Porcine Research

# Evaluation of oxidative stress during hot dry and hot humid environmental periods in indigenous pigs from arid tracts in India

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**Abstract**. To explore whether hot environmental period can cause oxidative stress in the healthy indigenous pigs from arid tracts in India, blood samples were collected to harvest the serum, when environmental conditions were moderate, hot dry and hot humid. To assess the existence of possible oxidative stress, serum markers like catalase, monoamine oxidase and glutathione reductase were determined. The mean value of each marker obtained during moderate environmental period was considered as control value. Serum catalase, monoamine oxidase and glutathione reductase activities increased significantly ($p \leq 0.05$) during hot dry and hot humid environmental periods as compared to moderate period. Hot humid mean values were 17.64%, 6.63% and 50.00% higher, respectively as compared to respective hot dry mean value. In each environmental period, mean value of each serum marker was significantly higher ($p \leq 0.05$) in male animals than in female. Extent of increase was maximum in animals of 10-12 months of age group and minimum in animals of 6-8 months of age groups. It was assumed that oxidative stress developed both during hot dry as well as hot humid periods in the indigenous pigs. This assumption was drawn on the basis of significant changes observed in the status of the serum markers. The modulation of physiological mechanisms in response to free radicals was evident. Magnitude of changes was greatest during hot humid period. This visibly illustrated that hot humid environment was more stressful to animals than hot dry. Extent of development of oxidative stress was greater in elder animals of both sexes and in male animals during hot humid environment. The evaluation of the degree of oxidative stress in the form of values can be useful to redefine the role of oxidative stress in different pathologies and can be used for clinical diagnosis and in health management of indigenous pigs in the arid tract. On the basis of findings, the use of antioxidants can be recommended during hot dry and hot humid environmental periods.

**Key Words**: catalase, environmental condition, glutathione reductase, monoamine oxidase, serum.

**Introduction**. In arid regions, pigs encounter frequently the environmental temperatures above the zone of thermoneutrality or comfort zone. These drastic changes in temperature and humidity may not result in death losses, but they can cause reduced growth and reproduction performance in the breeding herd. High humidity combined with high temperatures can enhance the negative effects of the high temperatures. In view of the fact that the pigs have to depend largely on evaporative heat loss to strive to keep on cool when it is hot, humidity level is very important. The greater the humidity level in the air, the less effective is the process of evaporative cooling. Pig farming faces many challenges like heat stress, disease, poor nutrition etc. which can effectively reduce the production performance. Research to find out physiological variations in hot climates in pigs is gaining importance as a primary focus point to facilitate expansion of pig industry

in harsher climatic areas. Heat stress results from the animal's inability to dissipate sufficient heat to maintain homeothermy. Animals respond to heat stress with a series of reactions. Studies with chronic heat stress have demonstrated altered physiological, metabolic, biochemical and cellular responses in animal models (Kataria et al 2014).

Under heat stress conditions, the aim is to decrease heat transfer from the surroundings to the animal and to amplify the process of heat transfer out of the animal. These mechanisms may help in reducing the effective temperature of the animal. This is important on arid tracts where in natural conditions it is impossible to check the encounter of animal with higher environmental temperatures. Environmental variations cause threats to living organisms all through their life span. Alterations in the echelon of oxygen, redox state and temperature may activate molecular concerns in an organism facilitating survival, reproduction and adaptation (Kagias et al 2012). Oxidative stress has been implicated in the pathogenesis of several acute and chronic diseases (Oyinloye et al 2016).

Alteration in environmental temperature is contemplated like stressor rooting oxidative stress in animals (Kataria et al 2010a). Progression of oxidative stress in indigenous pigs belonging to arid tracts due to high environmental temperature have been reported by researchers (Kataria et al 2013a). Antioxidant system maintains the concentration of reactive oxygen species (ROS) at ebb due to reactive characteristics and as a consequence to the event where the concentration of former is exceeded by latter, oxidative stress develops. Heat stress influences antioxidant status to a greater extent (Kataria et al 2013b). Since oxidative stress does not exhibit any symptom, various markers are used to evaluate oxidative stress in the laboratories (Kataria et al 2010c). Antioxidant enzymes are deemed to be effective markers of oxidative stress as latter brings about changes in their levels (Kataria et al 2010b). Scientists have observed changes in oxidative status during growth period of piglets including weaning effects (Degroote et al 2015). It is imperative to enrich antioxidative/oxidative balance by increasing antioxidative potential in pigs (Szczubiał 2015).

There is rareness of research work regarding hot dry and hot humid environmental temperature related oxidative stress in indigenous pigs. Therefore the present study was launched and the objective of our work was to evaluate the impact of oxidative stress induced due to hot dry and hot humid environmental periods on the antioxidant status of indigenous pigs. As markers, we chose serum antioxidant enzymes and determined their levels during moderate, hot dry and hot humid environmental periods.

**Material and Method**. The study was carried out in 180 apparently healthy indigenous pigs of both sexes, between 6 months to 12 months of age during moderate (maximum temperature ranged from 27 to 30$^{o}$C with corresponding relative humidity from 35 to 30%); hot dry (maximum temperature ranged from 44 to 49$^{o}$C with corresponding relative humidity from 15 to 8%) and hot humid (maximum temperature ranged from 39 to 42$^{o}$C with corresponding relative humidity from 63 to 52%) environmental periods. Blood samples were collected during slaughtering from private slaughter houses (Bikaner, Rajasthan, India) where all the animals were kept in similar conditions of management. In each ambience, 60 blood samples were obtained to harvest the serum samples and the animals were categorized regarding sexes as males (30) and non-pregnant females (30) and regarding age as 6-8 months (10 males and 10 females); 8-10 months (10 males and 10 females) and 10-12 months (10 males and 10 females). Serum markers of oxidative stress analyzed included catalase, monoamine oxidase and glutathione reductase.

Serum catalase was determined by the method as described by Goth (1991). The assay involved a combination of spectrophotometric assay of hydrogen peroxide with an optimized serum catalase determination. Serum was incubated with hydrogen peroxide and sodium-potassium phosphate buffer. One unit catalase decomposes 1 µ mol of hydrogen peroxide per 1 minute under these conditions. After stopping the reaction hydrogen peroxide was assayed spectrophotometrically.

Serum monoamine oxidase was determined by the colorimetric method of Green & Haughton (1961) with slight modification. Serum (0.1 mL) was processed with reagents as described in the method and extinction was measured at 450 mμ. Stock solution was prepared as 1 mg/mL, which was diluted 10 fold so as to give 0.01 mg/0.1 mL with 0.05 units. One mg was equal to 5 units. A series of standards was prepared by taking concentrations of monoamine oxidase enzyme in mg in an increasing order. A graph was plotted between units of enzyme ($UL^{-1}$) and optical densities and values of serum samples were obtained directly. Amount of enzyme was taken as 0.4, 0.8, 1.2, 1.6 and 2.0 mL and total quantity was made 2 mL by adding phosphate buffer. This gave the $UL^{-1}$ as 100, 200, 300, 400 and 500.

Serum glutathione reductase was determined by the colorimetric method as described by King (1965) with slight modification. Serum was treated with coenzyme solution for reduction of endogenous substrates. Then substrate was added and enzyme activity was determined by change in extinction.

$$\text{Activity (kU L}^{-1}\text{)} = \text{Change in OD per minute at 340 m}\mu \text{ x 1000 x 0.5 x 2}$$

Where: 1000 is the dilution factor, 0.5 is the quantity of serum and 2 is the time in minutes for first reaction).

Statistical significance for individual parameter between moderate and hot periods was analysed (Kaps & Lamberson 2004).

**Results**. The mean values of serum enzyme markers of oxidative stress viz. catalase, monoamine oxidase and glutathione reductase are presented in Table 1. The mean value of each marker during moderate environmental period was considered as control value. The mean value of each marker obtained during hot dry and hot humid environmental period was compared from respective control.

For each serum marker, mean values during hot dry and hot humid environmental periods were significantly higher ($p \leq 0.05$) than respective moderate mean value. Mean value during hot humid environmental periods was significantly higher ($p \leq 0.05$) than respective hot dry mean value for each serum marker. Percent changes in hot dry mean values of catalase, monoamine oxidase and glutathione reductase were 98.70%, 174.02% and 150.00%, respectively as compared to respective moderate mean value. Percent changes in hot humid mean values were 133.76%, 192.20% and 275.00%, respectively as compared to respective moderate mean value. Percent changes in hot humid mean values were 17.64%, 6.63% and 50.00%, respectively as compared to respective hot dry mean value. In each environmental period, mean value of each marker was significantly higher ($p \leq 0.05$) in male than in female individuals. Similarly, mean value of each marker was highest in animals of 10-12 months of age group and lowest in animals of 6-8 months of age groups. Differences were significant ($p \leq 0.05$) for each marker in every environmental period.

Table 1

Mean ± SEM values of serum enzyme markers of oxidative stress in indigenous pigs

| *Serum enzyme markers of oxidative stress* | *Environmental period* | *Overall* | *Gender* | | *Age groups* | | |
|---|---|---|---|---|---|---|---|
| | | | *Male (30)* | *Female (30)* | *6-8 month (20)* | *8-10 month (20)* | *10-12 month (20)* |
| Catalase $kUL^{-1}$ | Moderate (60) | $77.00 \pm 0.90^{b}$ | $88.00 \pm 0.30^{bc}$ | $66.00 \pm 0.25^{bc}$ | $63.00 \pm 0.12^{bd}$ | $75.00 \pm 0.20^{bd}$ | $93.00 \pm 0.13^{bd}$ |
| | Hot dry (60) | $153.00 \pm 0.50^{b}$ | $165.00 \pm 0.31^{bc}$ | $141.00 \pm 0.24^{bc}$ | $133.00 \pm 0.10^{bd}$ | $154.00 \pm 0.13^{bd}$ | $172.00 \pm 0.12^{bd}$ |
| | Hot humid (60) | $180.00 \pm 0.52^{b}$ | $200.00 \pm 0.28^{bc}$ | $160.00 \pm 0.20^{bc}$ | $158.00 \pm 0.11^{bd}$ | $179.00 \pm 0.12^{bd}$ | $203.00 \pm 0.14^{bd}$ |
| Monoamine oxidase ($UL^{-1}$) | Moderate (60) | $163.00 \pm 1.33^{b}$ | $174.00 \pm 0.10^{bc}$ | $152.00 \pm 0.10^{bc}$ | $150.00 \pm 0.10^{bd}$ | $162.00 \pm 0.10^{bd}$ | $177.00 \pm 0.10^{bd}$ |
| | Hot dry (60) | $422.00 \pm 1.52^{b}$ | $442.00 \pm 0.17^{bc}$ | $400.00 \pm 0.15^{bc}$ | $390.00 \pm 0.12^{bd}$ | $427.00 \pm 0.13^{bd}$ | $449.00 \pm 0.11^{bd}$ |
| | Hot humid (60) | $450.00 \pm 1.50^{b}$ | $470.00 \pm 0.15^{bc}$ | $430.00 \pm 0.14^{bc}$ | $427.00 \pm 0.14^{bd}$ | $451.00 \pm 0.11^{bd}$ | $472.00 \pm 0.12^{bd}$ |
| Glutathione reductase $kUL^{-1}$ | Moderate (60) | $8.00 \pm 0.10^{b}$ | $10.00 \pm 0.09^{bc}$ | $6.00 \pm 0.09^{bc}$ | $4.50 \pm 0.05^{bd}$ | $8.50 \pm 0.07^{bd}$ | $11.00 \pm 0.06^{bd}$ |
| | Hot dry (60) | $20.00 \pm 0.11^{b}$ | $25.00 \pm 0.08^{bc}$ | $15.00 \pm 0.07^{bc}$ | $12.00 \pm 0.06^{bd}$ | $21.00 \pm 0.05^{bd}$ | $27.00 \pm 0.06^{bd}$ |
| | Hot humid (60) | $30.00 \pm 0.12^{b}$ | $39.00 \pm 0.07^{bc}$ | $21.00 \pm 0.08^{bc}$ | $17.00 \pm 0.04^{bd}$ | $31.00 \pm 0.05^{bd}$ | $42.00 \pm 0.06^{bd}$ |

i. Figures in the parenthesis indicate number of indigenous pigs considered for evaluation;
ii. Overall value is the mean value obtained from indigenous pigs in an environmental period irrespective of sexes and age;
iii. 'b' marks significant ($p \leq 0.05$) differences among moderate, hot dry and hot humid mean values for a marker;
iv. 'c' marks significant ($p \leq 0.05$) differences between mean values of males and females within an environmental period;
v. 'd' marks significant ($p \leq 0.05$) differences among mean values of age groups within an environmental period.

**Discussion**. The control mean values of serum catalase, monoamine oxidase and glutathione reductase were in agreement with the earlier recordings in animals (Kataria et al 2010a,b,c). Studies pertaining to above stated parameters in serum are few in the literature. Results indicated that serum catalase, monoamine oxidase and glutathione reductase activities increased significantly ($p \leq 0.05$) during hot dry and hot humid environmental periods as compared to moderate environmental conditions. Maximum percent change during hot humid was observed in the value of serum glutathione reductase.

***Catalase (CAT)***. Environment associated variations in the catalase activities have been reported by many researchers (Marti et al 2007; Kataria et al 2010b,c) attributing it to higher rate of formation of hydrogen peroxide. The observations of present study regarding changes due to age authenticated the earlier findings (Kataria et al 2012; Pandey et al 2012). Higher serum catalase activity of elder group showed the effect of age. De & Darad (1991) conducted a study in rats and observed that catalase activity increased with the advancement in age. Higher catalase was to decompose hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani et al 1996; Chelikani et al 2004) as a part of antioxidant defense system. Catalase activity in animals has been related to reactive oxygen species generation (Phua 2004). This unambiguously illustrated the retort of the physiological mechanisms to the oxidative stress (Kataria et al 2010c). Serum catalase can be used effectively as marker of oxidative stress (Halliwell & Gutteridge 1999). The increased activity of serum catalase during hot ambience insinuated the power to impart defense to counter free radicals as a rejoinder of the body to treat the oxidative stress (Kataria et al 2010a,c). Many workers have recommended the use of catalase in the situations where free radicals are formed (Seekamp et al 1988). Enhanced catalase activity can be associated with the presence of oxidative stress in pigs of both genders and all age groups during hot dry and hot humid periods.

***Monoamine oxidase (MAO)***. There is scantiness of research on serum MAO activity in pigs. Higher activity of serum MAO during hot dry and humid environmental periods denoted the existence of oxidative stress (Weyler et al 1990). Age effects on MAO activities have been reported by previous studies (Tryding et al 1969; Ellis et al 1972). Values expressing MAO activity are reasonably patchy in mammalian species (Penttila 2005). Researchers have linked the increased activity of serum MAO with the development of oxidative stress (Kataria et al 2010a). The findings on the basis of levels of serum MAO elicited the conjecture that hot dry and hot humid periods produced oxidative stress in indigenous pigs. The range and mean value of serum MAO in present study was close to the earlier recordings in different species (Kataria et al 2010b). Use of serum MAO activity as a biomarker of oxidative stress is progressively acquiring place in the field of veterinary medicine. In recent times, determination of monoamine oxidase activity has come forward as an important tool to assess mitochondrial sources of oxidative stress (Danila et al 2015). However, there is dearth of research on this aspect in pigs. Many earlier researchers laid emphasis on the relationship of variation in activity of MAO to stress and oxidative stress (Veral et al 1997; Kataria et al 2010b) and suggested it as a marker of environmental temperature associated oxidative stress (Kataria et al 2010c). Oxidative deamination of several monoaminesis catalyzed by MAO can result in significant reactive oxygen species production contributing to oxidative stress (Weyler et al 1990). Higher activity in the present study during hot dry and hot humid periods signified the presence of oxidative stress.

***Glutathione reductase (GR)***. Higher activity of glutathione reductase can be related to hot environment induced oxidative stress, as it is a well known cellular antioxidant. Probably, its increased activity provided a protective effect against environmental stress. On the basis of earlier work on this enzyme in humans (Ohtsuka et al 1994) and animals (Kataria et al 2010a), it can be theorized that hot humid and dry environmental periods produced oxidative stress. Findings of present investigation corroborated the earlier recordings in animals (Kataria et al 2010a; Kataria et al 2013b). Kataria & Kataria (2012)

evaluated oxidative stress in pigs affected with classical swine fever by observing higher levels of GR in the serum. Based on blood GR activity, scientists have tried to find out the effect of various supplementations to ameliorate the antioxidant status (Zou et al 2016). High temperature stress is an important form of abiotic stress affecting all the aspects of physiology of the animals. Body can utilize systems to guard against the consequent impairment due to free radicals. Antioxidant enzyme system participates actively in body defense. Enzyme glutathione reductase is one of the contrivances of this system, which works as a tool to uphold against apparent environmental threats. Glutathione metabolism is associated with protection to counter oxidative stress (Ohtsuka et al 1994). It was observed that elder animals were more susceptible to oxidative damage than young as indicated by increased serum GR activity.

Based upon the findings and supported literature it was reiterated that the pigs suffered with oxidative stress during hot dry and humid environmental periods. Enhanced quantities of oxygen intake and its utilization contribute to the bumped up degree of oxidative stress. Impact of hot humid environmental period was greater on the serum biomarkers suggesting the presence of oxidative stress of larger magnitude than hot dry period.

**Conclusions**. It was concluded that hot environmental periods produced the oxidative stress in the indigenous pigs, which was reflected in the form of altered status of the markers studied in the serum. The modulation of physiological mechanisms in response to free radicals was evident. Magnitude of changes was more during hot humid period than hot dry as compared to moderate environment. This clearly showed that hot humid environment was more stressful to animals than hot dry. Elder and male animals showed the development of oxidative stress of greater magnitude during hot humid environment. The evaluation of the extent of oxidative stress in the form of values can be useful to define the role of oxidative stress in different pathologies and can be used for clinical diagnosis and in health management of indigenous pigs in the arid tract. On the basis of findings, the use of antioxidants can be recommended during hot environmental periods.

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