

## Interrelation of aldosterone with oxidative stress and electrolytes in hot climate in pigs from arid tracts

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**Abstract.** A study was conducted to find out the interrelation of aldosterone levels with oxidative stress along with modulations in electrolytes in hot climate in indigenous local swine's from arid tracts in Rajasthan, India. Sera were obtained to determine aldosterone, oxidative stress marker and electrolytes in moderate and hot climates. Serum aldosterone levels were determined by radioimmunoassay. Mean value of aldosterone in moderate climate was  $0.24 \pm 0.001$  nmol L<sup>-1</sup> which showed a 33.33% rise in hot climate. Maximum percent increase was observed in the value of serum gamma glutamyl transferase enzyme (GGT). As GGT is considered to be an effective marker of oxidative stress, its increase confirmed the development of oxidative stress in pigs. A positive significant ( $p \leq 0.01$ ) correlation was observed in the values of aldosterone and GGT in hot climate. Serum sodium and chloride showed positive correlations ( $p \leq 0.01$ ) with aldosterone whereas potassium, calcium, phosphorus and magnesium showed negative correlation ( $p \leq 0.01$ ) with the aldosterone. Similar patterns of correlations existed between different electrolytes and GGT value. Results showed effective modulations in the values of electrolytes due to heat stress in hot climate. The present research has assessed the interrelation of aldosterone with the oxidative stress as well as with electrolytes. It can be concluded that extreme hot climate produced the oxidative stress in pigs. Interpretation of these variations will help provide a perceptive approach of various unseen mechanisms during the progress of oxidative stress. Based on the results obtained, it can be stated that, besides diseases, stress conditions can also be associated with biochemical alterations. Formulation for adequate nutrient intake is challenging as mineral losses in hot climates and changes in blood acid-base chemistry are important components to be taken care of.

**Key Words:** Gamma glutamyl transferase, radioimmunoassay, hot climate, heat stress, pig, serum.

**Introduction.** 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. Aldosterone is a mineralocorticoid which regulates electrolyte and fluid balance. Aldosterone is a part of the renin-angiotensin system and drastic variations can result in problems of the vital organs. Stress and strenuous exercise can increase aldosterone levels. Physiological modulations in the aldosterone levels are important to help the body overcome immediate threats. It is challenging for a clinician to differentiate between the transient rise and chronic elevations, so that corrective measures can be taken in due time.

A rise in aldosterone levels does not mean adrenal pathology each time. Substantial scientific effort is made to link up the function of aldosterone with the

development of oxidative stress. Exposure to hot climate can result in heat stress. Heat stress may also culminate in oxidative stress (Kataria & Kataria 2013). Therefore, studies that correlate aldosterone concentration with oxidative stress in hot climate become important for managing pigs native to arid tracts. It is noteworthy that minerals in the form of electrolytes play a pivotal role in all biological functions of mammals, including expression and regulation of genes, enzyme actions, homeostasis, detoxification systems, bone metabolism etc. Electrolyte variation in blood shows physiological adjustments (Kataria et al 2013a). Contents of sodium and chloride in the diet of pigs may affect feed and water intake, performance, sodium and potassium balance and plasma aldosterone concentration (Chittavong et al 2013). Determination of serum electrolyte levels in animals is important to assess the fluid and electrolyte levels of the body, particularly in hot climate. This can also reflect on the proper renal functioning and hormonal influences. Lack of data in the scientific literature about various physiological parameters of indigenous pigs in this regard was sufficient to provoke the launch of an endeavor with a clear mandate of generating some baseline values, which can be used for the laboratory diagnosis of clinical conditions. Looking towards the important role played by the aldosterone in homeostatic mechanisms related with the salt and water balance and the significance of oxidative stress in higher ambient temperature periods, the present investigation was set to find an interrelation of aldosterone with electrolytes. This study will also help understanding the electrolyte balance in indigenous pigs.

**Material and Methods.** Serum aldosterone along with marker of oxidative stress and electrolytes were determined in indigenous pigs grown in the arid tracts of Rajasthan state, India. The animals were maintained under similar management and feeding regimens by private raisers. Blood samples were collected from private slaughter houses, in sterile tubes for harvesting sera, without anticoagulants, during periods of the year when the maximum environmental temperature ranged between 27°C and 29°C (moderate climate) and between 45°C and 46°C (hot climate). These animals were free from endo- and ecto-parasites as assessed by routine faecal and skin examination, respectively. The samples were collected from adult male animals (25 in each period).

Serum aldosterone was determined by immunoradiometric assay using Radio immuno assay kit (DiaSorin) in the RIA laboratory of Department of Veterinary Physiology, CVAS, Bikaner, Rajasthan, India as per the manufacturer's protocol. The assay is based on the competition between the labeled aldosterone and the aldosterone contained in the calibrators and serum samples, assayed for a fixed and limited number of antibody binding sites. After the incubation, the amount of labeled aldosterone bound to the antibody on the tube walls is inversely related to the concentration of unlabelled aldosterone present in the calibrators or samples. For the purpose of this assay, antibody coated tubes were used. All reagents were brought to room temperature before testing. The tubes were arranged as per the protocol provided by the manufacturer. Coated tubes were labeled for 0, 50, 100, 250, 500 and 1000 pg/mL calibrators starting from A to F, respectively. For the serum samples, coated tubes were labeled accordingly. Samples and reagents were dispensed in the bottom of the tubes by appropriate micropipettes as per the protocol. The contents of the tubes were vortexed except T tube. All the tubes (except T tubes) were incubated at 26-28°C for 18-22 hrs. The incubation mixture was aspirated carefully, leaving no trace of dye, except for the T tube. Then, the radioactivity of each tube, including the T tube, was measured using Gamma Counter (<sup>125</sup>I Gamma counter, ECIL, India).

Serum gamma glutamyl transferase (GGT) was determined by a spectrophotometric method (Wolf & William 1973). Serum electrolytes included sodium, potassium, chloride, calcium, phosphorus and magnesium. Sodium and potassium in serum were determined by the standard method using a flame photometer (Oser 1976). Serum chloride was determined by the titrimetric method of Schales & Schales (Oser 1976). Serum calcium was determined by the titrimetric method of Clark & Collip, modification of the Kramer & Tisdall (Oser 1976). Serum phosphorus was determined by the spectrophotometric method of Fiske & Subarrow (Oser 1976). Serum magnesium was determined by the titan yellow method (Varley 1988). Statistical significance for

individual parameters between moderate and hot periods was analysed (Kaps & Lamberson 2004). The mean value in moderate climate for each parameter was considered as control.

## Results and Discussion

The mean±SEM values of serum aldosterone and electrolytes along with per cent variations in the values due to the hot climate as compared to moderate climate in pigs are presented in Table 1. All the comparisons for each parameter for the hot climate were made from the respective moderate climate values, keeping them as control. There is a paucity of scientific literature containing information on serum aldosterone levels in indigenous pigs.

Table 1  
Serum levels of aldosterone, gamma glutamyl transferase and electrolytes in indigenous pigs in moderate and hot climate (n=25, mean±SEM values)

S. No.	Serum Parameters	Climate		Percent change
		Moderate	Hot	
1	Aldosterone (nmol L <sup>-1</sup> )	0.24±0.001*	0.32±0.001*	33.33%
2	Gamma glutamyl transferase (UL <sup>-1</sup> )	44.00±2.31*	298.00±2.99*	577.27%
2	Sodium (mmol L <sup>-1</sup> )	128.11±1.00*	149.00±1.34*	16.30%
3	Potassium (mmol L <sup>-1</sup> )	5.44±0.001*	5.10±0.001*	-6.25%
4	Chloride (mmol L <sup>-1</sup> )	105.12±1.11*	131.00±1.45*	24.61%
5	Calcium (mmol L <sup>-1</sup> )	2.95±0.01*	1.91±0.01*	-35.25%
6	Phosphorus (mmol L <sup>-1</sup> )	1.99±0.001*	1.12±0.001*	-43.71%
7	Magnesium (mmol L <sup>-1</sup> )	1.32±0.001*	0.90±0.001*	-31.80%

\* = (p≤0.05).

**Aldosterone.** The mean value of serum aldosterone was significantly (p≤0.05) higher in hot climate. Earlier research in animals has shown the increase in aldosterone levels due to the hot climate (Kataria et al 2000; Kataria & Kataria 2006), stress (Kataria & Kataria 2004) or oxidative stress (Ojeda-Cervantes et al 2013). Higher aldosterone levels probably help absorb more salt and water from the gastrointestinal tract. These underlying mechanisms can help figure out the water retention process in the body, which is instrumental in maintaining body temperature and to overcome threats cropping up due to water deficit in higher ambient temperatures.

Chronic stress over an expanded period can disturb the internal regulatory mechanisms which control aldosterone. Aldosterone increases sodium absorption by the kidneys and helps control the homeostasis of blood sodium levels. Recent studies in native pig breeds from arid tracts have shown development of oxidative stress during higher ambient temperature periods (Kataria et al 2013b). The results of the present investigation regarding aldosterone status are also pointing towards its role as one of the contributing factors in the development of oxidative stress. It is interesting to note that a positive significant correlation (p≤0.01) of aldosterone was observed with GGT, a biomarker of oxidative stress. The direct effect of aldosterone on oxidative stress has been shown through its ability to increase the levels of an important subunit of NADPH oxidase, essential for superoxide anion generation (Calò et al 2004). Reduced nitric oxide bioavailability can be involved in the development of oxidative stress by aldosterone. The above discussion has given a direction to appreciate the additional role of aldosterone as a stress hormone, through its involvement in the development of oxidative stress.

**Gamma glutamyl transferase.** The mean value of serum GGT was significantly (p≤0.05) higher in hot climate. Increased serum GGT activity caused the development of oxidative stress (Onat et al 2006). In clinical physiology, its levels can be used effectively as markers of hepatic involvement and oxidative stress. Researches in animals have correlated higher GGT activity to environmental stress (Kataria & Kataria 2007). Kataria

& Kataria (2012) suggested that an increased quantity of serum GGT should be looked at in terms of oxidative stress along with liver problems.

**Sodium.** The mean value of serum sodium was significantly ( $p \leq 0.05$ ) higher in hot climate, pinpointing water retention due to increased aldosterone (Kataria et al 2000). Modulation in sodium value could be due to environmental temperature variation (Kataria et al 2002b) and oxidative stress mechanisms. Higher sodium intake is correlated with the increased serum concentration of inflammation and oxidative stress markers in human medicine (Gallardo et al 2007). In the present study, an increase was observed in the level of serum GGT, providing significant evidence to suggest the role of sodium in the development of oxidative stress.

**Potassium.** The mean value of serum potassium was significantly ( $p \leq 0.05$ ) lower in hot climate. The lower value during the hot period indicated the effect of increased aldosterone levels (Kataria et al 2000). van der Molen et al (1986) found the relation of aldosterone with sodium and potassium in pigs. Enzyme systems, metabolic functions and performance measures depend on the homeostasis. During the hot period, potassium loss increases, probably under the influence of higher aldosterone.

**Chloride.** The mean value of serum chloride was significantly ( $p \leq 0.05$ ) higher in hot climate. Consideration of physiological changes due to various factors like environmental temperature is important in order to understand mineral metabolism. The changes in the electrolyte metabolism are reported in animals during high environmental temperature periods (Shebaita & Pfau 1983). The higher value during the hot period indicated the retention of water in the body. In the body, chloride follows sodium ions in the maintenance of acid-base balance (Leaf 1962). In the present study, serum sodium levels were significantly ( $p \leq 0.05$ ) higher in hot climate.

**Calcium.** The mean value of serum calcium was significantly ( $p \leq 0.05$ ) lower in hot climate. Influence of the season on serum calcium was studied by many early researchers in different animal species (Kataria et al 2002a). Probably the decrement in serum calcium level potentate the oxidative stress in hot climate. The mechanism could be explained on the basis of the hypothesis that oxidative stress can disrupt normal physiological pathways and cause cell death (Ermak & Davies 2002). Oxidative stress causes calcium influx into the cytoplasm from the extra cellular environment, then its rising concentration in the cytoplasm causes its influx into mitochondria, disrupting normal metabolism and leading to cell death. Probably, the movement of the calcium ions from plasma into the cells lowered the calcium levels in extreme hot climate. Studying the levels of serum calcium in varying climates can help determine prophylactic mineral supplementation. The results of all the electrolytes at one platform can help in the formulation of supplementing mixtures and can help the veterinarians in making clinical interpretations.

**Inorganic phosphorus.** The mean value of serum inorganic phosphorus was significantly ( $p \leq 0.05$ ) lower in hot climate. Season is known to have an effect on serum inorganic phosphorus (Dias et al 2008). Phosphorus plays an important role in many metabolic processes, such as muscle contraction to deliver oxygen to tissues, bone integrity, and energy metabolism. Serum phosphorus values principally reflect current dietary intake. This could be due to differences in the chemical composition of the feed ingested. Phosphorus resumption increase during physiological states like growth, lactation and pregnancy. It has a close connection with calcium. In the present study, serum calcium levels were lower in hot climate as compared to moderate climate. This could be the reason for low phosphorus levels. Low feed intake could also be one of the possible reasons for decreased serum phosphorus levels. The formation of free radicals alters the oxidative metabolism, which leads to the disruption of the ionic environment of the cell. This causes low phosphorus levels (Walwadkar et al 2006). On this basis,

decreased phosphorus levels in extreme climates in the present study could be correlated with oxidative stress.

**Magnesium.** The mean value of serum magnesium was significantly ( $p \leq 0.05$ ) lower in hot climate. Researchers have correlated the variation in the levels of magnesium with oxidative stress. Variations in magnesium concentration may directly manipulate the cellular redox state, while its deficiency is coupled with the increased creation of reactive oxygen species, along with the initiation of immune and inflammatory mechanisms (Kataria et al 2012). The pattern of changes in serum magnesium levels in the present study is pointing towards the development of oxidative stress and the relationship of magnesium with antioxidant capacity in animals (Kataria et al 2012). The relationship of magnesium with other ions is also important in order to interpret the results, as hypomagnesaemia is often accompanied and complicated by hypocalcaemia and it can also result in hypokalemia. In the present study, low serum calcium and potassium levels were also observed in pigs in hot climate.

**Correlations.** Correlations among various parameters are presented in Table 2. The correlations among the levels of aldosterone, oxidative stress marker and electrolytes showed a significant ( $p \leq 0.01$ ) effect of hot climate. Serum gamma glutamyl transferase enzyme, sodium and chloride showed positive significant correlations with aldosterone, indicating development of oxidative stress and the role of aldosterone, sodium and chloride in the oxidative stress mechanisms. Positive correlations can be considered as the mirror images of responses obtained as a result of modulations in the mechanisms due to oxidative stress. Serum potassium, calcium, phosphorus and magnesium showed negative correlations with the aldosterone levels. Negative correlations also suggested a combination of the effect of the aldosterone hormone and the generation of free radicals. Variations in the electrolytes were probably generated in order to maintain ionic balance in adverse conditions. Serum sodium concentration is considered as an indirect indicative of aldosterone levels. Low serum potassium depicted the clear response of hormone aldosterone. Oxidative stress greatly affects the physiology of animals, particularly the haemopoietic and immune systems. Hence, the study of serum profile in extreme climate becomes important to evaluate stress in otherwise clinical healthy individuals.

Table 2

Correlations in the serum levels of aldosterone, gamma glutamyl transferase and electrolytes in indigenous pigs in hot climate (n=25)

S. No.	Serum parameter	ALD	GGT	Na	K	Cl	Ca	P	Mg
1	ALD	1.000**	0.998**	0.999**	-	0.976**	0.975**	0.977**	0.973**
2	GGT	0.995**	1.000**	0.996**	-	0.971**	0.941**	0.931**	0.921**
3	Na	0.999**	0.996**	1.000**	-	0.974**	0.980**	0.983**	0.980**
4	K	-	-	-0.974**	1.000**	-	0.977**	0.975**	0.979**
5	Cl	0.997**	0.992**	0.996**	-	1.000**	0.970**	0.971**	0.960**
6	Ca	-	-	-0.980**	0.977**	-	1.000**	0.977**	0.982**
7	P	-	-	-0.983**	0.975**	-	0.977**	1.000**	0.967**
8	Mg	-	-	-0.980**	0.979**	-	0.982**	0.967**	1.000**

ALD = Aldosterone, GGT = Gamma glutamyl transferase, Na = Sodium, K = Potassium, Cl = Chloride, Ca = Calcium, P = Phosphorus, Mg = Magnesium, \*\* = ( $p \leq 0.01$ ), - = negative correlation.

**Conclusions.** The present research work has assessed the interrelation of aldosterone with the oxidative stress as well as with electrolytes. It can be concluded that extreme hot climate produced the oxidative stress in pigs. This was based on the altered status of the aldosterone, oxidative stress marker and electrolytes. An interrelation was observed between aldosterone and oxidative stress marker and electrolytes. Interpretation of these physiological variations will help provide a perceptive approach of various unseen mechanisms during the progress of oxidative stress. The analysis of various electrolytes becomes significant as recent understandings in this field demonstrate their pivotal role in skeleton and metabolism. Based on the results, it may be possible to make reasonable predictions of alterations in acid-base balance and serum electrolyte concentrations when laboratory evaluations are not available. Numerous nutritional modifications are used for hot climate feeding in pigs, however, many need further investigation in order to achieve specific recommendations. Formulation for adequate nutrient intake is challenging, as mineral losses in hot climate conditions and changes in blood acid-base chemistry are important components to be taken care of during heat stress. The results of interaction of aldosterone with various electrolytes can provide a series of practical approaches to improve nutrition during heat stress. On the basis of the results obtained, it can be stated that, besides diseases, stress conditions can also be associated with biochemical alterations.

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