1.02b	128.00±0.98c 151.1o±0.95a	133.57±1.19B 141.00±1.77A
SOD Buffalo Sahiwal cow	1.45±0.08d 1.34±0.05d	2.55±0.04b 3.01±0.10a	2.00±0.10c 2.17±0.09c	2.00±0.09B 2.17±0.13A
Catalase Buffalo Sahiwal cow	1.21±0.05d 1.77±0.02c	2.54±0.07b 2.73±0.04a	2.47±0.05b 1.73±0.05c	2.07±0.12 2.08±0.09
MDA Buffalo Sahiwal cow	1.00±0.08c 1.27±0.05c	2.40±0.09a 1.77±0.05b	2.16±0.10a 2.40±0.06a	1.85±0.12 1.81±0.09

Dissimilar small alphabets within table differ significantly (P≤0.01)
Dissimilar capital alphabets within table differ significantly (P≤0.01)

According to our study serum SOD concentration was significantly high at stage II of lactation in both animals. Serum Catalase concentration was lower in first stage of lactation and then significantly increased in second stage and decrease in the third stage in buffalo and significantly high in lactation stage-II in cows (Table 2). The findings of our study are in corroboration with the reports of Sharma *et al.* **[24]** that catalase activity was not significantly higher in early lactating cows. Our study also supported by Cigliano *et al* **[25]**, who stated that SOD was significantly higher in mid lactating cows, both in bovine and buffaloes. According to Konvicna *et al.* **[26]** SOD activities were slowly elevated, which was possibly caused by a response of the organism to higher superoxide generation, though the SOD activity decreases after calving in dairy cows **[27]**. Several recent studies have shown that the antioxidant capacity after parturition dairy cows is insufficient to counteract the increase in reactive oxygen supply **[16, 18]**. In our study decrease antioxidants in early stage and rise in mid stage might be due to increased oxidative stress. The rise in SOD activity during lactation stage II is a marker of oxidative stress. Normally lipid peroxidation level in cells and tissues is low but in certain physiological conditions its level increased. The final product of lipid peroxidation is MDA and a marker for

oxidative stress **[28]**. Lipids are more disposed to peroxidative damage due to presence of unsaturated bonds and low energy necessary for the initiation of process **[29-35]**.

Table 2 revealed that in lactating buffalo serum MDA concentration were significantly lower at stage I of lactation and increased stage I to stage II then lowered stage II to stage third but that it is not significant, in sahiwal cow significantly increased level of MDA observed from lactation stage I to stage III. According to the study of Adela *et al.* **[15]** level of MDA was observed maximum in the early lactation as compared to the level in late lactation. The high level of MDA may be due low activity of antioxidant enzymes leads to oxidative stress in dairy cows. According to Sharma *et al.* **[24]** the lipid peroxidation (plasma MDA production) was significantly higher in early lactating cows than advanced pregnant cows. The determination of MDA allows for the estimation of intensity and evaluation of oxidative stress **[30, 36-40]**. In our study MDA was higher might be due to the reason that at that time milk production was maximum so more energy is needed ant there was more lipolysis and more free radicals produced thus slightly increased the MDA level.

CONCLUSION

Dairy animals seemed to have more oxidative stress and low antioxidant defence capacity during lactation which may contribute to the incidence of many metabolic diseases. Therefore regular monitoring of the antioxidant defence and supplementation of antioxidants can help to reduce the oxidative stress in the dairy animals.

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