



Seasonal changes in certain blood antioxidants in cattle and buffaloes

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Climatic change and high production demands can predispose animals to stresses of various kinds and of varying degrees. Oxidative stress in a living organism results from an imbalance between the production of reactive oxygen metabolites (ROM) and the capacity of the antioxidant mechanism to neutralize these ROM (Sies 1997). Mainly glutathione reductase (GSH), super oxide dismutase (SOD) and catalase (CAT) act as antioxidants in body and help in catalyzing respective free radical species or oxidants (Lykkesfeldt and Svendsen 2006). Lipid peroxides are represented by malonaldehyde (MDA) and it is one of the main oxidants produced during oxidative stress. Together, these antioxidants and oxidants constitute oxidative stress indices. Hence estimation of oxidative stress indices in the body will enable to predict the level of oxidative stress animal is undergoing under a particular environment and hence the present study was carried out with the same objective.

The study was carried out at Cattle and Buffalo farm of LPM section, of this Institute, from January to June 2013 with mean temperature of 10°C in winter (ranging from 4 to 16°C from January and February), 23°C in spring (ranging from 18 to 28°C between March and April) and 38°C in summer (ranging from 34 to 42°C between May and June) respectively. Selection of animals was made randomly from the farm herd of crossbred cattle (Tharparkar and Holstein Friesian cross) and crossbred buffalo kept and maintained on similar feeding and management practices. The animals were divided into 6 groups (group 1 to 6), from both cattle and buffalo each group having 6 animals (n=6). Groups 1 to 3 were based on lactation status and non-lactating groups 4 to 6 were based on age in months.

Blood samples (10 ml) were collected from the jugular vein in vials containing heparin or EDTA (1.0 mg/ml). The heparinized blood samples were preserved at normal refrigeration for estimation of oxidative stress indices within

12–48 h. Haemoglobin concentration was estimated by cyanomethaemoglobin method (Vankampen and Zinglstra 1961).

Measurement of blood oxidative stress indices: After centrifugation at 3,000 rpm for 10 min, the plasma and buffy coat were removed to harvest the red blood cells (RBC). Part of RBC pellet was diluted with chilled distilled water in 1:10 for the preparation of 10% stock haemolysate which was used for the estimation of SOD, CAT and LPO activities, and rest of the RBC pellet was diluted with chilled normal saline in 1:1 to get 50% RBC suspension for GSH estimation.

Catalase was estimated in the 10% RBC haemolysate after appropriate dilution following the method of Cohen *et al.* (1970). Briefly, the reaction was initiated by the addition of 50 µl of diluted sample to 2.950 ml of phosphate buffer–hydrogen peroxide solution. Initial absorbance at 240 nm was read after 20 s against reference cuvette in which instead of H₂O₂, same amount of PBS was added. Time(s) required for the fall in the initial absorbance by 0.050 was noted and catalase present in the assay mixture was calculated. The activity of the enzyme was expressed as units/mg of haemoglobin.

Superoxide dismutase activity was measured using nitro-blue tetrazolium as substrate as per Marklund and Marklund (1974) with certain modifications suggested by Menami and Yoshikawa (1979). Briefly, the assay mixture in a total volume of 3 ml consisted of 50 mM of TRIS cacodylic acid buffer (pH 8.2), 50 µl of the sample after suitable dilution and 0.2 mM of pyrogallol. In the blank, enzyme was substituted by equal quantity of distilled water. The increased absorbance due to auto-oxidation of pyrogallol was recorded at 420 nm using double beam UV–VIS Spectro-photometer. One unit of SOD activity was defined as the amount of enzyme, which inhibited the auto-oxidation of pyrogallol by 50% under the given experimental condition and the values were expressed as units/mg of haemoglobin.

The concentration of glutathione (GSH) in RBC suspension was estimated by 5, 5-dithiobis-(2-nitro-benzoic acid) (DTNB) method as per Prins and Loos (1969). GSH concentration in the test samples was calculated by employing

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the molar extinction coefficient of DTNB-GSH conjugate ($\mu\text{mol/mg Hb}$), 13600/M/cm.

Lipid peroxides level in the RBC haemolysate was determined following the methods of Placer *et al.* (1966). Briefly, 0.2 ml of RBC haemolysate was added to 1.3 ml of 0.2 M TRIS-KCl buffer of pH 7.4 and incubated at 37 °C for 30 min, after which 1.5 ml of thiobarbituric acid (TBA) was added and the mixture heated in boiling water-bath for 10 min using glass beads as condenser. After cooling, 3 ml of pyridine/*n*-butanol (3:1 v/v) and 1 ml of 1 N NaOH were added to it and mixed by shaking. Blank was prepared by taking 0.2 ml of distilled water instead of RBC haemolysate. The absorbance was read at 548 nm. The nmol MDA (malonaldehyde) per mg of RBC haemolysate was calculated by using 1.56×10^6 as extinction co-efficient. Lipid peroxides level in the erythrocytes was expressed in nmol of MDA per mg of haemoglobin.

Statistical analysis: Data were analyzed by one-way analysis of variance (ANOVA) using statistical software package SPSS 16.0. The comparison between values was established at level of significance, $P < 0.05$.

Mean $\pm$ SE of catalase (in $\mu\text{mol H}_2\text{O}_2$ decomposed/min/mg Hb), SOD (in $\mu\text{mol MTT formazan/mg Hb}$), GSH (in $\mu\text{mol conjugate/ml packed RBC}$) and MDA (in nmol MDA/mg Hb) in lactating and non-lactating groups of cattle and buffalo during winter, spring and summer is given in Table 1 and Table 2 respectively. Significantly ($P < 0.05$) higher levels of catalase during hot summer and cold winter than during spring indicated oxidative stress due to too high and too low temperature. Chandra and Aggarwal (2009) also reported increased catalase activity in crossbred cows during summer. Significantly ($P < 0.05$) higher levels of catalase in lactating cattle under different seasons than the non-lactating cattle may be due to the fact that lactation or production stress also causes oxidative stress (Lohrke *et al.* 2004) in addition to

climatic stress. Significantly ($P < 0.05$) increased levels of SOD during summer and winter may be due to oxidative stress under climatic stress. These findings are in corroboration with Bernabucci *et al.* (2002). Significantly ($P < 0.05$) increased levels of SOD in lactating cattle in all the 3 seasons than non-lactating cattle may be because of the oxidative stress due to milk production stress (Castillo *et al.* 2006). Significantly ($P < 0.05$) higher levels of GSH in both the lactating and non-lactating cattle during summer and winter were found than during the spring. Similar findings were made by Bernabucci *et al.* (2002) and Tanaka *et al.* (2011). This may be due to the oxidative changes under the climatic stress thereby elevating levels of GSH (Celi 2010). Significantly ($P < 0.05$) elevated levels of GSH in lactating cattle than the non-lactating cattle in all seasons may be due to the burden of lactation along with heat and cold stress (Bernabucci *et al.* 2002, Castillo *et al.* 2006). Significantly ($P < 0.05$) increased levels of MDA during hot summer and cold winter in both lactating and non-lactating animals than during spring and in lactating cattle in all seasons than non-lactating cattle is because of climatic stress (Bernabucci *et al.* 2002) in all animals and production stress (Castillo *et al.* 2006) in lactating animals.

In brief significantly ($P < 0.05$) higher levels of catalase, SOD, GSH and MDA were found in both the lactating and non-lactating cattle during summer and winter than during the spring. This may be due to the climatic stress, which increases oxidative stress indices (Celi 2010). In all the 3 seasons significantly ($P < 0.05$) higher levels of catalase, SOD, GSH and MDA were found in lactating cattle than the non-lactating cattle. This may be due to the overburden of lactation stress (Lohrke *et al.* 2004) in addition to climatic stress. Elevated oxidative stress indices in crossbred cattle during hot summer and cold winter and low levels during spring may reflect their better adaptation to lower temperature than

Table 1. Mean $\pm$ SE of catalase (in $\mu\text{mol H}_2\text{O}_2$ decomposed/min/mg Hb), SOD (in $\mu\text{mol MTT formazan/mg Hb}$), GSH (in $\mu\text{mol conjugate/ml packed RBC}$) and MDA (in nmol MDA/mg Hb) in different groups of cattle during winter and summer

Cattle		Winter				Summer			
		Catalase	SOD	GSH	MDA	Catalase	SOD	GSH	MDA
Lactating	Group 1 (1st lactation)	7.92 $\pm$ 0.640	215.89 $\pm$ 5.52	72.24 $\pm$ 1.82	2.20 $\pm$ 0.24	8.38 $\pm$ 0.720	316.14 $\pm$ 15.61	110.12 $\pm$ 3.34	3.89 $\pm$ 0.43
	Group 2 (2nd lactation)	7.41 $\pm$ 0.612	211.43 $\pm$ 4.99	65.12 $\pm$ 1.13	1.89 $\pm$ 0.15	8.28 $\pm$ 0.711	311.21 $\pm$ 11.12	108.54 $\pm$ 2.92	3.67 $\pm$ 0.32
	Group 3 (3rd lactation)	7.52 $\pm$ 0.632	213.32 $\pm$ 5.01	71.76 $\pm$ 1.71	2.12 $\pm$ 0.21	8.01 $\pm$ 0.699	317.54 $\pm$ 16.23	109.43 $\pm$ 3.05	3.59 $\pm$ 0.31
Non-lactating	Group 4 (12–18 month)	7.74 $\pm$ 0.651	210.19 $\pm$ 4.54	60.73 $\pm$ 1.10	1.86 $\pm$ 0.14	8.11 $\pm$ 0.701	314.53 $\pm$ 13.27	106.42 $\pm$ 2.43	3.50 $\pm$ 0.29
	Group 5 (18–24 month)	7.54 $\pm$ 0.633	207.65 $\pm$ 3.98	62.25 $\pm$ 1.12	1.69 $\pm$ 0.12	7.99 $\pm$ 0.689	310.11 $\pm$ 10.26	104.75 $\pm$ 2.11	3.47 $\pm$ 0.25
	Group 6 (24–30 month)	7.43 $\pm$ 0.623	208.32 $\pm$ 4.03	159.64 $\pm$ 1.09	1.78 $\pm$ 0.13	7.87 $\pm$ 0.657	309.34 $\pm$ 10.19	103.45 $\pm$ 1.99	3.45 $\pm$ 0.21

$P < 0.05$.

Table 2. Mean±SE of catalase (in $\mu\text{mol H}_2\text{O}_2$ decomposed/min/mg Hb), SOD (in $\mu\text{mol MTT formazan/mg Hb}$), GSH (in $\mu\text{mol conjugate/ml packed RBC}$) and MDA (in nmol MDA/mg Hb) in different groups of buffalo during winter and summer

Buffalo		Winter				Summer			
		Catalase	SOD	GSH	MDA	Catalase	SOD	GSH	MDA
Lactating	Group 1 (1st lactation)	6.91±0.591	202.23±3.91	63.97±1.53	1.98±0.21	7.72±0.613	312.34±12.25	104.54±1.98	3.66±0.32
	Group 2 (2nd lactation)	6.78±0.562	199.65±2.94	60.42±0.98	1.95±0.16	7.55±0.599	308.65±9.78	102.47±1.74	3.14±0.29
	Group 3 (3rd lactation)	6.59±0.491	200.18±3.02	61.67±1.19	1.98±0.19	7.63±0.601	306.95±7.96	106.64±2.92	3.42±0.30
Non-lactating	Group 4 (12–18 month)	6.82±0.573	198.87±2.68	59.92±0.89	1.42±0.12	7.33±0.578	302.86±5.32	100.32±1.42	2.98±0.22
	Group 5 (18–24 month)	7.31±0.501	199.34±3.35	60.20±1.01	1.27±0.11	8.27±0.662	303.43±6.09	98.87±1.10	2.86±0.20
	Group 6 (24–30 month)	7.12±0.492	198.45±2.54	58.97±0.85	1.56±0.12	8.01±0.641	305.75±6.91	101.24±1.53	3.01±0.29

hot or cold temperature under tropical climate (Banerjee and Ashutosh 2011).

In both lactating and non-lactating buffaloes, significantly ($P<0.05$) higher levels of catalase during summer and winter than the spring may be due to the heat and cold stress under too high and too low temperature (Table 2). Increased oxidative stress indices occur in buffaloes during summer (Lallawmkimi 2009). Significantly ($P<0.05$) elevated levels of catalase levels in lactating buffaloes than the non-lactating buffaloes under different seasons may be due to oxidative stress because of production stress. Lallawmkimi (2010) also reported significantly higher levels of catalase lactating Murrah buffaloes. Significant ($P<0.05$) increase in SOD levels during summer and winter may be due to climatic stress (Megahed *et al.* 2008) and during lactation may be due to the oxidative stress under high demands of production (Lallawmkimi *et al.* 2010). During summer and winter significant ($P<0.05$) increase in GSH levels is related to heat or cold stress, which causes oxidative stress (Sarwar *et al.* 2009). Significantly ($P<0.05$) higher levels of GSH in lactating buffaloes than the non-lactating buffaloes during different seasons indicated oxidative changes due to high milk yielding (Lallawmkimi 2009). Significantly ($P<0.05$) higher levels of MDA during summer and winter suggest oxidative damage under unfavorable temperatures (Megahed *et al.* 2008). Significantly ($P<0.05$) elevated levels in lactating buffaloes may be due to production stress along with climatic stress (Kumar *et al.* 2010).

In brief significantly ($P<0.05$) higher levels of catalase, SOD, GSH and MDA during summer and winter than in the spring in both the lactating and non-lactating buffaloes is related to the climatic stress, which causes oxidative damage thereby elevating levels of oxidative stress indices (Lallawmkimi 2009). Significantly ($P<0.05$) higher levels of catalase, SOD, GSH and MDA found in lactating buffaloes than the non-lactating buffaloes during all the seasons may

be due to lactation stress because increased milk production predispose to oxidative stress (Lallawmkimi *et al.* 2010).

Oxidative stress indices of crossbred cattle were higher than the buffaloes during summer whereas buffaloes showed higher values of oxidative stress indices during winter than the crossbred cattle. This may be due to inherent adaption of buffaloes to high temperature and their susceptibility to low temperature or alternatively susceptibility of crossbred cattle to higher temperature in tropical countries and their better adaptation to lower temperature. Banerjee and Ashutosh (2011) also reported higher levels of stress indices in crossbred cattle during hot summer whereas Megahed *et al.* (2008) reported higher levels of stress indices in buffalo cows during winter.

Under the changing climatic scenario and the increasing demands for high milk production from dairy animals, oxidative stress is inevitable. This may be a reason for increased levels of oxidative stress indices during summer and winter in lactating cattle and buffaloes. Oxidative stress may predispose animals to disease. Further research will help in devising the strategies that will combat oxidative stress.

SUMMARY

The aim of this study was to find out the impact of climate change and production stress on oxidative stress indices in lactating and non-lactating cattle and buffalo under tropical conditions. Oxidative stress (OS) indices like catalase (CAT), super oxide dismutase (SOD), glutathione reductase (GSH) and lipid peroxidase (LPO) were estimated from the blood samples during winter, spring and summer. Oxidative stress indices were significantly elevated in both cattle and buffalo during hot summer and cold winter than during spring season indicating stress due to climatic stress. Significantly elevated levels of oxidative stress indices were recorded in lactating animals under different seasons than non-lactating animals indicating production stress in addition to climatic stress in

lactating animals. Climate change and demand for heavy milk production predisposes animals to oxidative stress. Strategies to combat climate change induced oxidative stress need to be devised to prevent animal sufferings and production loss.

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