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Heat Stress Associated Alterations in Water-Deficit Markersin Sheep from Western Rajasthan

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ABSTRACT

A study was conductedduring 2018-2019 (Approximately one year) at College of Veterinary and Animal Science, Bikaner, Rajasthanto access the heat ambience associated alterations in water-deficit markers in sheep from western Rajasthan. Appraisal of environmental elements was carried out on the basis of recording of heat load index during intervening, dry-hot, humid-hot and cold Environmental Periods from Sri Ganganagar and Churu districts of Rajasthan. The mean values among EPs varied significantly ($p \leq 0.05$) for minimum, maximum and average Temperature Humidity Index (THI). During humid-hot EP, the % variation in the values of plasma bicarbonate, urine bicarbonate, Fractional Excretion of Bicarbonate ions (FE_{Bicarb}), plasma anion gap and urine anion gap were found to be maximum (+32.73%, +112.78%, +168.75%,-45.74% and +23.17, respectively. On the basis of study it was concluded that the humid hot was the most effective season among all ambiences. The female sheep were affected more than male sheep. Along with that it was also observed that 15–19 age group was affected the most among all 4 age groups. Among all ambiences, humid hot environmental condition becomes very significant for unorganized farm animals. In study of stress caused by hot environment bicarbonate ions appeared to be the most perceivable analyte representing fluid, electrolyte and acid-base status. It could also be concluded that environmental stress, disturbs the ionic balance so the nutritional and management remedies are required for better health and production of sheep under at unorganised farms.

KEYWORDS: Heat ambience, sheep, water-deficit markers and hormone

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

The arid and semi arid tracts of Rajasthan are the most awful sufferer of seasonal changes. The sketch of physiological organization in the body of the animal during stress enunciates to amass proper tactics for the comfort and security of livestock. Environment elements surrounding an animal must be explored wisely to deduce impact on animal physiology. There are several components of environment, however, temperature humidity index (THI) of a particular area ig gaining wide spread importance Kataria et al. (2016 a, b, c). Animal interface with the environmenttakes in physiological variations owing to stress factors originating by virtue of the environment. Hence, it is befitting to appreciate attributes of homeokinesis which may help in providing the scaffolding for building up of the gambits to decline stress impinging on the animals. Animal welfare needs scientific focus on the ways by which animals react to their environment using physiological tools like metabolic aspects, hydration statusadaptive responses, and psycho-physiological reactivityetc. Kataria and Kataria (2004 a). The thermal environment has a strapping bang on animals with environmental temperature having the peak stimulus. Environmental periods have in general a burly swing on production of animals along with management and reproduction. Attentive medley of physiological parameters in serum concern to water deficit in body are of serious concern for evaluation of organ triumphs Joshi et al. (2017a,b). Water stress is gaining significance in the explorations associated with homeostasis. Extreme environmental temperature has tremendous potential to lower down the hydration status, therefore producing lurid transformations in blood indices and glomerular filtration rate Katariaand Kataria (2004a). Ultimaely affect the physiological parameters like blood bicarbonate level and anion gap in serum and urine, Comprehension of stress is crucial requiring befitting laboratory tools and as the first step of staircase Katariaet al. (2005a). During study plasma and urine bicarbonate, fractional excretion of bicarbonate, plasma and urine anion gap were assessed as water deficit markers. Abhishek et al. (2022a) reported the significant changes in osmolality and tonicity in calves during humid-hot as compared to dry-hot. This exhibited that hydration status was affected to a greater extent during humid-hot. Water stress is gaining significance in the explorations associated with homeostasis. According to Bhavsar Tanvi et al. (2020) reported the increased serum level of oxidative stress during humid hot ambience, they reported the role of abiotic stressors and data can be employed for clinical diagnosis and in making health supervision strategies of calves.Extreme environmental temperature has tremendous potential to lower down the hydration status, therefore producing lurid transformations in blood indices and glomerular filtration rate Kataria et al. (2001c). The stress caused by ambient heat may be ameliorated by better nutritional management.Measurement of concentrations of electrolyte is an old and well established tool; however, its importance is worth discussing in the contemporary physiology. Researchers are seriously screening to change the dietary cation-anion divergence. Anionic salts are used to check hypocalcaemia by escalating calcium movement from bone and raised uptake of calcium from the intestine. With the foundation of values, any laboratory can come up with strong diagnostic outputs Kataria and Kataria (2005b). The arid and semi arid tracts of Rajasthan are the most awful sufferer of seasonal changes. The sketch of physiological organization in the body of the animal during stress enunciates to amass proper tactics for the comfort and security of livestock Kataria et al. (2000b). Comprehension of stress is crucial requiring befitting laboratory tools and as the first step of staircase Kataria and Kataria (2010b). During study plasma and urine bicarbonate, fractional excretion of bicarbonate, plasma and urine anion gap were assessed as water deficit markers.Abhishek et al. (2022) reported the significant changes in osmolality and tonicity in calves during humid-hot as compared to dry-hot. This exhibited that hydration status was affected to a greater extent during humid-hot.Water stress is gaining significance in the explorations associated with homeostasis. Extreme environmental temperature has tremendous potential to lower down the hydration status, therefore producing lurid transformations in blood indices and glomerular filtration rate.

2. MATERIALS AND METHODS

The study was conducted during 2018-2019 (Approximately one year) at College of Veterinary and Animal Science, Bikaner, Rajasthan to access the heat ambience associated alterations in water-deficit markers in sheep from western Rajasthan. To accomplish the aim of the investigation, blood and urine samples were collected from the *Nali* sheep belonging to owners of private slaughter houses. Collection of blood samples was accomplished during the process of slaughtering. Non-invasive techniques were utilized to collect urine samples from the *Nali* sheep at the time of voiding before slaughtering. The blood samples were collected from Sri Ganganagar (29.9094°N and 73.8800°E) and Churu (28.2925°N and 74.9707°E) during April, May-June (dry hot), July, August-September (humid hot), October-November (moderate period) and December-January months (cold environmental periods) of 2017–2018. The whole research work was executed with the permission of Institutional Animal Ethics Committee (IAEC), College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan. Total 1280 apparently healthy male and female *Nali*sheep of varying age groups were inspected. Collection of blood and urine samples was undertaken during intervening, dry-hot, humid-hot and cold periods (EPs). In each environmental period (EP), 320 blood and urine samples each were congregated in the morning hours from clinically healthy *Nali* sheep. Obtaining of the blood samples was done adding anticoagulant (dipotassium EDTA) for the whole blood and for plasma. During experiment intervening period comprised of months of October and November; dry-hot period comprised of months of April, May and June; humid-hot period consisted of months of July, August and September and cold environmental period consisted of months of December and January. Intervening period was considered as controlperiod. In each EP, 320 *Nali* sheep were distinguished (160 males and 160 females) to collect samples of blood and urine. The male and female *Nali* sheep were classified as 3–7 months (40 male and 40 female); 7–11months (40 male and 40 female); 11–15 months (40 male and 40 female) and 15–19 months (40 male and 40 female) of age groups in each EP. In study plasma and urine bicarbonate, were determined as described by Varley (1988) method, fractional excretion of bicarbonate, plasma and urine anion gap were determined as described by Bagga et al. (2005). All three hormones were determined by chemiluminescence assay (CE, SIEMENS, ADVIA, Centaur (R) (CP), Immunoassay system, Siemens Healthcare Diagnostics Ltd., UK) method.

Special computer programmes were used to compute means and standard error (Anonymous 01) and analyses of variance (Anonymous 02) to verify the significance of the effects. The changes in the means were evaluated by Duncan's new multiple ranges test Duncan (1955).

3. RESULTS AND DISCUSSION

The mean values among EPs varied significantly ($p \leq 0.05$) for minimum, maximum and average THI. Humid-hot EP displayed maximum values of all the three elements of THI as compared to respective values during other EPs. During humid-hot EP, maximum THI range was 83–96.11. Average THI mean values were 71.97±0.14, 85.90±0.16, 86.90±0.18 and 62.98±0.17, respectively during intervening, dry-hot, humid-hot and cold EPs from Churu and SriGanganagar districts of Rajasthan. The values among EPs varied significantly ($p \leq 0.05$). Humid- hot showed maximum value. Average heat load index mean values were 70.99±0.60, 76.95±0.36, 83.86±0.61 and 46.60±0.64, respectively during intervening, dry-hot, humid-hot and cold EPs. It can be stated that intensity of environmental elements was maximum during humid-hot EP.

3.1. Portrayal of changes in values of water-deficit markers during varying EPs

A highly significant ($p \leq 0.01$) effect of extreme EPs i.e. dry-hot, humid-hot and cold was observed by analysis of variance. Level of plasma bicarbonate, urine bicarbonate, FE_{Bicarb}were observed maximum during humid-hot EP. During humid-hot EP, the % variation in the values of plasma bicarbonate, urine bicarbonate and FE_{Bicarb} were found to be maximum (+32.73), (+112.78) and (+168.75), respectively. In each EP, overall mean value of female sheep was significantly ($p \leq 0.05$) higher than the respective overall mean value of male sheep. In male and female categories, in each group, the maximum mean values of urine bicarbonate were observed in humid-hot EP. In each gender, in each EP, minimum value was observed in 3–7 months age group and maximum value was observed in 15–19 months age group. All the changes were significant ($p \leq 0.05$), table1, 2 and 3.

Chauhan et al. (2015) revealed the bang of heat load on balance of acid-base in sheep. Under heat stress, increase of blood pH and decrease of bicarbonate was noted. A study by Trefz et al. (2017) concluded that hyperkalemia is a regularly noticed imbalance of electrolyte in neonatal dehydrated calves having diarrhoea. Further advantages of infusions of hypertonic sodium bicarbonate were discussed. In a study, Joshi (2018) investigated effect of extreme ambiences in *Rathi* cattle; he observed the changes in plasma bicarbonate level due to heat stress. In a study, Promila (2018) estimated plasma bicarbonate as an indirect analyte of hydration status in sheep. A significant change was observed in the mean value during hot ambience reflecting bang of environmental temperature. A study by Singh (2018) revealed plasma bicarbonate as an indirect analyte of hydration status in goats. A significant change was observed in the mean value of bicarbonate during hot ambience shows the effect of heat stress on body physiology.

3.2. Portrayal of changes in values of plasma anion gap and urine anion gap during varying EPs

During humid-hot EP plasma anion gap level was observed minimum while urine anion gap was observed maximum during same period. During humid-hot EP, the % variation in the values of plasma anion gap and urine anion gap were found to be maximum (-45.74) and (+23.17), respectively, whileduring in each EP, overall mean value of female sheep was significantly ($p \leq 0.05$) higher than the respective overall mean value of male sheep. All the changes were significant ($p \leq 0.05$). In each gender, in each EP, minimum value was observed in 3–7 months age group and maximum value was observed in 15–19 months age group. The changes according to age groups, irrespective of gender, divulged an increasing pattern of the mean values which were found to be minimum in 3–7 months age group and maximum in 15–19 months age group. Analysis of variance also indicated significant ($p \leq 0.05$) differences. A study by Mellor (1970) attempted to investigate sheep and goat for exploring ion distribution. A study by Kutas (1965) attempted to explore net acid base excretion in the urine of cattle. This study was for the estimation of acid base equilibrium.Screening

Table 1: Mean±SEM values of plasma bicarbonate (PBicarb, mmol l^{-1}) in the *Nali* sheep during varying environmental periods (EPs)

Sl. No.	Effects	Mean±SEM values during environmental periods			
		Intervening	Dry-hot	Humid-hot	Cold
Environmental period overall values (320)		22.18^{b}±0.15	25.54^{b}±0.18	29.44^{b}±0.23	23.67^{b}±0.17
Overall mean values of males (160)		19.68^{bc}±0.02	22.68^{bd}±0.01	25.74^{bd}±0.04	20.96^{bd}±0.01
a.	3-7 months (40)	18.15^{bd}±0.016	21.17^{bd}±0.016	23.49^{bd}±0.139	19.17^{bd}±0.016
b.	7-11 months (40)	19.17^{bd}±0.016	22.19^{bd}±0.014	25.20^{bd}±0.016	20.18^{bd}±0.015
c.	11-15 months (40)	20.21^{bd}±0.034	23.17^{bd}±0.018	26.14^{bd}±0.011	21.22^{bd}±0.010
d.	15-19 months (40)	21.21^{bd}±0.014	24.19^{bd}±0.018	28.16^{bd}±0.016	22.19^{bd}±0.015
Overall mean values of females (160)		26.01^{bc}±0.010	28.18^{bc}±0.010	32.12^{bc}±0.012	26.21^{bc}±0.010
e.	3-7 months (40)	24.10^{bd}±0.001	26.16^{bd}±0.006	30.11^{bd}±0.009	25.20^{bd}±0.006
f.	7-11 months (40)	25.24^{bd}±0.001	27.16^{bd}±0.004	32.17^{bd}±0.003	26.16^{bd}±0.006
g.	11-15 months (40)	26.10^{bd}±0.001	29.14^{bd}±0.003	34.10^{bd}±0.002	27.14^{bd}±0.006
h.	15-19 months (40)	28.02^{bd}±0.001	31.16^{bd}±0.005	36.14^{bd}±0.006	28.97^{bd}±0.007

Table 2: Mean±SEM values of urine bicarbonate (UBicarb, mmol l^{-1}) in the *Nali* sheep during varying environmental periods (EPs)

Sl. No.	Effects	Mean±SEM values during environmental periods			
		Intervening	Dry-hot	Humid-hot	Cold
Environmental period Overall values (320)		27.84^{b}±0.15	47.12^{b}±0.15	59.24^{b}±0.16	36.62^{b}±0.13
Overall mean values of males (160)		26.86^{bc}±0.00	45.62^{bd}±0.00	57.62^{bd}±0.00	27.37^{bd}±0.00
a.	3-7 months (40)	23.58^{bd}±0.02	42.61^{bd}±0.01	54.62^{bd}±0.00	32.60^{bd}±0.01
b.	7-11 months (40)	25.6^{bd}±0.01	44.62^{bd}±0.00	56.61^{bd}±0.00	34.62^{bd}±0.00
c.	11-15 months (40)	27.64^{bd}±0.00	46.62^{bd}±0.00	58.64^{bd}±0.00	36.62^{bd}±0.02
d.	15-19 months (40)	30.62^{bd}±0.00	48.64^{bd}±0.00	60.63^{bd}±0.00	38.64^{bd}±0.00
Overall mean values of females (160)		28.83^{bc}±0.01	48.60^{bc}±0.00	60.86^{bc}±0.00	37.63^{bc}±0.00
e.	3-7 months (40)	25.6^{bd}±0.01	45.64^{bd}±0.00	57.63^{bd}±0.00	34.62^{bd}±0.00
f.	7-11 months (40)	27.64^{d}±0.00	47.64^{bd}±0.00	59.62^{bd}±0.00	36.62^{bd}±0.00
g.	11-15 months (40)	29.50^{bd}±0.02	49.54^{bd}±0.02	61.56^{bd}±0.01	38.64^{bd}±0.00
h.	15-19 months (40)	32.60^{bd}±0.01	51.61^{bd}±0.01	64.64^{bd}±0.00	40.64^{bd}±0.00

Table 3: Mean±SEM values of fractional excretion of bicarbonate (FE_{HCO3},%) in the *Nali* sheep during varying environmental periods (EPs)

Sl. No.	Effects	Mean±SEM values during environmental periods			
		Intervening	Dry-hot	Humid-hot	Cold
Environmental period Overall values (320)		0.032^{b}±0.040	0.081^{b}±0.043	0.084^{b}±0.045	0.058^{b}±0.041
Overall mean values of males (160)		0.029^{bc}±0.010	0.078^{bc}±0.010	0.082^{bc}±0.012	0.057^{bc}±0.010
a.	3-7 months (40)	0.026^{bd}±0.0005	0.076^{bd}±0.0006	0.080^{bd}±0.0006	0.051^{bd}±0.0006
b.	7-11 months (40)	0.030^{bd}±0.0004	0.079^{bd}±0.0005	0.082^{bd}±0.0005	0.054^{bd}±0.0006
c.	11-15 months (40)	0.033^{bd}±0.0005	0.078^{bd}±0.0004	0.083^{bd}±0.0005	0.058^{bd}±0.0005
d.	15-19 months (40)	0.035^{bd}±0.0004	0.079^{bd}±0.0005	0.084^{bd}±0.0005	0.059^{bd}±0.0005

Table 3: Continue...

Sl. No.	Effects	Mean±SEM values during environmental periods			
		Intervening	Dry-hot	Humid-hot	Cold
Overall mean values of females (160)		0.035^{bc}±0.011	0.084^{bd}±0.011	0.086^{bd}±0.011	0.059^{bd}±0.011
e.	3-7 months (40)	0.031^{bd}±0.0005	0.078^{bd}±0.0006	0.082^{bd}±0.0006	0.055^{bd}±0.0006
f.	7-11 months (40)	0.034^{bd}±0.0004	0.081^{bd}±0.0005	0.083^{bd}±0.0005	0.056^{bd}±0.0006
g.	11-15 months (40)	0.036^{bd}±0.0005	0.085^{bd}±0.0004	0.085^{bd}±0.0005	0.059^{bd}±0.0005
h.	15-19 months (40)	0.038^{bd}±0.0004	0.086^{bd}±0.0005	0.088^{bd}±0.0005	0.063^{bd}±0.0005

of anionic salts influence on acid-base status and urinary calcium excretion in dairy cows were done by Oetzel et al. (1991). Sendag et al. (2011) determined net acid base excretion which are imperative markers the acid-base balance in ewes. Joshi (2018) investigated cattle to find effect of extreme ambiences on urine anion gap and significant changes were observed, table 4 and 5.

Table 4: Mean±SEM values of plasma anion gap (PAG, mmol l^{-1}) in the *Nali* sheep during varying environmental periods (EPs)

Sl. No.	Effects	Mean±SEM values during environmental periods			
		Intervening	Dry-hot	Humid-hot	Cold
Environmental period overall values (320)		22.96^{b}±0.40	16.03^{b}±0.43	13.05^{b}±0.45	20.96^{b}±0.41
Overall mean values of males (160)		21.33^{bc}±0.10	13.73^{bc}±0.10	11.42^{bc}±0.12	18.45^{bc}±0.10
a.	3-7 months (40)	19.94^{bd}±0.05	10.37^{bd}±0.06	8.64^{bd}±0.06	13.16^{bd}±0.06
b.	7-11 months (40)	20.60^{bd}±0.04	12.48^{bd}±0.05	9.73^{bd}±0.05	17.36^{bd}±0.06
c.	11-15 months (40)	21.50^{bd}±0.05	14.73^{bd}±0.04	10.04^{bd}±0.05	18.62^{bd}±0.05
d.	15-19 months (40)	25.73^{bd}±0.04	16.90^{bd}±0.05	13.20^{bd}±0.05	19.07^{bd}±0.05
Overall mean values of females (160)		24.60^{bc}±0.10	20.48^{bd}±0.11	17.54^{bd}±0.12	23.88^{bd}±0.10
e.	3-7 months (40)	23.44^{bd}±0.05	18.89bd±0.06	15.63^{bd}±0.06	22.61^{bd}±0.06
f.	7-11 months (40)	24.60^{bd}±0.04	19.97^{bd}±0.05	17.01^{bd}±0.05	23.84^{bd}±0.06
g.	11-15 months (40)	25.55^{bd}±0.05	20.50^{bd}±0.04	18.18^{bd}±0.05	24.90^{bd}±0.05
h.	15-19 months (40)	26.82^{bd}±0.04	22.11^{bd}±0.05	19.34^{bd}±0.05	25.02^{bd}±0.05

Table 5: Mean ± SEM values of urine anion gap (UAG, mmol l^{-1}) in the *Nali* sheep during varying environmental periods (EPs)

Sl. No.	Effects	Mean±SEM values during environmental periods			
		Intervening	Dry-hot	Humid-hot	Cold
Environmental period overall values (320)		28.01^{b}±0.040	31.50^{b}±0.043	34.59^{b}±0.045	31.63^{b}±0.041
Overall mean values of males (160)		27.75^{bc}±0.010	28.51^{bd}±0.012	32.82^{bd}±0.011	28.67^{bd}±0.010
a.	3-7 months (40)	23.34^{bd}±0.005	26.35^{bd}±0.006	31.36^{bd}±0.006	25.27^{bd}±0.006
b.	7-11 months (40)	27.19^{bd}±0.004	28.28^{bd}±0.005	32.30^{bd}±0.005	27.16^{bd}±0.006
c.	11-15 months (40)	28.20^{bd}±0.005	29.29^{bd}±0.005	33.39^{bd}±0.004	29.31^{bd}±0.005
d.	15-19 months (40)	30.19^{bd}±0.004	30.21^{bd}±0.005	34.37^{bd}±0.005	31.21^{bd}±0.005
Overall mean values of females (160)		30.26^{bc}±0.10	36.10^{bc}±0.10	38.17^{bc}±0.12	35.02^{bc}±0.10
e.	3-7 months (40)	30.23^{bd}±0.005	35.23^{bd}±0.006	35.99^{bd}±0.006	32.32^{bd}±0.006
f.	7-11 months (40)	31.28^{bd}±0.004	36.22^{bd}±0.005	37.26^{bd}±0.005	36.27^{bd}±0.006
g.	11-15 months (40)	33.27^{bd}±0.005	37.39^{bd}±0.004	38.21^{bd}±0.005	37.32^{bd}±0.005
h.	15-19 months (40)	36.29^{bd}±0.004	38.56^{bd}±0.005	39.14^{bd}±0.005	39.18^{bd}±0.005

Figures in the parenthesis: Number of Nalisheep; EP: Environmental period; 'b': Significant ($p\leq0.05$) differences among mean values for a row; 'c': Significant ($p\leq0.05$) differences between overall mean values of males andfemales for an EP; 'd': Significant ($p\leq0.05$) differences among mean values of different genderspecific age groups for an EP

4. CONCLUSION

Humid hot ambience was the mostharsh ambience causing bang on animal physiology. The female sheep were affected more than the male sheep. The reference values of wellbeing and contentment index of *Nali* sheep belonging to Churu and Sri Ganganagar districts, Rajasthan were based upon the environmental elements and physiological analytes, since animal population greatly suffer the impact of higher environmental temperatures in arid tracts.

5. FURTHER RESEARCH

In future the effect of heat stress could be extended on molecular level and the role of heat shockproteis could be explored in sheep in same area.

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