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ALDOSTERONE ASSAY TO EVALUATE OXIDATIVE STRESS IN *MARWARI* LAMBS DURING EXTREME HOT AMBIENCE

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ABSTRACT

A study was undertaken to assess the function of aldosterone as oxidative stress marker in lambs of *Marwari* breed during extreme hot ambience. Radio immunometric assay was used to determine serum aldosterone. Results revealed significant ($p \leq 0.05$) increase in serum aldosterone, γ-glutamyl transferase (GGT), sodium and chloride contents during hot ambience as compared to moderate ambience. Serum potassium, calcium, phosphorus and magnesium showed reverse trend and the values were significantly lower during hot ambience as compared to moderate ambience. Maximum per cent change was observed in serum GGT, thus establishing the oxidative stress. A relationship of aldosterone with oxidative stress was observed. Results visibly validated that besides diseases, ambient stress can put in variations in aldosterone with the establishment of oxidative stress.

Key words: Aldosterone, electrolytes, hot ambience, *Marwari* lambs, oxidative stress

INTRODUCTION

Past decade has witnessed ever-emerging evidence that implicates oxidative stress in various patho-physiological expressions while the research correlating oxidative stress with environmental temperature variations has received little attention. Livestock encounter vast variations in ambient temperatures which may put their health at risk. Understanding the physiological modulations of body reactions in response to environmental changes may prove beneficial in veterinary clinical science. Endocrine system plays an important role in stress conditions. Exposure to hot ambience causes heat stress which may culminate in oxidative stress (Kataria and Kataria, 2013).

It is vital to find significant parameters which may serve as biomarkers of stress. Aldosterone is a mineralocorticoid regulating electrolyte and fluid balance as a part of renin-angiotensin system. Most studies on aldosterone have been directed to hypoadrenocorticism, however, its new facet is coming out as its possible role in oxidative stress. Physiological modulations in aldosterone levels are vital to help the body in overcoming the immediate threats. Presently the focus of research of clinician is to distinguish between short-lived rise and chronic rise in the levels of aldosterone. An increase in aldosterone does not mean adrenal pathology every time. Higher aldosterone levels are associated with systemic evidence of oxidative stress in human medicine (Kotlyar *et al.,* 2006).

Marwari is an important breed of sheep in arid tracts. Lambs are considered as back bone of sheep industry. Therefore, studies to correlate oxidative stress during hot ambience have become essential for the scientific management of young animals to enhance survival rate. Determination of serum electrolyte levels in animals is important to assess the fluid and electrolyte levels of body particularly during hot ambience. This also reflects the proper renal functioning and hormonal influences. Schedules for health maintenance require protection from stress particularly oxidative stress. Owing to the paucity of research associating aldosterone with oxidative stress in young sheep, the present study was conducted to assess the role of aldosterone as an oxidative stress marker in lambs during hot ambience.

MATERIALS AND METHODS

The present study was carried out on 24 healthy lambs of *Marwari* breed of either sex, maintained by private owners with almost similar type of management conditions. Blood sample were collected in sterile tubes to harvest sera during moderate ambience when mean maximum ambient temperature ranged from 27 to 29^{o}C and during extreme hot ambience when maximum temperature ranged from 45 to 47°C. In each ambience twelve animals were screened. Serum aldosterone along with GGT as marker of oxidative stress and electrolytes *viz.*, sodium, potassium, chloride, calcium, phosphorus and magnesium were determined. Serum aldosterone was determined by immune-radiometric assay using radio-immuno-assay kit (DiaSorin) in RIA Laboratory, Department of Veterinary Physiology, CVAS, Bikaner, Rajasthan as per the manufacturer's protocol using Gamma Counter (^{125}I Gamma counter, ECIL, India). Serum γ-glutamyl transferase (GGT) was determined by spectrophotometric method (Wolf and Williams, 1973). Serum sodium and potassium were determined by standard method using a flame photometer, serum chloride by titrimetric method of Schales and Schales, serum calcium by titrimetric method of Clark and Collip, modified by Kramer & Tisdall and serum phosphorus by spectrophotometric method of Fiske & Subarrow (Oser, 1976). Serum magnesium was determined by titan yellow method (Varley, 1988). All the comparisons for each parameter during hot ambience were made from respective moderate ambience value, keeping it as control. Statistical significance for each parameter between moderate and hot periods was analysed and mean values during moderate ambience for each parameter was considered as control (Kaps and Lamberson, 2004).

RESULTS AND DISCUSSION

A significant increase was observed in serum aldosterone, GGT, sodium and chloride contents during hot ambience than in moderate ambience. Mean serum potassium, calcium, phosphorus and magnesium values showed reverse trend and the values were significantly lower in hot ambience than moderate ambience. Maximum change was observed in serum GGT, followed by serum aldosterone. Highest change in serum GGT indicated the development of oxidative stress (Lee *et al*., 2004). The changes in aldosterone concentration reflected its association with oxidative stress.

Aldosterone favours sodium reabsorption by the kidney and helps homeostasis. Increase in aldosterone is perhaps essential to support absorption of more salts and water from gastrointestinal tract. Higher environmental temperature modulates many physiological mechanisms for better

water retention process in body, which is helpful in maintaining body temperature and to overcome water scarcity challenges. Earlier workers have shown association of higher levels of aldosterone with hot ambience, stress and oxidative stress (Kataria *et al*., 2000; Kataria and Kataria, 2006; Ojeda-Cervantes *et al*., 2013). The chronic stress over an expanded period disturbs internal regulatory

Table 1: Serum levels of aldosterone, γ-glutamyl transferase and electrolytes in lambs (n = 12, mean ± SEM values)

Serum parameters	Ambiences		Per cent change
	Moderate	Hot	
Aldosterone (nmol L^{-1})	0.20 ± 0.001	0.34 ± 0.001 ^{d}	70.0
Gamma glutamyl transferase (UL^{-1})	30.00 ± 0.008	167.00 ± 0.01 ^{d}	123.3
Sodium (mmol L^{-1})	120.00 ± 0.13	141.00 ± 0.11^{d}	17.5
Potassium (mmol L^{-1})	5.00 ± 0.001	4.22 ± 0.001 ^{d}	-15.6
Chloride (mmol L^{-1})	105.00 ± 0.10	125.00 ± 0.10 ^{d}	19.0
Calcium (mmol L^{-1})	2.80 ± 0.001	1.89 ± 0.001 ^{d}	-32.5
Phosphorus (mmol L^{-1})	1.93 ± 0.001	1.41 ± 0.001 ^{d}	-26.9
Magnesium (mmol L^{-1})	1.31 ± 0.001	1.01 ± 0.001 ^{d}	-22.9

Superscript 'd' indicates that a given parameter differs significantly ($p \leq 0.05$) from the respective moderate mean value.

mechanisms which control aldosterone. Studies have shown that aldosterone-induced organ injuries are associated with increased expression of NADPH oxidase components and reactive oxygen species levels (Nishiyama *et al*., 2007). The results of present study emphasize its role as one of the contributing factors in the development of oxidative stress. Direct effect of aldosterone on oxidative stress has been shown through its ability to increase the levels of an important subunit of NAPH oxidase, essential for superoxide anion generation (Calò *et al*., 2004). This indicates the additional role of aldosterone as stress hormone due to its involvement in oxidative stress development.

Earlier research in animals has correlated higher GGT activity to environmental stress (Kataria and Kataria, 2007). Serum GGT can be used efficiently as markers of oxidative stress. Increased serum GGT values reflect the conditions of oxidative stress along with liver problems (Kataria and Kataria, 2012). Enhanced serum GGT activity suggests more oxidative stress. Serum GGT may prove useful in studying oxidative stress-related issues in clinical settings. Probably serum GGT is an early sensitive enzyme related to oxidative stress (Lee *et al*., 2004).

Oxidative stress can promote sodium retention by increasing its reabsorption (Banday *et al*., 2011).Variations in sodium could be due to environmental temperature variation (Kataria *et al*., 2002) and oxidative stress mechanisms. Increase in sodium concentration during hot ambience indicated water retention due to increased aldosterone (Kataria *et al*., 2000). The lower value of serum potassium during hot period indicated the effect of increased aldosterone level (Kataria *et al*., 2000). Aldosterone favours sodium retention and potassium excretion. Enzyme systems, metabolic functions and performance measures depend on homeostasis. The changes in electrolyte metabolism are reported in animals during hot ambience (Shebaita and Pfau, 1983). Chlorides in body follow sodium ions in maintenance of acid base balance. The higher value during hot period indicated the retention of water in body. In present study, serum sodium level was significantly higher during hot ambience. Decrease in serum calcium is known to be related with oxidative stress during hot ambience. The oxidative stress has been ascribed to intracellular Ca^{2+} excess, especially because mitochondrial matrix Ca^{2+} overload enhanced the generation of reactive oxygen species (Brookes *et al.,* 2004). Probably movement of calcium from plasma into the cells lowered the calcium levels in extreme hot ambience. Serum calcium levels during varying ambiences can help in provide valuable information and help in assessing stress status. Decreased phosphorus levels during hot ambience could be due to the oxidative stress. Formation of free radicals alters oxidative

metabolism which leads to the disruption of ionic environment of cell. This causes low phosphorus levels (Walwadkar *et al*., 2006). In present study, serum calcium level was lower during hot ambience as compared to moderate ambience. Perhaps, this could be the reason of low phosphorus.

Hyperaldosteronism has been demonstrated to be associated with magnesium loss in urine and hypomagnesemia (Horton and Biglieri, 1962). Research in rats has shown that aldosterone infusion results in hypocalcemia and hypomagnesemia associated with the enhanced production of reactive oxygen species (Chhokar *et al.*, 2005).Variations in magnesium concentration may candidly manipulate the cellular redox state and its deficiency is coupled with the increased creation of reactive oxygen species (Kataria *et al.*, 2012) along with the initiation of immune and inflammatory mechanisms. The present study revealed the relation of lowered magnesium with high temperature associated oxidative stress.

Conclusion: The study revealed that extreme hot ambience produced oxidative stress in lambs. Elucidation of the changes will facilitate insightful advances to divulge mechanisms required in the expansion of oxidative stress. Results visibly validated that besides diseases, ambient stress can put in variations in aldosterone with the establishment of oxidative stress.

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