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**Anandita Srivastava**

M.V.Sc. Scholar Department of Veterinary Physiology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

**Nalini Kataria**

Professor and Head of Department, Department of Veterinary Physiology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

## Contemplation of metabolomics during extreme hot ambience in non-descript sheep from arid tracts

Anandita Srivastava and Nalini Kataria

### Abstract

An endeavor was launched to contemplate metabolomics during extreme hot ambience in non-descript sheep from arid tracts. Blood samples were collected to yield serum from healthy male and female sheep of 4-13 month age groups during slaughtering from private slaughter houses in Bikaner, Rajasthan during moderate and extreme hot ambiances. To study metabolomics, serum glucose ( $\text{mmol L}^{-1}$ ), total proteins ( $\text{g L}^{-1}$ ) and cholesterol ( $\text{mmol L}^{-1}$ ) were considered having moderate mean values  $3.77 \pm 0.03$ ,  $78.00 \pm 1.00$  and  $3.67 \pm 0.03$  respectively. The mean values of serum metabolites were found significantly ( $p \leq 0.05$ ) lower during extreme hot ambience. Age effect showed a significant ( $p \leq 0.05$ ) decrease in the mean values in hot ambience being highest in the sheep of 4-7 months of age and lowest in of 10-13 months. Effect of extreme hot ambience was evident on each group with great impact. The upshots of work will immensely contribute in monitoring the health status of sheep.

**Keywords:** Ambience, antioxidants, non-descript sheep, cholesterol, serum

### 1. Introduction

Investigation to find out physiological variations in extreme hot climate in sheep has become the burning matter before researchers. It is required to facilitate spreading out of sheep raising in harsher climatic areas. Suitable review of thermal stress is crucial to be relevant in using appropriate management lines for non-descript sheep. Heat stress outcome from the animal's inability to dissipate sufficient heat to maintain homeothermy. Reactive oxygen species (ROS) are formed by regular metabolic processes including oxidative metabolism. Many of them are mandatory for cell functions like signaling. Outcome of the process when the level of reactive oxygen species surpasses that of antioxidant system is oxidative stress.

Metabolomics is the technical investigation of chemical methods involving metabolites. It also incorporates the regulators of metabolism. Several arid tract animals have to forage under extremely hot environmental periods to maintain energy and water balance. Chief problem of urban regions is higher temperature along with adjustment of thermal fruition in dwelling in population. Changes in environmental temperature can bring about stress to the animals along with extreme variations in the metabolites in blood (Kataria *et al.* 2000 a,b) [9, 10]. The non-descript sheep plays an important role in the economy of marginal owners and poor sheep raisers. Looking towards the scantiness of research to associate oxidative stress with extreme ambient temperature in sheep, the present endeavour was carried out with the aim to determine serum metabolites in non-descript sheep from arid tracts during extreme hot ambience.

### 2. Materials and Methods

One hundred twenty blood samples of apparently healthy male and female non-descript sheep from arid tracts in Bikaner district, Rajasthan, ageing 4 months to 13 months were collected from private slaughter houses during moderate and extreme hot ambiances to harvest serum. Blood samples were obtained during slaughtering from the private slaughter houses under the permission of Institutional Animal Ethics Committee (IAEC), College of Veterinary and Animal Science, Bikaner, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan. The samples were analyzed to determine metabolites of serum. Moderate (control) ambience encompassed the months of October-November and extreme hot ambience included May and June. The sheeps were also be classified as per age as 4-7 months, 7-10 months, 10-13 months of age.

### Correspondence

**Anandita Srivastava**

M.V.Sc. Scholar Department of Veterinary Physiology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Each group was comprised of 20 animals. In each ambience, 60 blood samples were collected. Serum metabolites included serum glucose, total proteins and cholesterol, determined by the Folin-Wu method as described by Oser (1976) [15]. Then cholesterol was determined in the petroleum ether by employing the Liebermann-Burchard reaction. It was calculated as follows: Optical density of serum Cholesterol ( $\text{mg dL}^{-1}$ ) = (optical density of serum / average K value of cholesterol) X 500. K value was obtained by observing optical density of standard and dividing it by concentration of standard. Value 500 was the factor used in formula. Then the value was converted to  $\text{mmolL}^{-1}$  (SI unit). Classification of the main effects were as ambiances, sex and age groups. Further, the subsets of ambiances were moderate and extreme hot periods and of age groups were 4-7 months, 7-10 months, 10-13 months. For each subset data were expressed as mean $\pm$ SEM and the significance of the influences of ambiances and age groups was tested (Kaps and Lamberson, 2004) [8] with the variations in the means (Duncan, 1955) [4].

### 3. Results and Discussion

The present endeavour was launched to determine serum metabolites in non-descript sheep from arid tracts during moderate and extreme hot ambience.

**3.1 Serum Glucose:** The mean values of serum glucose were significantly ( $p \leq 0.01$ ) lower during extreme hot ambience as compared to moderate overall mean value (Table 1). Lowered value of serum glucose observed during extreme hot ETP

attempted to express the reduced energy balance in sheep. The effect of stress due to hot environment was evident. Blood glucose level in ruminants depends upon the rate of gluconeogenesis. Environmental temperature is known to influence the rate of glucose formation especially higher environmental temperature (Bhartendu, 2017) [2]. The findings collected in the present endeavour agreed to the earlier observations Kataria *et al.* (2010b) [12] in goats; Joshi *et al.* (2012) [7] in buffaloes; Thori (2015) [19] in calves, Bhartendu (2017) [2] in goat and Srivastava (2018) [17] in sheep. Serum glucose mean values exhibited significant ( $p \leq 0.05$ ) alterations in both moderate and extreme hot ETPs due to sex and age. Analysis of variance also substantiated the significance ( $p \leq 0.01$ ) of alterations due to sex and age. Age effect showed a significant ( $p \leq 0.05$ ) increase in the mean values being highest in the non-descript sheep of 4-7 months of age. Findings collected in the present endeavour evidently exhibited the reduction in serum glucose in non-descript sheep of all age groups divulging the fact of depletion of serum glucose. It was ostensible that influence of extreme hot ambience on serum glucose level was higher in younger sheep of 4-7 months of age than other older age groups. Oldest age group in the investigation revealed highest percent alteration during extreme hot ETP and 7-10 months of age group exhibited minimum percent alteration during extreme hot ETP. Joshi (2012) [6] found age related alterations in serum glucose levels in buffaloes. Researchers surmised that changes in gluconeogenesis during hot environments can produce alterations in glucose levels (Soren, 2013) [16].

**Table 1:** Mean  $\pm$  SEM values and percent changes of serum glucose ( $\text{mmol L}^{-1}$ ) of non-descript sheep during moderate and extreme hot ambiances.

Effects	Moderate ambience Mean $\pm$ SEM (60)	Extreme hot ambience Mean $\pm$ SEM (60)	Percent Changes
Ambience overall value mean value (120)	3.77 <sup>b</sup> $\pm$ 0.03	3.13 <sup>b</sup> $\pm$ 0.02	-16.97
Male (30)	3.89 <sup>bd</sup> $\pm$ 0.003	3.23 <sup>bd</sup> $\pm$ 0.004	-16.96
Female (30)	3.65 <sup>bd</sup> $\pm$ 0.004	3.03 <sup>bd</sup> $\pm$ 0.003	-16.98
4-7 Months age (20)	3.99 <sup>bf</sup> $\pm$ 0.001	3.32 <sup>bf</sup> $\pm$ 0.001	-16.79
7-10 Months age (20)	3.72 <sup>bf</sup> $\pm$ 0.002	3.16 <sup>bf</sup> $\pm$ 0.001	-15.05
10-13 Months age (20)	3.60 <sup>bf</sup> $\pm$ 0.001	2.91 <sup>bf</sup> $\pm$ 0.001	-19.16

- i. Figures in the parenthesis indicate number of non-descript sheep.
- ii. <sup>b</sup> marks significant ( $p \leq 0.05$ ) differences between moderate and hot ambience mean values for a row.
- iii. <sup>d</sup> marks significant ( $p \leq 0.05$ ) differences between male and female mean values within an ambience.
- iv. <sup>f</sup> marks significant ( $p \leq 0.05$ ) differences between mean values of the age groups within an ambience.

### 3.2 Serum Total Proteins

The mean values of serum total proteins were significantly ( $p \leq 0.05$ ) lower during extreme hot ambience as compared to moderate overall mean value (Table 2). In present endeavor, may be expansion of oxidative stress during extreme hot ambience concluded in decrement in serum total proteins in non-descript sheep of all age groups. Age effect showed a significant ( $p \leq 0.05$ ) increase in the mean values being highest in the non-descript sheep of 10-13 months of age group. Changes due to extreme ambiances were lowest in 4-7 months of age of animals. The index of serum proteins present in circulating blood is the total serum proteins. Decrement in total serum proteins in non-descript sheep of all age groups was evident. It was perceptible that effect of extreme ambience on serum TP was elevated in older sheeps of 10-13

months of age. Probably pressure of ambient temperature modulated the values in older group to a greater extent thus lessening the gap in the values.

Reduced contents of serum proteins in summer than winter was reported in sheep by Sykes and Russel (1979) [18] and Latif *et al.* (1997) [14] and in dromedaries in Kataria *et al.* (2002) [11]. Shifting of proteins and changes in contents due to heat stress is well texted by several workers (Kataria *et al.*, 2002) [11]. Disease and stress situations may cause oxidative alterations in proteins producing greater yielding in blood of free radicals and give rise to alterations in several proteins of the blood. Therefore, scientists have surmised that total serum proteins can also be of immense significance as biomarker of oxidative stress (Joshi, 2012) [6].

**Table 2:** Mean  $\pm$  SEM values and percent changes of serum total proteins (g L<sup>-1</sup>) of non-descript sheep during each ambiances

Effects	Moderate ambience Mean $\pm$ SEM(60)	Extreme hot ambience Mean $\pm$ SEM(60)	Percent Changes
Ambience overall value (120)	78.00 <sup>b</sup> $\pm$ 1.00	68.00 <sup>b</sup> $\pm$ 1.00	-12.82
Male (30)	80.00 <sup>bd</sup> $\pm$ 0.80	70.00 <sup>bd</sup> $\pm$ 0.87	-12.50
Female (30)	76.00 <sup>bd</sup> $\pm$ 0.85	66.00 <sup>bd</sup> $\pm$ 0.86	-13.15
4-7 Months age (20)	75.70 <sup>bf</sup> $\pm$ 0.57	65.00 <sup>bf</sup> $\pm$ 0.53	-13.33
7-10 Months age (20)	78.20 <sup>bf</sup> $\pm$ 0.53	67.00 <sup>bf</sup> $\pm$ 0.54	-14.32
10-13 Months age (20)	80.10 <sup>bf</sup> $\pm$ 0.54	72.00 <sup>bf</sup> $\pm$ 0.53	-10.11

- Figures in the parenthesis indicate number of sheep.
- <sup>b</sup> marks significant ( $p \leq 0.05$ ) differences between moderate and hot ambience mean values for a row
- <sup>d</sup> marks significant ( $p \leq 0.05$ ) differences between male and female mean values within an ambience.
- <sup>f</sup> marks significant ( $p \leq 0.05$ ) differences between mean values of the age groups within an ambience

**3.3 Serum cholesterol:** The mean values of serum cholesterol were significantly ( $p \leq 0.01$ ) lower during extreme hot ambience as compared to moderate overall mean value (Table 3). In present investigation, influence of extreme hot ambience was recorded to be significant ( $p \leq 0.05$ ) on serum cholesterol value in non-descript sheep when matched up to moderate (control) value. Scientists have shown that reduced contents of cholesterol in blood of heat stressed animals could be due to low thyroid activity hence revealing the decreased metabolic rate of animals (Kataria *et al.*, 2000b) [10]. Earlier research has also thrown light on the fact that heat stress can cause accountable variations in the phytochemistry of the healthy grazing flora. This could be one of the factors causing reduction in plasma lipids (Al-Qarawi, 1999) [1]. Contemporary progress in the arena of clinical medicine has attempted to associate cholesterol contents with oxidative stress (Joshi, 2012) [6]. The data produced in the present endeavour can assist in the potential research in the area of oxidative stress as changes in serum cholesterol can be found in an array of situations other than the thyroid disorders (Kataria *et al.*, 2000b) [10]. Summer influence had significantly decreased blood cholesterol. This could be due to variation thyroid activity (Kataria *et al.*, 2000b) [10]. Similar kind of studies are required to check the monumental mismanagement in monitoring the health status of the sheep.

However, there is paucity of research on this aspect in non-descript sheep. A mean values of cholesterol were recorded to be significantly ( $p \leq 0.05$ ) higher in male sheep as compared to female sheep in both the ETPs. Magnitude of percent lowering due to extreme hot ETP was more in female sheep than male sheep for cholesterol. Age effect showed a significant ( $p \leq 0.05$ ) decrease in the mean values being lowest in the non-descript sheep of 10-13 months. Percent variation was found to be maximum in non-descript sheep of oldest age group. These variations showed lowering of values during extreme hot ambience. Variations with advancement of age were comparatively lower during extreme hot ambience in comparison to moderate ambience. This showed the clear effect of ambiances on the non-descript sheep of different age groups. Carroll *et al.* (1973) [3] measured plasma cholesterol in young ones of animals like suckling and weaned lambs, calves and colts. Kiran *et al.* (2012) [13] investigated effect of sex and age on cholesterol in healthy small ruminants. Scientists are of the opinion that age and sex consideration should be carried out for a parameter like cholesterol, otherwise biasness may be observed in the interpretation (Heitmann, 1992) [5]. It can be inferred that hot ETP influenced animals of all age group and sexes.

**Table 3:** Mean  $\pm$  SEM values and percent changes in serum cholesterol (mmolL<sup>-1</sup>) of non-descript sheep during moderate and extreme hot ambiances.

Effects	Moderate ambience Mean $\pm$ SEM(60)	Extreme hot ambience Mean $\pm$ SEM(60)	Percent Change In Hot ambience
Ambience Overall mean value (60)	3.67 <sup>b</sup> $\pm$ 0.03	3.03 <sup>b</sup> $\pm$ 0.02	-17.43
Male (30)	3.79 <sup>bd</sup> $\pm$ 0.003	3.33 <sup>bd</sup> $\pm$ 0.004	-12.13
Female (30)	3.55 <sup>bd</sup> $\pm$ 0.004	2.73 <sup>bd</sup> $\pm$ 0.003	-23.09
4-7 Months age (20)	3.89 <sup>bf</sup> $\pm$ 0.001	3.22 <sup>bf</sup> $\pm$ 0.001	-17.22
7-10 Months age (20)	3.62 <sup>bf</sup> $\pm$ 0.002	3.06 <sup>bf</sup> $\pm$ 0.001	-15.46
10-13 Months age (20)	3.50 <sup>bf</sup> $\pm$ 0.001	2.81 <sup>bf</sup> $\pm$ 0.001	-19.71

- Figures in the parenthesis indicate number of sheep.
- <sup>b</sup> marks significant ( $p \leq 0.05$ ) differences among moderate and hot ambience mean values for a row.
- <sup>d</sup> marks significant ( $p \leq 0.05$ ) differences between male and female mean values within an ambience.
- <sup>f</sup> marks significant ( $p \leq 0.05$ ) differences between mean values of the age groups within an ambience

#### 4. Conclusion

Manner of changes of data implied the line of approach in physiological tactics by sheep during the periods when possible development of oxidative stress was there. Variation in serum metabolites reflected danger to stability of health and production values during extreme hot ambience as metabolites plays a major role in normal body functioning. Bang of effect was bigger during extreme hot ambience. According to age, sheep of 4-7 months of age were found to be more affected. It was appraised that classical tactics were present in the non-descript sheep to concur unsympathetic

conditions. In a succinct way, it can be proposed that nutrition of non-descript sheep is required to be supplemented with appropriate nutrients. The upshots of endeavour will immensely contribute in monitoring the health status of non-descript sheep to improve indirectly the monitory status of marginal sheep raisers.

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