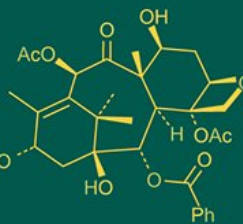
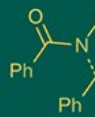


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## Impact of seasonal transitions on the hematological panel of indigenous Nagavali sheep

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### Abstract

The Nagavali sheep is a recently recognized breed native to the North Coastal region of Andhra Pradesh. Screening various serum parameters serves as a valuable tool to advance small ruminant research. This study spanned three seasons (summer, monsoon, and winter) with blood samples collected from 100 sheep of both sexes during each period. The hematological indices, including Packed cell volume (PCV/hematocrit), Mean corpuscular volume (MCV), and Mean corpuscular hemoglobin (MCH), showed significantly higher levels in summer compared to the monsoon and winter seasons across rams, ewes, and the total herd. This elevation is hypothesized to be an adaptive mechanism during summer to maintain hydration and aid in thermoregulation. Conversely, substantially lower Mean corpuscular hemoglobin concentration (MCHC) levels were observed in summer than in the monsoon and winter seasons. Regarding leukocyte differentials, the percentage of lymphocytes in rams and the overall herd showed no significant seasonal variation; however, ewes exhibited markedly higher lymphocyte levels in summer compared to winter, though values remained within the normal physiological range. Monocyte percentages were significantly higher in winter compared to summer and monsoon across rams, ewes, and the total herd. Among granulocytes, neutrophils exhibited no significant seasonal differences, whereas lower eosinophil percentages were recorded in summer compared to the other two seasons.

**Key words:** Nagavali sheep - haematological parameters - season - THI

### Introduction

Sheep is an agriculturally important species in India, making a significant contribution to food, fibre, rural employment and GDP. Andhra Pradesh is second in sheep population in India, with a population of 17.6 million (20th Livestock census, 2020). Nagavali sheep is widely renowned for its resilience and is found in north coastal districts and represents about 4.5% of Andhra Pradesh's total sheep population. It is mostly reared for the purpose of meat. Climate change occurring across the world is posing major challenge to animal husbandry especially in third world countries. Climate parameters such as air temperature, relative humidity, direct and indirect sunlight and wind speed shows impact on feed and water availability, fodder quality and disease incidence and THI levels gradually increase from January, peak in June or July, and then decrease until December. Rising Temperature-humidity Index (THI) levels can adversely effect the health of the animal by increasing the physiological parameters of rectal temperature (RT), respiration rate (RR), and pulse rate (PR) in Munjal sheep reared in a subtropical climate (Kumar S *et al.*, 2025) <sup>[2]</sup>. Among these environmental factors, variations in ambient temperature have the greatest influence on livestock productivity and animal welfare (Rathwa *et al.*, 2016) <sup>[1]</sup>. Continuous heat stress impairs animal development, milk and meat production and reproduction. The present study was therefore designed to establish a Comprehensive haematological profile which can serve as a base line data and also to compare the changes in these parameters over different seasons in rams and ewes, with an objective to assess the impact of climate change on serum biochemical profiles in Nagavali sheep.

## Materials and Methods

The present investigation was carried out Nagavali sheep of Andhra Pradesh maintained at Livestock Research Station, College of Veterinary Science, Garividi, S.V.V.U, Vizianagaram, Andhra Pradesh. The geographical coordinates of the study area of the study area are Latitude: 18.28946° N (18° 17' 22" N) Longitude: 83.55393° E (83° 33' 14" E). Blood collection was done in the months of May, August and December for the seasons Summer, Monsoon and Winter respectively.

## Experimental design

A total of 100 apparently healthy Nagavali sheep aged one year and above with similar physiological status were randomly selected for the current study at the Livestock Research Station, College of Veterinary Science, Garividi, SVVU, Vizianagaram district. These animals were divided into 2 groups of 50 (on gender basis) each; Group-1(Ewes) and Group- 2(Rams)

## Chemicals, glassware and equipment

High-grade reagents (HiMedia, Thermo Fisher, Sigma-Aldrich) and standard consumables (Borosil, Tarsons) were used. Centrifugation and spectrophotometry were performed via a REMI RM-12C and a Thermo Scientific Multiskan Sky, respectively.

## Temperature-humidity index (THI)

Daily microclimatic data including maximum, minimum, dry-bulb Temperature {db}, and wet-bulb Temperature {wb} alongside relative humidity were recorded at 1:00 PM

using a digital thermohygrometer. The Temperature-Humidity Index (THI) was calculated according to Johnson (1965):  $\{THI\} = 0.72(T_{db}) + T_{wb} + 40.6$

## Blood sample collection

Jugular blood samples (5 mL) were collected seasonally (summer, monsoon, winter) between 07:30 and 08:30 h. Samples were processed into whole blood K3-EDTA and serum (separated at 5,000 rpm for 5 min) before storage at -20°C.

## Statistical analysis

Results are expressed as mean  $\pm$  standard error (SE). Data were analyzed using a Student's t-test in Microsoft Excel 2007 following the statistical methods described by Snedecor and Cochran (1989) [3].

## Results and discussion

### Temperature-Humidity Index (THI)

The mean values of Temperature Humidity Index (THI) prevailing during the experimental period are presented in Table No.1. The mean temperature humidity index in summer was 80.01. In monsoon, the mean temperature humidity index recorded was 70.5. In winter, the mean temperature humidity index recorded was 67.89. The most significant factor impacting the growth performance of indigenous breeds of sheep is thermal stress (heat stress), especially in tropical, subtropical, and arid regions. While indigenous breeds generally possess superior natural adaptation to harsh local conditions compared to exotic breeds, prolonged or severe heat stress still negatively affects their growth.

**Table 1:** Average Temperature-Humidity Index (THI) observed during Summer, Monsoon and Winter seasons of the experimental period

| Parameter | Seasons          |                  |                  |
|-----------|------------------|------------------|------------------|
|           | Summer           | Monsoon          | Winter           |
| THI       | 80.02 $\pm$ 0.43 | 70.51 $\pm$ 0.41 | 67.89 $\pm$ 0.27 |

$P \leq 0.05$ : Significant

Our results were in agreement with Abdelatif and Alameen (2012) and Tej *et al.* (2018) where higher THI values were observed during summer season. This high THI indicates a significant risk of heat stress, especially for livestock. Sheep and goats are thought to cope with extreme climatic conditions, especially high temperatures (Sevi and Caroprese, 2012) [5]. However, Finocchiaro *et al.* (2005) [6] observed an effect of high temperature-humidity index (THI) values in dairy sheep. One approach for studying animal responses to heat stress is to study the correlation between bioclimatic indicators such as THI and production and reproduction characteristics (Serradilla *et al.*, 2018). The THI value of 72 and below was considered as lack of heat stress, 73 to 77 as mild heat stress, 78 to 89 as moderate and above 90 as severe heat stress (Srikandakumar *et al.*, 2003) [8]. The current work's findings indicate that the summer season of the observation period had moderate heat stress. In the current study, the other two seasons, monsoon and winter, were spared of heat stress, as suggested by Silanikove's (2000) [9] findings, who reported that a THI score of 70 or less was considered comfortable.

## Total erythrocyte count (TEC)

Our findings agree with Nedeva *et al.*, (2019) [10], who found that seasonal changes did not significantly affect total

erythrocyte count (TEC) in high-producing Lacaune sheep. Iyiola-Tunji *et al.*, (2015) [11] similarly reported no major differences in TEC between the dry and wet seasons in Nigerian lambs. Although our study found no statistically significant differences, TEC values during the summer and monsoon were slightly higher than in winter. Fadare *et al.*, (2012) [12] also observed higher red blood cell counts in sheep, which can be linked to higher oxygen demands during heat stress. This minor elevation in summer may stem from increased oxygen demands during panting, or from dehydration leading to hemoconcentration, as noted by Chaudhary *et al.*, (2015) [13] and Rathwa *et al.*, (2016) [1]. In contrast, Selvaraj *et al.*, (2004) [14] and Banerjee *et al.*, (2015) [15] reported higher red blood cell concentrations in sheep and goats during the winter season.

## Total leucocyte count / white blood cell count

In the present study, the mean values of TLC (103/ $\mu$ l) had no significant difference among summer, monsoon and winter seasons in ewes, whereas in rams, significantly higher TLC values were recorded in summer when compared to winter. Similar trend was observed in the total herd mean values of sheep where summer values were significantly higher than winter. In the current study, TLC levels were significantly higher in summer compared to

winter in rams and in total herd. Similar findings were reported by Suhair and Abdalla (2013) <sup>[16]</sup> who evaluated the influence of seasonal changes and shearing on the regulation of body temperature and blood components in desert rams. They observed higher count of WBC during high ambient temperature in sheep. However, lower TLC values were recorded during high ambient temperature by Wojtas *et al.*, (2014) <sup>[17]</sup> in sheep and Banerjee *et al.*, (2015) <sup>[15]</sup> in goats. Ghosh *et al.*, (2013) <sup>[18]</sup> reported contradictory results where TLC counts were increased during winter season in goats. In contrary, Nafisat *et al.*, (2021) <sup>[19, 31]</sup> who conducted a study

to assess the effect of breed, sex, age and season on the haematological parameters revealed higher TLC values in wet season than summer in the sheep of Bauchi state, Nigeria. In the current study, the higher WBC count in the summer relative to the winter season was mostly due to the hot season. As reported by Okab *et al.*, (2008) <sup>[20]</sup> hot climatic conditions which are amiable for insect vectors may attribute to sub clinical infections. These infections in hot seasons may contribute to blood viscosity which results in leucocytosis.

**Table 2:** Effect of Season on Haematological Parameters of Rams and Ewes (Mean  $\pm$  SE)

| Parameter                        | Rams                      |                            |                           | Ewes                      |                            |                           |
|----------------------------------|---------------------------|----------------------------|---------------------------|---------------------------|----------------------------|---------------------------|
|                                  | Summer<br>(Mean $\pm$ SE) | Monsoon<br>(Mean $\pm$ SE) | Winter<br>(Mean $\pm$ SE) | Summer<br>(Mean $\pm$ SE) | Monsoon<br>(Mean $\pm$ SE) | Winter<br>(Mean $\pm$ SE) |
| TEC (106/ $\mu$ l)               | 10.75 $\pm$ 0.19a         | 10.46 $\pm$ 0.23a          | 10.58 $\pm$ 0.25a         | 10.64 $\pm$ 0.18a         | 11.09 $\pm$ 0.19a          | 10.63 $\pm$ 0.21a         |
| TLC (103/ $\mu$ l)               | 7.62 $\pm$ 0.10a          | 7.35 $\pm$ 0.13ab          | 6.91 $\pm$ 0.17b          | 7.78 $\pm$ 0.12a          | 7.61 $\pm$ 0.12a           | 7.72 $\pm$ 0.10a          |
| Haemoglobin concentration (g/dl) | 10.09 $\pm$ 0.14a         | 9.60 $\pm$ 0.10b           | 9.35 $\pm$ 0.11b          | 9.70 $\pm$ 0.13a          | 9.44 $\pm$ 0.10a           | 9.66 $\pm$ 0.11a          |
| Packed Cell Volume (%)           | 37.76 $\pm$ 0.53a         | 30.56 $\pm$ 0.44b          | 30.84 $\pm$ 0.64b         | 36.18 $\pm$ 0.63a         | 31.18 $\pm$ 0.47b          | 34.44 $\pm$ 0.62a         |
| MCV (fL)                         | 36.59 $\pm$ 0.93a         | 30.30 $\pm$ 1.18b          | 29.22 $\pm$ 0.85b         | 34.68 $\pm$ 0.99a         | 28.50 $\pm$ 0.64b          | 32.82 $\pm$ 0.83a         |
| MCH (pg)                         | 9.70 $\pm$ 0.17a          | 9.50 $\pm$ 0.34ab          | 8.85 $\pm$ 0.20b          | 9.23 $\pm$ 0.14a          | 8.62 $\pm$ 0.16b           | 9.20 $\pm$ 0.19a          |
| MCHC (g/dl)                      | 27.01 $\pm$ 0.55a         | 31.64 $\pm$ 0.48b          | 30.69 $\pm$ 0.43b         | 27.16 $\pm$ 0.55a         | 30.44 $\pm$ 0.34b          | 28.30 $\pm$ 0.32a         |
| Lymphocytes(%)                   | 38.76 $\pm$ 0.97a         | 40.78 $\pm$ 0.80a          | 40.20 $\pm$ 0.96a         | 41.30 $\pm$ 0.71a         | 39.30 $\pm$ 0.66ab         | 38.86 $\pm$ 0.64b         |
| Monocytes                        | 5.14 $\pm$ 0.50ab         | 3.68 $\pm$ 0.32a           | 5.92 $\pm$ 0.50b          | 4.96 $\pm$ 0.34a          | 3.94 $\pm$ 0.3             | 6.54 $\pm$ 0.46b          |
| Neutrophils                      | 52.82 $\pm$ 0.77a         | 51.90 $\pm$ 0.77a          | 52.00 $\pm$ 0.84a         | 50.26 $\pm$ 0.74a         | 52.24 $\pm$ 0.75a          | 51.60 $\pm$ 0.66a         |
| Eosinophils                      | 1.90 $\pm$ 0.27a          | 3.64 $\pm$ 0.35b           | 3.36 $\pm$ 0.32b          | 3.08 $\pm$ 0.36a          | 4.58 $\pm$ 0.35b           | 3.64 $\pm$ 0.36ab         |

$P \leq 0.05$ : Significant Means with similar superscripts within a row do not differ significantly

### Haemoglobin

In the present study, in ewes, the mean values of Haemoglobin concentration were not significantly different among summer, winter and monsoon seasons. Rams showed significantly higher mean Haemoglobin concentration in summer than monsoon and winter. The present study results are in accordance with the findings of Rathwa *et al.* (2016) <sup>[1]</sup> who conducted a research to evaluate haematological and physiological parameters in indigenous sheep during summer and winter seasons and reported significantly higher Haemoglobin concentration in sheep in summer season than in winter season. Similar variations in haemoglobin concentration was also reported by Sejian *et al.*, (2012) <sup>[21]</sup> in a study conducted to determine the effect of multiple stressors on growth and adaptive capability of Malpura ewes under semi-arid tropical environment. Indu *et al.* (2014) <sup>[22]</sup> reported that the haemoglobin content in heat stress group was significantly higher than the control group. Suhair and Abdalla (2013) <sup>[16]</sup>, on contrary, reported a drop in Hb content in sheep at high ambient temperatures. Banerjee *et al.*, (2015) <sup>[15]</sup> noted a marked increase in Haemoglobin concentration during winter season compared to summer in goats. Increased haemoglobin levels in summer season observed in this research might be attributed to the rise in environmental temperature. As suggested by Al-Eissa (2011) enhanced blood oxygen carrying capacity during the summer season alters erythrocyte counts and haemoglobin concentrations. Another cause of elevated Hb levels in sheep might be dehydration, which causes hemoconcentration during heat stress.

### Packed cell volume/ hematocrit

In the present study, in ewes, mean PCV (%) values were significantly higher during summer than in monsoon. Rams

showed significantly higher PCV levels in summer when compared to monsoon and winter. Significantly different PCV values were observed among summer, monsoon and winter seasons in the total herd of sheep with highest values in summer.

Our packed cell volume (PCV) results align with Sejian *et al.* (2012) <sup>[21]</sup>, who studied Malpura ewes under semi-arid conditions, and Indu *et al.* (2014) <sup>[22]</sup>, who observed significantly higher PCV levels in heat-stressed groups compared controls. Rathwa *et al.* (2016) <sup>[1]</sup> also reported significantly higher mean PCV values during summer than winter in indigenous sheep, a trend similarly observed by Kumar *et al.* (2011) in Beetal goats and Babe *et al.*, (2015) <sup>[24]</sup> in Iraqi black goats. Conversely, Suhair and Abdalla (2013) found lower PCV levels in sheep at high temperatures, and Banerjee *et al.* (2015) <sup>[15]</sup> noted higher winter PCV levels in goats. The elevated PCV readings during summer in our study are likely driven by higher ambient temperatures causing dehydration and hemoconcentration, or by an increased red blood cell count. This rise in PCV acts as an adaptive mechanism, helping supply the water needed for evaporation and body cooling, as noted by El-Nouty *et al.*, (1990).

### Mean corpuscular volume

In the current study, the mean MCV (fL) values of ewes were significantly higher in summer than monsoon and there was no significant difference between summer and winter values. Mean values of MCV were significantly higher in summer when compared to monsoon and winter. Similar trend was observed in the total herd mean values of MCV in the Vizianagaram sheep where summer mean values were significantly higher than in monsoon and winter.

The findings of the present study were in conformation with Iyiola-Tunji *et al.*, (2015) <sup>[11]</sup> who studied the effect of season on haematological parameters of lambs and recorded higher MCV values in the early dry season compared to early wet season. Similar pattern of values were reported by Tej *et al.*, (2018) who evaluated seasonal variation in crossbred female calves and reported non-significant higher MCV values in summer than in winter. Tej and co-workers attributed these higher MCV values to the superior adaptive capability of the calves. However, Abdelatif *et al.*, (2009) <sup>[4]</sup> observed an increased level of MCV in Nubian goats during winter compared to the dry and wet seasons.

### Mean corpuscular haemoglobin

In the present study, MCH (pg) levels of ewes in summer were significantly higher when compared to monsoon. The mean values of MCH were significantly higher in summer than in winter in rams. The current study values were in accordance with Iyiola-Tunji *et al.*, (2015) <sup>[11]</sup> who studied the effect of season on haematological profiles of lambs in Northern Guinea Savannah Zone of Nigeria and revealed that MCH values were higher in the early dry season than in the early wet season. Similar findings were observed by Al-Saeed *et al.*, (2009) <sup>[25, 28]</sup> who evaluated the seasonal variation of blood parameters in local cattle and discovered higher MCH levels in the summer than the winter season, which they attributed to high erythrocyte-stimulating factor release, which relieves the animal from internal heat induced by temperature stress. However, Abdelatif *et al.* (2009) <sup>[4]</sup> observed a higher level of MCH during the winter season compared to dry and wet summer in Nubian sheep.

### Mean corpuscular haemoglobin concentration

In the present study, the mean values of MCHC in ewes were significantly higher in monsoon than summer and winter. In rams, significantly lower MCHC values were observed in summer when compared to monsoon and winter. Significant difference was observed between the MCHC values of total herd of Vizianagaram sheep among summer, monsoon and winter.

The current study findings were in accordance with Al-Haidary *et al.*, (2004) who investigated the haematological responses of Naimey sheep to heat stress in semi-arid conditions and reported a decrease in MCHC value during summer season. In contrary, higher MCHC values observed during summer season were reported by Kumar and Pauchauri (2000) <sup>[27]</sup> who evaluated the haematological profile of crossbred dairy cattle to monitor herd health status at medium elevation in Central Himalayas.

### Lymphocyte (%)

In this study, ewes showed significantly higher lymphocyte percentages in summer than in winter. However, lymphocyte levels in rams and the total herd did not change significantly across the summer, monsoon, and winter seasons. These findings align with Nafisat *et al.*, (2021) <sup>[19, 31]</sup>, who observed higher lymphocyte counts in sheep during the dry season. A similar non-significant increase in summer lymphocytes was noted in Vizianagaram sheep, matching outcomes reported by Al-Saeed *et al.*, (2009) <sup>[25, 28]</sup> in local cattle. This slight rise in lymphocytes during summer may be attributed to mild, subclinical infections in apparently healthy sheep.

### Monocyte (%)

In the current study, significantly higher percentages of monocytes were observed in winter than in monsoon and summer seasons in ewes. In rams, significantly higher levels of Monocytes (%) were noticed in winter when compared to monsoon. There was a significant difference between the levels of monocyte in the total herd of sheep among summer, monsoon and winter seasons, with the highest values in winter. In accordance with the present study, Naik *et al.*, (2013) <sup>[29]</sup> reported higher mean monocyte in Punganur cattle in winter compared to summer season. In contrast to the present study the findings of Giri *et al.*, (2017) <sup>[30]</sup> revealed higher monocyte in summer than in winter season.

### Granulocytes (%)

Neutrophil percentages in rams, ewes, and the total herd showed no significant variations across the summer, monsoon, and winter seasons, though a non-significant trend toward higher values was observed during the monsoon. These findings align with Nafisat *et al.* (2021) <sup>[19, 31]</sup>, who reported significantly higher mean neutrophil percentages during the rainy season compared to the dry season in sheep. Conversely, eosinophil percentages demonstrated clear seasonal differences, being significantly higher in the monsoon than in the summer. Both rams and the total herd exhibited their lowest eosinophil percentages during the summer season compared to both the monsoon and winter periods.

The granulocyte percentages recorded in this study align with the findings of Aengwanich *et al.* (2009) <sup>[32]</sup> regarding seasonal variations in crossbred beef cattle. Furthermore, the elevated eosinophil percentages observed during the monsoon season can be attributed to a higher incidence of parasitic infections typical of heavy rainy periods, which provoke allergic immune responses and stimulate increased eosinophil production..

### Conclusion

Our Temperature-Humidity Index (THI) data indicate that the summer season imposed moderate heat stress on the sheep. While total erythrocyte count (TEC) showed no significant seasonal variation, white blood cell (WBC) counts were higher in rams and the total herd during summer and monsoon, likely due to subclinical infections prevalent in hot conditions. Hemoglobin levels remained stable in ewes but showed elevated means in summer for rams and the total herd, pointing toward heat-stress-induced dehydration and haemoconcentration. Furthermore, hematological indices such as packed cell volume (PCV/hematocrit), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were significantly higher in summer across all groups, acting as an adaptive mechanism to supply necessary water for thermoregulation and evaporative cooling. Conversely, mean corpuscular hemoglobin concentration (MCHC) dropped substantially during summer. Leukocyte differentials revealed that lymphocyte percentages remained unchanged in rams and the overall herd across seasons, though ewes exhibited higher summer values within normal physiological limits. Monocyte percentages peaked significantly in winter, while neutrophils remained constant and eosinophils dipped during summer. Ultimately, hematological parameters showed no significant differences between the rainy and

winter seasons, with major variations concentrated in summer. This divergence highlights the physiological allostasis driven by summer heat stress and underscores the adaptive capacity of the animals to harsh climatic shifts and seasonally exacerbated pathogens.

#### author's contribution

Sai Srinivas and Gangu Naidu performed experiments and analysis, K Aswani kumar, A Sampath Ganesh and T Srinivasa rao conceptualized and organized the research.

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#### ethical statement

In the present study, saliva samples were collected under the approval of SVVU Institutional Animal Ethics Committee (281/go/ReBi/S/2000/CPCSEA/CVSc/TPTY/010).

#### Declaration of competing interest

The authors declare no competing or financial interests

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