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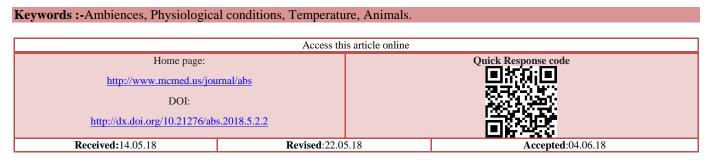
ALTERATIONS IN SERUM 5' NUCLEOTIDASE ACTIVITY OF RATHI FEMALE CALVES, HEIFERS AND COWS FROM ARID TRACTS DURING EXTREME AMBIENCES

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ABSTRACT

An exploration was conducted to appraise physiological strategies in *Rathi* female calves, heifers and cows from arid tracts implying alterations in serum 5' nucleotidase (5'NT) activity during extreme ambiences. Break-up of the endeavour included sampling of animals during moderate, extreme hot, rainy and extreme cold ambiences in the arid tract of Bikaner district, Rajasthan, India. The cattle were classified into calves, heifers and cows. The overall mean value of serum 5'NT during moderate ambience was 58.00±0.40UL⁻¹, which was obtained from 300 Rathi cattle incorporating calves, heifers and cows. The overall mean values of serum 5'NT were significantly ($p \le 0.05$) higher during extreme hot, rainy and extreme cold ambiences in comparison to moderate mean overall value. During rainy ambience, the per cent variation in the value of serum 5'NT was found to be maximum (+56.89). It can be deduced that bang of rainy ambience was huge on overall serum 5'NT value followed by extreme hot and cold ambiences. Among physiological states, cows had higher overall value of serum 5'NT as compared to overall value of calves and overall value of heifers during moderate conditions. This pattern was maintained during all the three extreme ambiences with respective notable elevations during rainy ambience. Degree of influence of ambiences and physiological states on serum 5'NT was assessed by measuring per cent variations. Overall value revealed highest per cent variation during rainy ambience as compared to extreme hot and cold ambiences indicating that serum 5'NT was modulated maximally during rainy ambience followed by extreme hot and cold. Among calves, heifers and cows, per cent variations were highest in overall values of cows. This pattern was maintained in all the three extreme ambiences, extent being higher, respectively during rainy ambience. This precedent exhibited that calves were distressed largely and rainy ambience had greater impression on the animals. Archetype of per cent variation exhibited that among calves, pre-ruminant group revealed maximum modulations. Post-pubertal heifers were influenced largely in heifer group revealing maximum per cent variation during rainy ambience. Among cows, non-pregnant milch and primipararevealed superior effect as compared to other members of respective group. Rainy ambience revealed maximum temperature humidity index values. Extreme ambiences affected the liver functions of the animals.



INTRODUCTION

Animals function most competently within their thermo-neutral zone. Above the upper and the lower

critical temperatures, animals feel stressed. Harsh environment restricts the production and reproduction. It

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has been observed that the critical temperatures given are not fixed features for any species and they may vary with age, sex and physiological conditions. While observing these aspects, eco-physiological characteristics should be considered. Integrative evaluations have been developed to assess surrounding environment of animals in hot environmental conditions. Exploratory examination of the stress and productivity responses of animals incorporates management strategies which can be modified for welfare practices. Scientific awareness on animal welfare must converge on the ways animals respond to their ambience with the individual multiplicity of adaptive responses and psycho-physiological reactivity to increase the production output and product quality [1].

The animal's environment is very multifaceted. Yet, researchers try to describe and gauge it in terms of a single analyte or a small cluster of analytes of primary significance. There are several measures of the thermal environment. However, dry-bulb temperature is in general regarded to be the major thermal correlate. Ambient temperature adds up heat to the body besides that is gained by the animal from metabolic processes. Dissipation of extra heat is immensely significant in order to sustain normal body temperature. Thermal radiation from surroundings of an animal like walls of house or ground during extreme hot conditions can intensify impact of hot temperature, particularly in arid tracts. High humidity can also worsens the impact of high temperature. Therefore, it is also taken up with the ambient temperature as an important correlate. High humidity decreases the probable potential of evaporation by the animal. Wind can decrease adverse impacts of high ambient temperatures. Stress responses extend in a wider scale of analytes, starting from hormones to the target responses [2, 3].

Measurement of 5'NT is an important component of liver function tests. 5'-nucleotidase catalyzes the phosphorylytic cleavage of 5'nucleotides. This enzyme is mainly located in the plasma membrane and plays an important role in metabolism of nucleotides. The blood level of 5'nucleotidase can be employed to assess liver functions and increased serum 5'nucleotidase activity may divulge hepatitis, ischemia or liver insult [4]. Certain physiological conditions like pregnancy can increase the levels.

A demanding element for researchers is to safeguard these animals and to employ health programmes so that deterioration in number of these animals can be prevented with real-time efforts to enhance the number of *Rathi* animals. It is inevitable to turn away the association of the cattle to extreme ambiences under natural husbandry conditions. Extreme ambiences situate negative force on the dairy animals moving back the positive processes like growth, production, reproduction and health. Animals carry out physical activity during extreme hot and humid conditions which can put their physiological processes in jeopardy. Longer association of animals with extreme hot ambient temperature linked with supercilious relative humidity can bargain the ability of animals to drive out surplus body heat which affects physiological elements of animal routinely like feed intake, growth, production of milk and reproduction. Sooner or later, failure is of farmers in terms of reduction in prosperity from animal produces. Vast economical trouncing due to abiotic stress has changed the scientific attention towards measurement outlooks which can be influential in rearing of *Rathi* animals in an advanced way [5]. Based upon above deliberations, the object of this investigation was to assess the physiological gambits in *Rathi* female calves, heifers and cows entailing alterations in serum 5'NT activity during extreme ambiences of a year by measuring temperature humidity index values.

MATERIALS AND METHODS

To accomplish the objectives of the study, 1200 apparently healthy Rathifemale calves, heifers and were screened from private dairies located in and around Bikaner district, Rajasthan. To achieve the goals of the study, Rathi female animals ageing two weeks old to 12 years of age were sampled during moderate, extreme hot, extreme cold and rainy ambiences. Samples for experiment were comprised of blood to harvest serum. Clean and dried test tubes were employed for blood collection without any anticoagulant to harvest the serum. In each ambience, 300 blood samples were collected in the morning hours from clinically healthy animals. Experiment was carried out with the permission of Institutional Animal Ethics Committee (IAEC), College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India. Temperature humidity index (THI) values of sample collection periods in and around Bikaner district, Rajasthan, India during moderate, extreme hot, extreme cold and rainy ambiences were measured [6]. Moderate ambience comprised of October-November, extreme cold comprised of December- January, extreme hot ambience of May and June and rainy ambience comprised of July-August-September. Animals were grouped according to age in three categories as category I, II and III in each ambience. Category I included Rathi female calves ageing from two weeks to one year. This was based on the physiological basis [7] of involving type of digestion [8] and weaning practices opted by private dairy owners of the area [9]. Therefore, grouping included 2 to 3 weeks old (Pre-ruminant phase), 3 to 8 weeks old (Transitional phase), 8 to 16 weeks old (Pre-weaning), 16 to 32 weeks old (Post- weaning) and 32 to 48 weeks old (Calf-yearling transition) female *Rathi* calves. Each group was consisted of 30 animals. Though pre-ruminant phase involves the calves from birth to 3 weeks of age, however, in the study only 2-3 weeks old calves were incorporated. Category II incorporated female animals (heifers) ageing from one year to 3.5 years of age. This classification was based on the observation of time of onset of puberty in

Rathi heifers [10, 11] and reproductive pattern followed by private dairies and marginal owners in and around Bikaner district, Rajasthan, India. Grouping of female animals included 1-2.5 years and 2.5 to 3.5 years of age group. This classification was purely on the basis of behavioural and other observations associated with the onset of puberty [12] and history from the animal owners. These animals were categorised as prepubertal and postpubertal, respectively [13, 14]. All post pubertal animals were non pregnant. Each category comprised of 30 animals. Category III incorporated Rathi cows ageing 3.5-12 years. They were broadly divided into group A and group B according to physiological states [15]. Animals of group A involved non-pregnant milch; pregnant milch and pregnant dry cows. All milch animals were sampled between 3 and 4 months of gestation period to maintain similarity. Animals of group B were classed as primipara and multipara cows. This was irrespective of states like pregnancy and milch. All primipara were between 3.5 and 6 years whereas all multipara were between 6 and 12 years of age. To accomplish the objectives regarding dynamics of environmental correlates vis-a-vis appraisal of physiological strategies in Rathi female calves, heifers and cows implying modulations in endocrine, organ and tissue functions, energy metabolism and cellular oxidative stress responses, the result of various parameters analyzed were compared with those analyzed during moderate months serving as control.

It was determined by method of Campbell [16]. Hydrolysis of nucleotides with a phosphate group on carbon atom 5' of the ribose is carried out by this enzyme. As an example, adenosine 5'-phosphate which is hydrolyzed to adenosine and inorganic phosphate. Since, nucleotides are also hydrolyzed by alkaline phosphatase, the difference of hydrolysis carried out with and without added nickel gives the 5'-nucleotidase activity (UL⁻¹).

In the present exploration, primary analytes were to study dynamics of environmental correlates vis-a-vis appraisal of physiological strategies in female Rathi cattle implying modulations in endocrine, organ and tissue functions, energy metabolism and cellular oxidative stress responses. Establishment of the main effects were as ambience overall values, overall values of calves, overall values of heifers and overall values of cows. For each overall value of effect, mean values were observed during moderate, extreme hot, rainy and extreme cold ambiences. Additionally, the subgroups of overall values of calves were pre-ruminant, transitional, pre-weaning, post-weaning and calf-yearling transition; overall values of heifers were pre-pubertal and post-pubertal; and overall values of cows were pregnancy and milch status (non-pregnant milch, pregnant milch and pregnant dry) and parity (primipara and multipara). For each sub group data were expressed as mean ± SE of mean. Special computer programmes were employed to compute means and standard error (http://www.miniwebtool.com) and analyses

of variance (www.danielsoper.com) to verify the significance of the impacts [17]. The adaptations in the means were evaluated by Duncan's new multiple range test. Interactions were measured for each parameter as Cows group A X Cows group B; Ambiences X Calves; Ambiences X Heifers; Ambiences X Cows group A; Ambiences X Cows group B; and Calves X Heifers X Cows group A X Cows group B. They have been shown in the analysis of variance table of each parameter in results and discussion section. Per cent variations were also worked out in comparison with respective moderate value. The data have been presented in table for each parameter. A per cent variation quantitatively explains a change in a specific parameter. An absolute variation is merely the difference between the control (moderate) value and the new one for example any extreme ambience value. Per cent variation is a ratio of the absolute variation to the control or moderate value. Researchers have observed it to be an appropriate means to assess specific changes in a parameter [18].

RESULTS AND DISCUSSION

Mean \pm SEM values of serum 5'nucleotidase of *Rathi* female cattle i.e. calves, heifers and cows during moderate, extreme hot, rainy and extreme cold ambiences are presented in table 1. Table 2 reveals the per cent variations in serum 5'nucleotidase of *Rathi* female cattle during extreme hot, rainy and extreme cold ambiences as compared to moderate ambience. Portrayal of variations in the mean values of serum 5' nucleotidase are done in figures 1 and 2.

The overall mean value of serum 5'nucleotidase during moderate ambience was 58.00 ± 0.40 UL⁻¹ which was obtained from 300 *Rathi* cattle incorporating calves, heifers and cows. The range was 53.82-62.21 UL⁻¹ during moderate ambience. The range and overall mean value of serum 5'nucleotidase obtained during moderate ambience in present exploration were more or less in agreement with the earlier findings [19]. Studies pertaining to serum 5'nucleotidase in different age groups of cattle are few in the literature and there is dearth of research work on *Rathi* breed.

Description of changes in values of serum 5'NT during varying ambiences

The overall mean values of serum 5'NT were significantly (p \leq 0.05) higher during extreme hot, rainy and extreme cold ambiences in comparison to moderate mean overall value. During rainy ambience, the per cent variation in the value of serum 5'nucleotidase was found to be maximum (+56.89). Rainy ambience revealed maximum temperature humidity index (THI) values. At average environmental temperature, THI values obtained were 71.07± 0.10, 85.00± 0.15, 86.00± 0.15 and 62.32± 0.10, respectively during moderate, extreme hot, rainy and extreme cold ambiences.

An increase in the serum 5'nucleotidase levels have been observed by earlier workers in animals due to ambience stress. Early investigations in the field of 5'nucleotidase correlated it with bile duct functions. Later on researchers have started giving a new insight to the functions of this enzyme. The relation of 5'NT with the indices of oxidative stress was discussed by DiSilvestro [19]. Oxidant insult to liver can raise the serum levels [4]. Stressed animals can show higher activity of serum 5'NT [20]. Thompson et al. [21] observed the production of extracellular adenosine in mice which was involved in phospho hydrolysis of adenine nucleotide intermediates and was controlled by the terminal enzymatic step catalyzed by 5'-nucleotidase. Ambience associated variations in the activities of 5'-nucleotidase in animals have been related with oxidative stress. 5'-NT catalyzes the phosphorylytic cleavage of 5'nucleotides. It plays a chief role in metabolism of nucleotides [22]. Extreme ambience related increase serum 5'-NT in present study indicated liver stimulation. In an investigation, hepatocellular insult consequenced in an increased serum 5'-nucleotidase activities [23]. The upshot of present study regarding 5'nucleotidase status is also pointing towards its role as one of the markers to detect oxidative stress. It is appealing to be aware of the role of 5'nucleotidase in this direction. Results of present study demonstrated that impact of rainy ambience was maximum in terms of 5'nucleotidase modulations followed by extreme hot and cold ambiences. Out of these three extreme ambiences, it can be stated that rainy and extreme hot ambiences were able to modulate 5'nucleotidase enzyme effectively as compared to cold ambience, which though showed a rise with low magnitude.

Effect of physiological states of *Rathi* female cattle on serum 5'nucleotidase

In the present investigation, Rathi female cattle had three major groups in all the four ambiences. In broader terms, animals were divided into three categories as calves, heifers and cows. Statistical analysis depicted significant ($p \le 0.05$) variations among all the three overall mean values (calves, heifers and cows) in each ambience. Since the moderate mean value in each case was considered as control, the variations due to extreme ambiences (extreme hot, rainy and extreme cold) were also significant (p≤0.05). Overall mean values of calves was minimum and of cows was maximum significantly (p < 0.05). This pattern was akin for all the ambiences. Per cent variation in the overall mean values of cows was maximum and was least in calves. This trend was similar for all the ambiences. Rainy ambience marked maximum per cent variation in each category. This pattern remained similar in extreme hot and cold ambiences. Mean values were also compared within each category. In each ambience, variations among all the type of calves were significant (p≤0.05). Pre-ruminants revealed maximum

serum 5'nucleotidase value and calf-yearling transition group showed minimum value in each ambience. All the types of calves revealed maximum per cent variation during rainy ambience as compared to respective moderate ambience mean value. This exhibited that effect of rainy ambience was greatest on all the calves followed by extreme hot and cold ambiences. Per cent variation in calfyearling transition group was maximum in rainy ambience.In each ambience, variations between heifers (pre-pubertal and post-pubertal) were significant ($p \le 0.05$). Post-pubertal had significantly ($p \le 0.05$) higher values of serum 5'nucleotidase in each ambience in comparison to pre-pubertal. In both the types, maximum mean values were observed in rainy ambience as compared to moderate ambience followed by extreme hot and extreme cold ambiences. In comparative terms, both the groups exhibited higher per cent variations during rainy ambience. However, maximum per cent variation was exhibited by post-pubertal animals.In each ambience, variations among cows were significant ($p \le 0.05$). In group A animals, pregnant-dry had significantly (p≤0.05) higher values of serum 5'nucleotidase in each ambience in comparison to others. In all the three types, maximum mean values were observed in rainy ambience as compared to moderate ambience followed by extreme hot and extreme cold ambiences. In comparative terms, all the types exhibited higher per cent variations during rainy ambience. Maximum per cent variations were exhibited by nonpregnant milch as compared to pregnant milch and pregnant dry. This pattern was same in extreme hot and cold ambiences. In group B animals, multipara had (p≤0.05) higher values significantly of serum 5'nucleotidase in each ambience in comparison to primipara. In both the types, maximum mean values were observed in rainy ambience as compared to moderate ambience followed by extreme hot and extreme cold ambiences. Primipara animals showed higher per cent variations in extreme hot, rainy and extreme cold ambiences as compared to multipara animals. Changes in serum 5'NT activities in the calves, heifers and cows clearly revealed the impact of physiological states. Earlier researchers have also shown the influence of physiological states on 5'NT activities [4, 5]. Kataria [24] advocated the relation of increased 5'NT activity with the metabolic adjustments in buffaloes. Ferrari et al. [25] hypothesized that oestrogen-dependent, sexual dimorphism found in the induction of priming was observed in the mechanisms incorporated in its expression as a controlling influence on ecto-5'NT.

Interactions of ambience with physiological states

The interactions were computed as Cows, group A XCows, group B;Ambiences X Calves; Ambiences X Heifers; Ambiences X Cows group A; Ambiences X Cows, group B; and Calves X Heifers X Cows, group A X Cows, group B. They were found to be highly significant $(p \le 0.01)$ (Table 7) which revealed the impact of extreme ambiences on the *Rathi* animals of all physiological states.

It can be inferred that brunt of rainy ambience was vast on overall serum 5'NT followed by extreme hot and cold ambiences, irrespective of physiological states. Among physiological states, cows had higher overall mean value of serum 5'NT as compared to overall value of calves and overall value of heifers during moderate. This pattern was maintained during all the three extreme ambiences with respective remarkable elevations during rainy ambience. Degree of bang of ambiences and physiological states on serum 5'NT was assessed by per cent variations. Overall value revealed highest per cent variation during rainy ambience as compared to extreme hot and cold ambiences indicating higher modulation. Among calves, heifers and cows, per cent variations were highest in overall values of cows during all the ambiences followed by heifers and calves. Extent of this pattern was greater, respectively during rainy ambience. These patterns indicated that cows exhibited maximum modulations in serum 5'NT activity and rainy ambience had greater impact on all the types of animals. Pattern of per cent variation revealed that among calves, pre-ruminant revealed maximum modulations. Post-pubertals were affected most and among cows, nonpregnant milch and primipara divulged greater effects.

S.	Effects	Mean ± SEM values during ambiences					
No.		Moderate	Extreme	Rainy	Extreme		
			hot		cold		
1.	Ambience Overall values (300)	58.00b ±0.40	80.00b ±0.43	91.00b ±0.45	70.00b ±0.41		
2.	Age group categorization (I, II &	ge group categorization (I, II & III categories)					
I.	Calves, 2-48 weeks (150), categorization as a, b, c, d & e						
	Overall values of calves (150)	56.00bg±0.10	75.00bg±0.11	86.00bg±0.12	66.00bg±0.10		
	Pre-ruminant (30)	58.00bc±0.05	81.00bc±0.06	92.00bc±0.06	70.60bc±0.06		
	Transitional (30)	57.00bc±0.04	78.00bc±0.05	89.00bc±0.05	68.00bc±0.06		
	Pre-weaning (30)	56.00bc±0.05	75.00bc±0.04	86.00bc±0.05	66.00bc±0.05		
	Post -weaning (30)	55.00bc±0.04	72.00bc±0.05	83.00bc±0.05	64.00bc±0.05		
	Calf-yearling transition (30)	54.00bc±0.04	69.00bc±0.04	80.00bc±0.05	62.00bc±0.04		
II.	Heifers, 1-3.5 years (60), categorization as a & b						
	Overall values of heifers (60)	58.00bg±0.10	80.00bg±0.10	91.00bg±0.12	70.00bg±0.10		
	Pre-pubertal (30)	57.00bd±0.08	78.00bd±0.08	89.00bd±0.08	68.00bd±0.08		
	Post-pubertal (30)	59.00bd±0.08	82.00bd±0.07	93.00bd±0.08	72.00bd±0.08		
III.	Cows, 3.5-12 years (90), categorization as group A & B						
	Overall values of cows (90)	60.00bg±0.11	85.00bg±0.10	96.00bg±0.10	74.00bg±0.13		
	Group A (90), Physiological states: Pregnancy and milch status						
	Non-pregnant milch (30)	58.00be±0.08	83.00be±0.09	94.00be±0.09	72.00be±0.07		
	Pregnant milch (30)	60.00 be±0.09	85.00 be±0.08	96.00 be±0.08	74.00 be±0.08		
	Pregnant dry (30)	62.00be±0.08	88.00be±0.09	99.00be±0.09	76.00be±0.07		
	Group B (90), Physiological states: Parity						
	Primipara (45)	59.00 bf±0.09	83.00 bf±0.08	94.00 bf±0.09	72.00 bf±0.08		
	Multipara (45)	61.00 bf±0.08	87.00 bf±0.07	98.00 bf±0.08	76.00 bf±0.09		

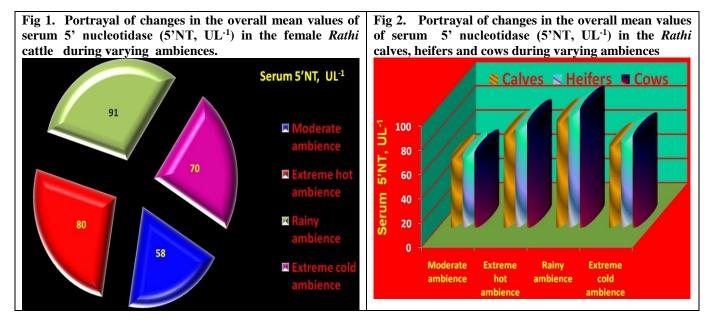
1. Figures in the parenthesis = Number of *Rathi* animals

- 2. 'b' = Significant ($p \le 0.05$) differences among mean values for a row.
- 3. 'c' = Significant ($p \le 0.05$) differences among mean values of calves for an ambience
- 4. 'd' = Significant ($p \le 0.05$) differences between mean values of heifers for an ambience
- 5. 'e' = Significant (p≤0.05) differences among mean values of Group A for an ambience
- 6. 'f' = Significant (p≤0.05) differences between mean values of Group B for an ambience
- 7. 'g' = Significant (p≤0.05) differences among overall values of calves, heifers and cows for an ambience

S.No.	Effects	Per cent variations				
		Extreme	Rainy	Extreme cold		
		hot				
1.	Ambience OverallVariations (300)	+37.93	+56.89	+20.68		
2.	Age group categorization (I, II & III categories)					
I.	Calves, 2-48 weeks (150), categorization as a,b,c,d&e					
	Overall values of calves (150)	+33.92	+53.57	+17.85		
a.	Pre-ruminant (30)	+39.65	+58.62	+20.68		
b.	Transitional (30)	+36.84	+56.14	+19.29		
c.	Pre-weaning8(30)	+33.92	+53.57	+17.85		
d.	Post -weaning (30)	+30.90	+50.90	+16.36		
e.	Calf-yearling transition (30)	+27.77	+48.14	+14.81		
II.	Heifers, 1-3.5 years (60), categorization as a&b					
	Overall values of heifers (60)	+37.93	+56.89	+20.68		
a.	Pre-pubertal (30)	+36.84	+56.14	+19.29		
b.	Post-pubertal (30)	+38.98	+57.62	+22.03		
III.	Cows, 3.5-12 years (90), categorization as group A & B					
	Overall values of cows (90)	+41.66	+60.00	+23.23		
	Group A (90), Physiological states: Pregnancy a	and milch status				
a.	Non-pregnant milch (30)	+43.10	+60.34	+24.13		
b.	Pregnant milch (30)	+41.66	+60.00	+23.33		
c.	Pregnant dry (30)	+41.93	+59.67	+22.58		
	Group B (90), Physiological states: Parity					
a.	Primipara (45)	+68.00	+88.00	+44.00		
b.	Multipara(45)	+42.62	+60.65	+24.59		

Table 2. Per cent variations in the serum 5' nucleotid	ase mean values in the female Rathi cattle during extreme hot,				
rainy and extreme cold in comparison to moderate ambience					

+= Increase in the value from respective moderate ambience mean value



CONCLUSION

Extreme ambiences influenced the *Rathi* animals of all physiological states in terms of serum 5'NT activity. Scale of stimulation was different. It can be inferred that extreme ambiences had a propensity to alter liver

functions in the *Rathi* animals, which appeared in the form of altered levels of 5'NT. Utmost impact was observed during rainy ambience. Temperature humidity index values were observed to be maximum during rainy ambience. Brunt of rainy ambience was maximum on cows. Interactions among animals of various physiological states were significant for serum 5'NT activity.

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CONFLICT OF INTEREST

No interest

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