

# Impact of The Ageing on Oxidant/Antioxidant Balance And Hematology of Indigenous Cow and Its Amelioration by Ascorbic Acid

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## Research Article

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# Abstract

Ageing is a complex biological process of all flora and fauna. It leads to a gradual reduction in the ability to maintain homeostasis under internal physiological and external environmental stresses, hence reducing the viability of individual's and increasing their vulnerability to diseases. The objective of this study was to investigate the alteration in the oxidant /antioxidant balance and hematology in four different age group of indigenous *Tharparkar* cattle and its amelioration by ascorbic acid. A total 24 female *Tharparkar* cattle were included and were categorized into 4 groups of six cows in each group. Animals below 1 year-age were kept in group I, between 1 to 8 year-age in group II, between 8-10 year-age in group III and above 10 year-age in group IV. The oxidant/antioxidant markers (LPO, SOD, GSH, GPX and catalase activity) and haematological panels (Hb, TEC, TLC, Platelet and DLC) were determined on day 0, before ascorbic acid supplementation, and on day 6, 12, 18 and 24 post ascorbic acid supplementation. In the present study LPO and SOD levels were significantly ( $P < 0.05$ ) increased with the ageing. GSH and GPX activities significantly ( $P < 0.05$ ) decreased with ageing in different age groups. Variations in hematological parameters were also observed with ageing in different age groups. The geriatric cattle (> 10 years) exhibited remarkable alteration in oxidative stress indices, and haematological panels when compared to other groups. Supplementation of ascorbic acid reduced the oxidative stress and improved hemoglobin, PCV, TEC and TLC levels. From the findings of this study, it can be inferred that administration of ascorbic acid is helpful in ameliorating altered oxidant/antioxidant balance and hematological parameters with ageing in various age groups of cattle.

# Introduction

The livestock sector plays a very important role in the welfare of Indian rural economy. As age progresses, most vital organs show a gradual deterioration in their functional capacity attributed to the marked reduction in the individual's reserve capacity. To understand the ageing process, several hypotheses have been postulated. But, the evidences suggest that the harmful and cumulative effects of the reactive oxygen species (ROS) during lifespan are the paramount factors of the ageing process (Knight, 2000; Junqueira et al., 2004). An elevated release of free radicals by aging mitochondria and reduced antioxidant defense of geriatric individuals probably attributed to the development oxidative stress (Harman, 1998; Finkel and Holbrook, 2000). Oxidative changes in lipids, proteins and DNA have been produced by excessive levels of ROS and other reactive oxidants in aged animals (Chen et al, 2000). However, the studies demonstrating the associations of oxidative stress with ageing are still not clear (Cini and Moretti, 1995). The role of free radical reactions is now widely recognized in the clinical pathology of many diseases of cattle; however, the potential role of these reactions in the ageing process of indigenous cows is not yet unraveled.

Hematology always be helpful to assess the animal's health status and values of haematological panels alter as the age advances. A decreased haemoglobin, PCV and leukocyte counts is observed with the advancement of the age of animals. Routine haematological appraisal of geriatric animals could assist in earlier detection of degenerative diseases (Dell'Osa and Jaensch, 2016).

Oxidative stress is characterized by the disproportion between the development of oxidants or free radicals and the capability of the body to use anti-oxidant protection mechanisms to eliminate its adverse effects. Oxidative parameters SOD, CAT and GSH, along with GPX, are the most essential intracellular antioxidant defense mechanisms to counteract the imminent oxidative damage caused by free radicals during inflammation or other triggering agents. As postulated by Harman (1998) in his free radical theory of ageing, lipid peroxidation plays a crucial role in ageing individuals. Increased oxidative stress is sequel to production and exposure to ROS and antioxidant defense mechanism by the host in turn will lead to senescence (Stadtman, 2004).

In both animal and human medicine, the role antioxidants in health and various disease conditions is demonstrated very well (Valko et al., 2007; Celi, 2011). Briefly, antioxidants (such as thiols, ascorbates and polyphenols) can act against ROS and hence prevent oxidation. In most mammalian species except human, non-human primates and guinea pigs, ascorbic acid, a six-carbon lactone is synthesized from glucose in the liver. In blood plasma, neutrophils and other body tissues, ascorbic acid is an important antioxidant nutrient and has numerous physiological functions by playing a significant role in controlling the immune functions and providing protection at several cellular and subcellular stages from oxidative damage. Levels of ascorbic acid usually decreased during stress and physiological dysfunction as reported by several researchers (Ali, 2000; Ranjan, et al. 2005).

Most of the studies focused on the use of immunomodulators and antioxidants in therapeutic and prophylactic management of infectious diseases. However, there is a scarcity of information on oxidant/antioxidant profile and use of antioxidants in apparently healthy geriatric animals. Moreover, gerontological studies in livestock farm animals are meager as compared to pet and laboratory animals. Keeping in view of these facts and research reports, the current study was undertaken to study age-related alterations in oxidant/antioxidant balance and hematological panels of apparently healthy Tharparkar cattle. The evaluation of beneficial effects of ascorbic acid supplementation against altered oxidant/antioxidant balance and hematological panels in ageing was also aimed.

## Materials And Methods

### Experimental design

The research was conducted at the LPM section of (cattle and buffalo farm) Indian Veterinary Research Institute, ICAR-IVRI, Izatnagar, Uttar Pradesh 243 122 India. Selection of animals was made randomly from the farm herd, Tharparkar breed was selected for the study which is maintained under common nutrition and managemental strategies. Animals were fed on balanced ration prepared at the Feed Technology Division of the Institute. There was no additional feeding of antioxidant such as vitamin E and selenium. The animals were categorized into 4 groups (groups 1 to 4), each group having 6 animals (n=6). Groups were based on age in years. Group I consisted of birth to less than one year, II consisted of 1 to less than 8 years, III consisted of 8 to less than 10 years and IV consisted of 10 years and above age (Geriatric). All 24 cows included in the research were visually healthy.

As age-related variations in the indices of oxidative stress and hematological parameters were observed, ameliorative steps were undertaken using ascorbic acid. All the groups were treated with commercial preparations of ascorbic acid @ 20mg/kg- SID i/m for 5 consecutive days. The dose of ascorbic acid was determined according to Roth and Kaeberle (1985) report.

### Sample collection and processing

Animals were evaluated for changes in oxidant-antioxidant profile and hematological parameters before the start of therapy (day 0) and post therapy (day 6, 12, 18 and 24) of the study. Blood samples (10 ml) were collected from the jugular vein puncture in vials containing heparin and EDTA. The heparinized blood was centrifuged at 3000 rpm for 10 min and plasma and buffy coat were removed. In Phosphate Buffered Saline (PBS, pH 7.4) solution, the RBC's were gently dissolved and centrifuged again at 3000 rpm for 10 min. This process was repeated twice after discarding supernatant. About 2/3rd of the RBC pellet was diluted with ice-cold distilled water in 1:10 ratio for the preparation of 10% hemolysate, which was used for the estimation of lipid peroxidation (LPO), superoxide dismutase (SOD) catalase (CAT) and glutathione peroxidase (GPX). Remaining 1/3rd of the RBC pellet was diluted with PBS solution in 1:1 ratio to get RBC suspension which was used for reduced glutathione (GSH) estimation.

Cyanohemoglobin procedure was used to measure haemoglobin concentration of hemolysate (Tentori and Salvati, 1981).

### Evaluation of oxidant-antioxidant profile

According to Placer et al., (1966) lipid peroxidation concentration in RBC hemolysate in terms of concentration of malondialdehyde (MDA) per mg haemoglobin was measured. As per Prins and Loos, (1969) reduced glutathione in RBC suspension was measured by DTNB (5, 5' - Dihydrobis-2-Nitro Benzoic acid) method. The levels of superoxide dismutase (SOD) were determined according to the Madesh and Balasubramanian (1998) method and the unit is expressed as  $\mu\text{mol MTT formazan}$  formed per mg of haemoglobin. As per Aebi, (1986) catalase levels in haemolysate were estimated using  $\text{H}_2\text{O}_2$  as a substrate. Glutathione peroxidase (GPX) was measured by Paglia and Valentine (1967) method.

### Evaluation of hematological profile

Haemoglobin concentration (gm/dl) in the whole blood was estimated by modified Sahlis acid hematin method (Berman, 1919). Jain (1986) method was used for TEC, TLC, DLC (Differential Leukocyte Count), PCV and Platelet count examination.

## Statistical analysis

Data were analyzed by two way repeated measures ANOVA. The values were expressed as mean  $\pm$  S.E. To study the effect of groups, periods and interaction of group & period for various parameters for the repeated observations on the same animals during different periods following general linear model was used.

$$y_{ijk} = \mu + G_i + ID(G_i) + P_j + (GP)_{ij} + e_{ijk}$$

Where,

$y_{ijk}$  = k-th observed value of the response variable for i-th group for the j-th period

$\mu$  = General mean effect

$ID(G_i)$  = Random effect

$G_i$  = Effect of i-th group

$P_j$  = Effect of the j-th period

$(GP)_{ij}$  = Interaction effect of i-th group and j-th period

$e_{ijk}$  = error term

The multiple comparisons between groups, periods, time intervals and their interactions for various parameters were done by using Tukey test at 5% level of significance. The analysis was done by JMP 9.0 software.

## Results

Marked oxidative stress was observed in groups 1 to 8 year, 8 to 10 year and above 10 year age group of cattle. Variation in oxidative stress indices with ageing in cattle during the study period is depicted in the table 1. LPO and SOD levels were found to be markedly increased with ageing, and it was significantly ( $P < 0.05$ ) altered in all studied groups of the cows. GSH level was estimated to be decreased with ageing; however significant difference between 8-10 years cows and cows above 10 years age was not estimated. Similarly GPX level also found to be decreased with ageing, and significant difference ( $P \leq 0.05$ ) in GPX level was estimated in 1 to 8 years, 8 to 10 years, and above 10 years age group cows as compared with the animals below one year. The catalase activity of 8 to 10 years and above 10 years cows was significantly ( $P \leq 0.05$ ) higher as compared to animals below one year age group.

Moreover, mild alterations in the hematological parameters of different age groups of cows were recorded. Values of haemoglobin, TEC, PCV, TLC, differential leukocyte and platelet count are depicted in Table 2 and 3. Below one year aged animals revealed significantly ( $P < 0.05$ ) higher levels of hemoglobin, PCV and TEC concentration compared to other age groups. Significant ( $P < 0.05$ ) reduction in hemoglobin levels with ageing was estimated between 8 to 10 years and above 10 years age in comparison to below one year aged animals. Similarly PCV, TEC and TLC levels were found to be significantly ( $P < 0.05$ ) lowered with ageing. Moreover, 8 to 10 years and above 10 years age group cows revealed significantly ( $P < 0.05$ ) lowered PCV, TEC and TLC values as compared with animals below one year age group. Even

though values were within the normal reference range decreasing trend was observed in haemoglobin, TEC, PCV and TLC counts with ageing.

Significant ( $P < 0.05$ ) increase in platelets count was found only in the cows above 10 years age group. In differential leukocyte count significant ( $P < 0.05$ ) reduction in lymphocyte percentage was noticed in 8 to 10 years and above 10 year age group cows, and significant ( $P < 0.05$ ) increase in neutrophils percentage was observed in 8 to 10 year and above 10 year age group animals. Whereas eosinophil percentage was significantly higher in above 10 year age group cows. Monocytes and basophil percentage were not found to be significantly altered among the various age groups.

After ascorbic acid administration, below one year aged animals did not exhibit any significant changes in oxidant/ antioxidant balance and hematological values on days 6, 12, 18 and 24 post-administration. In 1 to 8 year age group animals, significant ( $P < 0.05$ ) reduction in LPO levels was noticed on day 18 and day 24, and the values were comparable to below one year age group. The 8 to 10 years and above 10 years age group cows also exhibited significant ( $P < 0.05$ ) reduction in LPO levels on day 12 and at subsequent interval of collection compared to their day 0 values. In SOD activities, significant ( $P < 0.05$ ) reduction was noticed on day 6 in 1 to 8 years age group, on day 12 in 8 to 10 years and above 10 years age group.

The GSH levels of 1 to 8 years, 8 to 10 years and above 10 years age group cows significant ( $P < 0.05$ ) improvements on days 24, 12 and 6, respectively, was found when compared with their day 0 values. In the both 8 to 10 years and above 10 years age groups, GSH levels were comparable to 1 to 8 years age group cows on day 24. Significant ( $P < 0.05$ ) increment in GPX activities was recorded on day 6 in 1 to 8 years, 8 to 10 years and above 10 years age groups cattle. 1 to 8 year aged cattle revealed a comparable GPX activity as of below one year age group on days 18 and 24. Significant ( $P < 0.05$ ) reduction in catalase activity was observed on day 6 only in above 10 years age group cows.

After ascorbic acid administration, significant ( $P < 0.05$ ) improvement in hemoglobin concentration was observed in 8 to 10 years and above 10 years age group of cattle on day 24. However changes in PCV and TEC levels were non-significant, but improved values were observed in 1-8 years, 8 to 10 years and above 10 years age group of cows on day 24 with increasing trend. Significant ( $P < 0.05$ ) elevation in TLC counts was observed on day 12 in 8 to 10 years age and above 10 years age group of cows. The platelets counts were not significantly altered in different age groups even after ascorbic acid administration. Significant ( $P < 0.05$ ) increase in lymphocyte percentage was noticed in 8 to 10 years aged cows on day 18. Whereas, significant ( $P \leq 0.05$ ) reduction in neutrophil percentage in 8 to 10 years age cows was observed on day 24.

Analysis by repeated measure ANOVA method revealed significant ( $P < 0.05$ ) differences between different age groups, before and after ascorbic administration with respect to the values/activities of LPO, SOD, GSH and GPX. No significant alteration in catalase activity was revealed in below one year, 1 to 8 years, 8 to 10 years animals. Further analysis of data revealed significant ( $P < 0.05$ ) changes in hematological parameters such as hemoglobin concentration, PCV, TEC, TLC and DLC before and after

treatment. Changes in the parameters are predominant on 18th and on day 24 than on 6th and 12th day of treatment. Geriatric group of cattle (> 10 year) exhibited considerable alteration in oxidant/antioxidant and hematological parameters compared to below one year aged animals.

## Discussion

Oxidative stress is associated with ageing and various diseases, including neoplasia, neurological disorders, diabetes and skin disorder (Valko et al., 2007; Singh and Dimri, 2010). Cell components, especially mitochondria and DNA sequences are damaged mainly by accumulation of reactive oxygen species (ROS) that are released by oxidative metabolism over a time (Wang and Wang, 2012; Carri et al., 2015). High productive cows are usually prone to greater risk of health related issues mainly due to inability to combat ROS generated in highly active metabolic cells (Sordillo, 2009). In the present study, our findings support the hypothesis that the levels of oxidative stress increases, and hematological parameters get altered during the ageing process. Similar pattern of changes were observed in senescent animal during aging in oxidative stress indices (Cand and Verdeti, 1989; Navarro, et al., 2004).

In the current study, LPO and SOD levels were significantly ( $P < 0.05$ ) increased with ageing in all age groups. Free radicals production leads to oxidative damage of proteins, lipids and nucleotides which in turn may cause dysfunction of nervous system and ultimately neuronal death (Head, 2013). Gala *et al.* (1996) also reported higher levels of MDA and SOD in aged dogs than young group, greater exposure of old tissues and cells to free radicals and their greater peroxidation rate when cell homogenates are exposed to sudden oxidation also indicate that enzymatic activities in red blood cells are highly age dependent (Kaspoglu, *et al.*, 2001)

The GSH level and GPX activities were found to be decreased with ageing. Similarly low GSH levels were observed in aged dogs when compared to young dogs as reported by Gala *et al.* (1996). Enzyme activity is decreased in old tissues and changed with age, which may be one of the potential causes for decreased enzymatic activities (Remacle, et al., 1992). Another reason may be the in vivo oxygen radical production is depleted in aged animals, the reduction in antioxidant defenses might be considered as a decreased physiological compensatory mechanism rather than adverse change leading to additive oxidative damage (Kaspoglu, *et al.*, 2001).

In general, antioxidant vitamins improve various components of cellular and noncellular immune response. Effective antioxidants include naturally occurring free radical scavenging antioxidant enzymes and also products that potentially enhance the enzymatic activity of GPX, GSH, SOD and CAT enzymes. In adult ruminants ascorbic acid is not an important dietary nutrient; however, advantages have been seen by supplementing ascorbic acid for stressed animals in adverse weather conditions and in health related disorders (Kolb, 1984). Ascorbic acid administration is cost effective and intervene harmful effects of free radicals and also very effective against age related neurological disorders in human (Harrison, 2012).

In the present study, administration of vitamin C had significantly ( $P < 0.05$ ) decreased altered LPO and SOD levels at different intervals. Significant ( $P < 0.05$ ) improvement in GPx and GSH levels was noticed in 1 to 8 year, 8 to 10 year and above 10 year age group cattle. Ascorbic acid is a powerful antioxidant and reducing agent soluble in water that prevents protein, lipid and DNA oxidation (Padayatty et al., 2003). Block, et al. (2003) reported a direct correlation between increased lipid oxidation products and decrease of vitamin C & E levels with ageing. Paolisso, et al. (1998) and Mecocci, et al. (2000) also reported decrease vitamin C levels in plasma is associated with increasing age.

The hematological profile plays an important diagnostic tool as a bare minimum database module. This may be a good tool for monitoring response to therapy, or as a starting point for preparing a list of differential diagnoses and for finding the severity of the infection. However, data integration is important for the highest diagnostic output (Barger, 2003). The variations in biochemical and haematological parameters helps in the evaluation of the animal's physiological or pathological state (Ahmad et al., 2003).

In our study hematological parameters namely hemoglobin, TEC, Hct % and TLC level decreased with ageing, similar findings has been reported by Deepak kumar (2019), Lane and Campbell (1969). With ageing there may be reduced red blood cell production and more irreparable damage of red blood cell in cow has been reported. Efficiency and enzymatic activity of red cells reduces with ageing which may be the reason for reduced activity. Size of the RBC and the associated Hb composition (MCH) of red blood cell increases where as haematocrit and TEC decreases in aged animals (Shaffer et al., 1981).

In the present study, TLC and number of lymphocytes decreased where as neutrophil and eosinophilic counts increased with ageing, mild neutrophilia are predominant in 8 to 10 year and above 10 year age group cattle, which is similar to findings of Bedenicki et al. (2014). Reduced lymphocyte count may be one of the causes of immunosenescence. Reduced lymphocyte counts can be directly proportional to CD4 + T cells decline with ageing. Stress leukogram leads to reduction in lymphocyte counts (Radakovich et al., 2017).

After administration of ascorbic acid 8 to 10 year and above 10 year age group cattle exhibited significant ( $P < 0.05$ ) improvement in Hb, TEC and PCV levels. Vitamin C increases the haematocrit through enhanced iron absorption and as such helps in reversal of anemia (Atanasova et al., 2004). Significant ( $P < 0.05$ ) improvement in TLC after therapy in 8 to 10 year and 10 year age group animals were observed on day 12. Our finding is in agreement with Fraser et al. (1980) and Field et al. (2002) who reported animals administered with ascorbic acid have exhibited increased levels of total WBC count in blood circulation. White blood cell activity is enhanced by both vitamin C and E administration in healthy individuals (Jeng et al., 1996). This is at variance with reports, Owu et al. (2016) who reported vitamin C did not significantly alter the WBC count in the treated groups.

In DLC, neutrophil levels significantly ( $P < 0.05$ ) decreased in 8 to 10 year on day 24 where as lymphocyte levels significantly ( $P < 0.05$ ) enhanced in 8 to 10 year on day 18. These findings were similar to Babe, (2011) who reported vitamin C has the capability to decrease the percentage of neutrophils and increase

the lymphocyte percentage significantly in heat stressed sheep. An important function of vitamin C in immune system is to protect the body against foreign invaders. Vitamin C completes its important activity by increasing synthesis of WBC's especially neutrophils which helps to fight against microorganisms such as bacteria and virus. Ascorbic acid is very crucial for the immune system and enhances synthesis of prostaglandin, T-lymphocytes and other WBC's (Eberhard et al., 1989).

Even though platelet count increased with age, significant ( $P < 0.05$ ) enhancement was noticed in above 10 year age group cattle. High count can be due to more synthesis of erythropoietin due to greater demands for oxygen and  $CO_2$  transport mainly because of enhanced metabolic activity or faulty exchange of gases caused by respiratory membrane damage (Zaki et al., 2008).

Altered oxidant/antioxidant status and hematological profile signifies that 8 to 10 year and above 10 year age are in a state of significant oxidative stress. Ageing causes change in the cattle oxidant/ antioxidant balance leading to oxidative stress which is observed in the present study.

In conclusions, ageing is associated with a remarkable alteration in oxidative stress indices and hematological parameters. Supplementation of ascorbic acid enhanced the oxidant-antioxidant status and improved altered hematological parameters in different age groups. Hence, supplementation of ascorbic acid may be helpful in managing cattle of different age group for better response. Furthermore, large scale studies are needed in order to validate the potential use of antioxidant therapy with ageing.

## Declarations

This is to declare that the article is original, has not been submitted or published elsewhere, and has the approval of all authors.

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**Ethical approval:** This study is approved by Institute Animal Ethics Committee (IAEC) vide order No. F. 26-2/2019/JD(R) dated: 6/01/2020

**Consent to participate:** Not applicable

**Consent for publication:** Not applicable

**Availability of data and material:** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability: Not applicable

### Author's contribution

Dr. Umesh Dimri and Dr. Mahendran K designed the present study, which was executed by Dr. Kavitha, K., Dr. A.K. Chaudhary, Dr. Megha, P., Dr. Stanzin Angmoo and Dr. Harini K.R. Data analysis was performed by Dr. M.R. Verma. The manuscript was prepared by Dr. Kavitha, K, Dr. Shankar K Singh, Dr. Gaur, Dr. Vivek Joshi and Dr. Gyanendra Singh with inputs and final approval by all authors.

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