



Effect of Heat Stress on Water Deficit Markers in *Nali* Sheep

Saurabh Singh Singhal, Mamta Meena*, Vikas Kumar Sharma, O.P. Meena, Nalini Kataria and A.K. Kataria

Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, INDIA

*Corresponding author: M Meena; E-mail: drmamtameena04@gmail.com

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ABSTRACT

An exploration was launched to 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 (EPs) from Sri Ganganagar and Churu districts of Rajasthan. The mean values among EPs varied significantly ($p < 0.05$) 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 ambiances. The female sheep were affected more than male sheep. Along with that it was also observed that 15- 19 months age group was affected the most among all 4 age groups.

HIGHLIGHTS

- Study focused on water-deficit markers in sheep from Rajasthan altered due to heat stress.
- Varying environmental periods can cause stress to the animals at different levels.
- Arid areas stand the trouble of seasonal change and the arid tracts of Rajasthan are the most dreadful victim of seasonal variations.

Keywords: Environmental periods, heat ambience, sheep, stress and water-deficit markers

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. Comprehension of stress is crucial requiring befitting laboratory tools and as the first step of staircase (Kataria and Kataria, 2005; Kataria and Kataria, 2010). During study plasma and urine bicarbonate, fractional excretion of bicarbonate, plasma and urine anion gap were assessed as water deficit markers. Water stress is gaining significance in the explorations associated with homeostasis. According to Arora and Kataria (2021) high heat load index changes metabolic functions in body causing variation in metabolites production. 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.*, 2001). The stress caused by ambient heat may be ameliorated by better nutritional management (Kishan pal *et al.*, 2020).

MATERIALS AND METHODS

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

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voiding before slaughtering. 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 control period. 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). Special computer programmes were used to compute means and standard error (<http://www.miniwebtool.com>) and analyses of variance (www.danielsoper.com) to verify the significance of the effects. The changes in the means were evaluated by Duncan's new multiple ranges test (Duncan, 1955).

RESULTS AND DISCUSSION

The mean values among EPs varied significantly ($p<0.05$) 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 Sri Ganganagar districts of Rajasthan. The

values among EPs varied significantly ($p<0.05$). 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.

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

A highly significant ($p<0.01$) 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 ($p<0.05$) 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 ($p<0.05$).

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 ambiances 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.

Table 1: Mean \pm SEM values of plasma bicarbonate (P_{Bicarb} , mmolL⁻¹) in the *Nali* sheep during varying environmental periods (EPs)

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

Table 2: Mean \pm SEM values of urine bicarbonate (U_{Bicarb} , mmolL⁻¹) in the *Nali* sheep during varying environmental periods (EPs)

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

Table 3: Mean \pm SEM values of fractional excretion of bicarbonate (FE_{HCO_3} , %) in the *Nali* sheep during varying environmental periods (EPs)

Sl. No.	Effects	Mean \pm SEM values during environmental periods			
		Intervening	Dry-hot	Humid-hot	Cold
Environmental period Overall values (320)		0.032 ^b \pm 0.040	0.081 ^b \pm 0.043	0.084 ^b \pm 0.045	0.058 ^b \pm 0.041
Overall mean values of males (160)		0.029 ^{bc} \pm 0.010	0.078 ^{bc} \pm 0.010	0.082 ^{bc} \pm 0.012	0.057 ^{bc} \pm 0.010
	3-7 months (40)	0.026 ^{bd} \pm 0.0005	0.076 ^{bd} \pm 0.0006	0.080 ^{bd} \pm 0.0006	0.051 ^{bd} \pm 0.0006
	7-11 months(40)	0.030 ^{bd} \pm 0.0004	0.079 ^{bd} \pm 0.0005	0.082 ^{bd} \pm 0.0005	0.054 ^{bd} \pm 0.0006
	11-15 months (40)	0.033 ^{bd} \pm 0.0005	0.078 ^{bd} \pm 0.0004	0.083 ^{bd} \pm 0.0005	0.058 ^{bd} \pm 0.0005
	15-19 months (40)	0.035 ^{bd} \pm 0.0004	0.079 ^{bd} \pm 0.0005	0.084 ^{bd} \pm 0.0005	0.059 ^{bd} \pm 0.0005
Overall mean values of females (160)		0.035 ^{bc} \pm 0.011	0.084 ^{bd} \pm 0.011	0.086 ^{bd} \pm 0.011	0.059 ^{bd} \pm 0.011
	3-7 months (40)	0.031 ^{bd} \pm 0.0005	0.078 ^{bd} \pm 0.0006	0.082 ^{bd} \pm 0.0006	0.055 ^{bd} \pm 0.0006
	7-11 months (40)	0.034 ^{bd} \pm 0.0004	0.081 ^{bd} \pm 0.0005	0.083 ^{bd} \pm 0.0005	0.056 ^{bd} \pm 0.0006
	11-15 months (40)	0.036 ^{bd} \pm 0.0005	0.085 ^{bd} \pm 0.0004	0.085 ^{bd} \pm 0.0005	0.059 ^{bd} \pm 0.0005
	15-19 months (40)	0.038 ^{bd} \pm 0.0004	0.086 ^{bd} \pm 0.0005	0.088 ^{bd} \pm 0.0005	0.063 ^{bd} \pm 0.0005

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, while during in each EP, overall mean value of female sheep was significantly ($p<0.05$) higher than the respective overall mean value of male

sheep. All the changes were significant ($p<0.05$). 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 ($p<0.05$) differences. A study by Mellor (1970) attempted to investigate sheep and goat for exploring ion distribution. A study by Kutas (1965) attempted to explore

Table 4: Mean \pm SEM values of plasma anion gap (P_{AG} , mmolL⁻¹) in the *Nali* sheep during varying environmental periods (EPs)

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

Table 5: Mean \pm SEM values of urine anion gap (U_{AG} , mmolL⁻¹) in the *Nali* sheep during varying environmental periods (EPs)

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

Figures in the parenthesis = Number of *Nali* sheep; EP = Environmental period; 'b' = Significant ($p<0.05$) differences among mean values for a row.; 'c' = Significant ($p<0.05$) differences between overall mean values of males and females for an EP; 'd' = Significant ($p<0.05$) differences among mean values of different genderspecific age groups for an EP.

net acid base excretion in the urine of cattle. This study was for the estimation of acid base equilibrium. Screening 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 excretions which are imperative markers the acid-base balance in ewes. Joshi (2018) investigated cattle to find effect of extreme ambiances on urine anion gap and significant changes were observed.

CONCLUSION

It could be concluded that, in each EP, overall mean value of all studied water deficit markers were observed significantly ($p < 0.05$) higher in female sheep than the respective overall mean value in male sheep. In each gender, in each EP, minimum value of each water deficit marker was observed in 3-7 months age group and maximum value was observed in 15-19 months age group. It could be concluded that humid hot ambience was the harshest ambience causing bang on animal physiology. The one of the objectives of the present study was to set the reference values of wellbeing and contentment index of *Nali* sheep belonging to Churu and Sri Ganganagar districts, Rajasthan based upon the environmental elements and physiological analytes, since animal population greatly suffer the impact of higher environmental temperatures in arid tracts.

FURTHER RESEARCH

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

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