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Changes in the reactive oxygen species scavenging enzyme superoxide dismutase in pigs during varying ambient temperatures

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Abstract. A study was carried out in Indian indigenous pigs to assess the changes in the levels of antioxidant enzyme superoxide dismutase (SOD), during varying ambient temperatures. Blood samples were collected during moderate, extreme hot and cold ambiences to harvest sera. Mean value of serum SOD during the moderate period was 155.25 ± 1.47 kU L^{-1}. Moderate ambience mean value was considered control. The mean value of serum SOD was significantly ($p \leq 0.05$) higher during hot and cold ambiences as compared to the control. Mean SOD value was 2.87 times higher in hot ambience and 1.77 times higher in cold ambience as compared to the moderate ambience. Increased SOD activities during extreme ambiences showed the presence of oxidative stress. Sex and age effects were significant ($p \leq 0.05$) in all the ambiences and were consistent during extreme ambiences inferring that serum SOD activities showed the interactions of ambience with sex and age. Present study provided data of SOD in indigenous pigs, which can be used as reference values for future studies and for diagnostic purposes. The archetype of changes of SOD activity revealed modulations of the adaptive mechanisms aimed to shield the pigs from alterations in ambient temperatures. Using this foundation of knowledge, antioxidant administration is recommended during extreme ambiences.
Key Words: ambient temperatures, cold, hot, pigs, serum, superoxide dismutase.

Introduction. Physiological stress can be defined as any external or internal condition that challenges the homeostasis of a cell or an organism. It can be divided into three different aspects: environmental stress, intrinsic developmental stress, and aging. Throughout life, all living organisms are challenged by changes in the environment. Fluctuations in oxygen levels, temperature, and redox state trigger molecular events that enable an organism to adapt, survive, and reproduce (Kagias et al 2012). Reactive oxygen species are highly reactive in biological systems and include superoxide, hydroxyl radical, hydrogen peroxide, and fatty acid peroxides. They are produced via normal oxidative metabolism and few of them are essential for cell signalling pathways and other functions. Oxidative stress results when the concentration of reactive oxygen species exceeds that of antioxidant system. The antioxidant system keeps their concentration low due to their reactive properties. The ability of pigs to neutralize the reactive oxygen species plays a key role in their welfare and scientists have recommended the application of such methods for the assessment of farmed animals' welfare (Brambilla et al 2001). Scientists have worked with various parameters to assess oxidative stress at laboratory level as it does not exhibit any symptom (Kataria et al 2010a). Oxidative stress is known to be associated with various health disorders. Changes in ambient temperatures are considered as one of the stressors causing oxidative stress (Kataria et al 2010b).

During extreme ambiences pigs are exposed to great variations in environmental temperatures putting the physiological mechanisms of these animals to jeopardy, resulting in great financial burdens to marginal pig farmers. Heat stress is related with reduced growth, poor sow performance and decrease in carcass quality (Fernandez et al 2012). For optimum production in pigs it is essential to understand the environmental

needs of the pigs so that strategies can be developed to protect them from the hazards of the environment. Extreme cold ambience also puts these animals in trouble due to poor thermoregulatory mechanisms. Reaction to stress involves activation of an extensive network encompassing the central nervous system as well as endocrine and immune responses.

Antioxidant enzymes are considered as potent markers of oxidative stress since their levels are modified with development of oxidative stress. Superoxide dismutase (SOD) is considered such a marker, and is used in animals to assess oxidative stress (Kataria et al 2010c). SOD catalyses the dismutation of superoxide and has a significant role in the defence against oxidative stress (Halliwell & Chirico 1993).

Paucity of research work regarding ambience-related oxidative stress in pigs highlights the need to generate reference values of biomarkers of oxidative stress in indigenous breeds. It is not considered wise to extrapolate the values of other species or breed. Veterinary diagnostic laboratories can use these data for timely detection of oxidative stress in pigs. Paucity of research to relate oxidative stress with extreme ambient temperatures in pigs emphasised the need of a study to assess these relations. Therefore the present study was launched and the objective of our work was to evaluate the impact of oxidative stress induced due to extreme hot and cold ambient temperatures on the porcine system. As markers, we chose serum SOD and determined its levels during moderate, hot and cold ambiences. In that context, we also assessed the possibility of using SOD as a marker of oxidative stress in indigenous pigs.

Materials and methods. The study was carried out in 270 apparently healthy indigenous pigs of either sex, between 6 months to one year of age to determine serum SOD enzyme during moderate (mean maximum ambient temperature 28.22^{o}C), hot (mean maximum ambient temperature 45.30^{o}C) and cold (mean minimum ambient temperature 1.02^{o}C) ambiences. 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, 90 blood samples were obtained to harvest the serum samples and the animals were categorized sex wise as male (45) and non-pregnant female (45) and age wise as 6-8 months (15 male and 15 female); 8-10 months (15 male and 15 female) and 10-12 months (15 male and 15 female).

SOD was determined by the colorimetric method of Winterbourn et al (1975) with a slight modification. The method is based on the ability of SOD to inhibit the reduction of nitroblue tetrazolium (NTB) by superoxide. One unit is defined as that amount of enzyme causing half the maximum inhibition of NTB reduction. In the modification, instead of the Michaelis-Menten type of plot, a linear plot was obtained when the reciprocal values for the percentage inhibition of NBT reduction were plotted against the serum samples. This plot was found to give precise values for the volumes of samples required for 50% inhibition of NBT reduction. Statistical significance for individual parameter between moderate and hot periods was analysed (Kaps & Lamberson 2004).

Results and discussions. Mean ± SEM values are presented in Table 1. Moderate mean value of serum SOD was 155.2 ± 1.47 kU L^{-1}. The mean value corroborated earlier findings in healthy pigs (Kataria & Kataria 2012b). The mean value of serum SOD was significantly ($p \leq 0.05$) higher during hot and cold ambiences as compared to moderate or control. Mean value was 2.87 times higher in hot ambience and 1.77 times higher in cold ambience as compared to control. The sex and age effects were significant ($p \leq 0.05$) in all ambiences. The mean values were significantly ($p \leq 0.05$) higher in male animals than female animals. In each ambience the age effect showed a significant ($p \leq 0.05$) increase in the mean values being highest in pigs of 10-12 months of age.

Extreme hot and cold ambiences are known to increase the rate of reactive oxygen species production. Body activates its defence mechanism in terms of antioxidant enzyme activity to scavenge the reactive oxygen species (Kataria and Kataria 2012a). Probably this could be the reason of higher values of serum SOD during hot and cold ambiences in pigs. Researchers have assessed the higher activity of SOD in terms of a marker of

oxidative stress in various species during hot ambient temperature (Bernabucci et al 2002; Kataria et al 2010b; Kataria et al 2010c; Sakatani et al 2012).

Table 1

Mean ± SEM values of serum superoxide dismutase (SOD, kU L^{-1}) in indigenous pigs during extreme ambiences

Variable		N	Ambiences		
			Moderate	Extreme hot	Extreme cold
Ambience		270	155.25 ± 1.47 [b]	446.01 ± 4.00[b]	275.28 ± 1.39[b]
Sex	Male	45	180.21 ± 2.00 [d]	471.71 ± 5.00 [d]	292.43 ± 2.21 [d]
	Female	45	136.29 ± 1.93 [d]	420.32 ± 3.65[d]	258.14 ± 1.90[d]
Age	6-8 months	30	135.20 ± 2.11 [f]	384.10 ± 2.11 [f]	200.10 ± 1.39 [f]
	8-10 months	30	151.00 ± 2.11 [f]	431.19 ± 4.00	253.14 ± 1.54[f]
	10-12 months	30	188.55 ± 2.23 [f]	522.75 ± 4.01[f]	372.61 ± 2.00[f]

Notes: 'b' marks significant (p≤0.05) differences among ambience overall mean values of a parameter; 'd' marks significant (p≤0.05) differences between male and female mean values of a parameter within an ambience; 'f' marks significant (p≤0.05) differences among mean values of different age groups of a parameter within an ambience.

The role of various antioxidants in controlling diseases explains their involvement during the disease state (Kataria et al 2012). Under certain conditions, antioxidant mechanisms are impaired and reactive oxygen species are increased and antioxidant mechanisms may become insufficient to completely prevent oxidative damage. Mammalian cells may encounter oxidative stresses that causes destruction of macromolecules and abnormal function (Jang et al. 2008). The flux of reactive oxygen species in the vasculature results in the initiation and promotion of various pathological conditions (Yang 2006). Earlier workers have used serum SOD as an important parameter to find out biotic stress (Kataria et al 2010a; Kataria & Kataria 2012a; Kataria & Kataria 2012b; Kataria et al 2012). SOD is one of the main antioxidant enzymes in mammals, and could reduce hydrogen peroxide and organic hydro peroxides. Activities are commonly used to assess body antioxidative status (Knight & Sunde 1987).

Higher activities show physiological up-regulation of this enzyme in an attempt to mitigate superoxide radical challenge (Kahlon & Singh 2003). Higher activity in male pigs suggested higher rate of formation of reactive oxygen species. Influence of age on serum SOD activity was also observed by earlier workers (De & Durad 1993; Nazifi et al 2009). Above discussion helped in summarizing that extreme ambience generated reactive oxygen species to produce oxidative stress in pigs. As a defence mechanism serum SOD activity increased tremendously.

Conclusions. Changes were observed in the antioxidant enzyme SOD during varying ambient temperatures. The development of oxidative stress was deduced from the trend of variation and by comparing them with earlier reports in other animal species. Magnitude of increase was greater in hot ambience than cold ambience in pigs of both sexes and all age groups. Modulation of adaptive mechanisms was greater in male in comparison to female pigs and in animals of 10-12 months of age in comparison to comparatively younger animals. Data obtained in the study can be used as reference values for future studies and for diagnostic purposes. The archetype of changes of SOD activity reveals modulations of the adaptive mechanisms to shield the pigs from alterations in ambient temperatures and using this foundation of knowledge, antioxidant administration is recommended during extreme ambiences to combat ensuing oxidative stress.

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