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LA Pangaonkar
PhD Scholar, Department of Veterinary Physiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

VR Patodkar
Professor and Head, Department of Veterinary Physiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

PV Mehere
Assistant Professor, Department of Veterinary Physiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

MB Amle
Professor and Head, Department of Animal Reproduction Gynaecology and Obstetrics, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

AV Khanvilkar
Associate Professor and Head, Department of Livestock Production and Management, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

SB Lambate
Assistant Professor, Department of Veterinary Anatomy and Histology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

Corresponding Author:
PV Mehere
Assistant Professor, Department of Veterinary Physiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

Seasonal variations in the levels of plasma copper and zinc in Deccani sheep

LA Pangaonkar, VR Patodkar, PV Mehere, MB Amle, AV Khanvilkar and SB Lambate

Abstract
The present investigation was carried out to study the seasonal variations on plasma copper and zinc during the summer and rainy season in Deccani sheep. Plasma copper and zinc were were analyzed by Inductively Coupled Plasma Atomic Emission Spectroscopy. Statistical analysis of plasma copper during the summer and rainy season indicated non-significant results with numerically higher values in summer (1.20 ± 0.15) than rainy season (0.94 ± 0.06) whereas, significantly (P<0.01) higher levels of plasma zinc were reported during summer (1.81 ± 0.13) than rainy (1.10 ± 0.07) season. It was concluded that seasonal variation was reflected by a positive correlation with THI in the levels of plasma copper and zinc.

Keywords: THI, season, plasma copper and zinc, Deccani sheep

1. Introduction
India is that the third largest country within the sheep population contributory to 5.7% globally. The sheep population in India is 74.26 million raised by 14.1% over the previous nineteenth livestock census of 2012 that contributes to 3.8% within the total Indian livestock population. In geographic region of Maharashtra, sheep population is 2.7 million that is raised by 3.87% over the previous nineteenth livestock census of 2012 [1]. Environmental factors like ambient temperature, radiation and humidity have direct and indirect effects on animals [2]. Sheep are less heat tolerant than goats as they have shorter bodies and legs, short and thick ears, tight skin, and dense fleeces/wool [3]. Copper is a mineral element that activates several enzyme systems, and though in fewer numbers than Zn, it is considered an essential nutrient [4]. The physiological role of Cu in the organism is related to several functions, which include cellular respiration, bone formation, connective tissue development, and essential catalytic cofactor of some metallo-enzymes, among others [5]. The need for Zn by most animals is based on its influence on enzymes and proteins and their activities, which are linked to vitamin A synthesis, carbon dioxide (CO_2) transport, collagen fiber degradation, free radical destruction, membrane stability of red blood cells, metabolism of essential fatty acids, carbohydrate metabolism, protein synthesis, metabolism of nucleic acids, among others [6, 7]. The mineral level in the animal body depends on a large number of factors such as species, breed, age, sex, nutritional and health status, mineral supplementation, seasonal and physiological variations [8]. The seasonal variation of plasma copper and zinc in Deccani sheep has not been studied yet, hence it was proposed to investigate the effect of THI on the levels of plasma copper and zinc in Deccani sheep during the summer and rainy season.

2. Materials and Methods
The present investigation was carried out in the Department of Veterinary Physiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal-412801, Dist. Satara and Sophisticated Instrument Facility (SAIF) of Indian Institute of Technology, Powai, Mumbai - 400076.

2.1. Experimental period
The experiment was conducted during Summer (March, April and May) and Rainy (July, August and September) seasons the year 2019.

2.2 Experimental Animals and collection of samples
The experiment was conducted on Eight (08) healthy, nonpregnant, nonlactating adult Deccani sheep above two years of age, having similar body weight, maintained under loose housing condition and kept under similar managemental and nutritional regimen throughout period of study at Livestock Instructional and Research Demonstration Farm, KNP College of Veterinary Science, Shirwal. Dist. Satara. The whole blood samples (06 ml each) were collected aseptically from experimental animals by jugular veinipuncture containing K3EDTA vacutainers during Summer (March, April, May) and Rainy season (July, August and September) fortnightly.

2.3 Ethical permission
The protocol of this experiment was approved by the institutional Animal Ethics Committee of KNP College of Veterinary Science, Shirwal. Dist. Satara vide No. IAEC/15/KNPCVS/01/2019.

2.4 Temperature Humidity Index
The meteorological data available at IMD, Pune like temperature and relative humidity were used for the calculation of temperature humidity index (THI) and based on mean temperature and relative humidity, THI was calculated using the below mentioned formula [9].

$$THI = (0.8 \times Tdb) + [(RH/100) \times (Tdb-14.4)] + 46.4$$

Where, Tdb = dry bulb temperature and RH = relative humidity.

2.5. Analysis of Copper and Zinc
The whole blood samples collected as per standard procedure were centrifuged at 3000 rpm for 15 minutes and blood plasma was separated and stored at -20 ºC until analysis. One ml plasma sample was taken in a 25 ml volumetric flask, to which 5 ml of the di-acid mixture was added. The di-acid mixture contained 70% perchloric acid (one part) and concentrated nitric acid (four parts). The contents of the volumetric flask were boiled gently for 30-45 minutes to oxidize all the easily oxidizable material. The solution was boiled until it became nearly colorless. The precaution was taken while heating the solution not allowing it to go to dryness. The solution was cooled to which some distilled water was added. The solution was then diluted to 25 ml with deionized water. The prepared aliquot was used for the analysis of the minerals under study [10]. The prepared aliquot was stored at room temperature and analyzed for plasma copper and plasma zinc by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICPAES) at sophisticated Instrument Facility (SAIF) of Indian Institute of Technology, Powai, Mumbai -400076.

2.6. Statistical analysis
All the data of the present study was analyzed using computerized Web based Agricultural Statistics Software Package, WASP. 2.0 by applying a completely randomized design and simple correlation [11].

3. Results and Discussion
3.1 Temperature and Humidity Index: The (THI) has been widely used as an indicator of thermal stress in livestock and it involves both ambient temperature (wet/dry bulb) and relative humidity which is universally used as a heat stress index for livestock production [12]. A THI value of 70 or less is considered comfortable, 75-78 stressful and values greater than 78 cause extreme distress and the animals are unable to maintain the thermoregulatory mechanism or the normal body temperature [13]. Therefore in the present study, the values of THI indicated that Deccani sheep were in severe stress in the summer season than the rainy season. The home tract of Deccani sheep is the western region of Maharashtra especially Solapur, Pune, Satara, Sangli and Kolhapur districts with an average temperature of 40 ^{0}C, and it is common to increase upto 43 ^{0}C in the summer season. The meteorological data recorded during the present study were obtained from India Meteorological Department, Pune (MS) and the monthly calculated mean values of THI during the study period revealed the significantly (P<0.01) higher THI during Summer (77.81 ± 0.19) than Rainy season (74.92 ± 0.16). These findings of the present study are in close agreement with [14], who recorded the THI value during the peak summer (month of May), peak rainy (month of August) and peak winter (month of December) seasons as 76.91 ± 0.58, 73.52 ± 0.73 and 69.46 ± 1.02, respectively indicating significantly (P<0.01) higher THI during Summer than other seasons at Satara districts of Maharashtra. Other researchers also recorded THI during the summer and winter season (82.55 ± 1.52 and 59.36 ± 2.24) and reported extreme severe heat stress during summer than the winter season[15,16]. Significantly (P<0.05) higher THI values during Pre-monsoon /Summer (81.84 and 51.11 ± 6.15) than monsoon/rainy (78.36 and 22.97 ± 1.01) and post-monsoon/winter season (77.99 and 23.49 ± 0.97) in hot and humid tropics was revealed the increased heat stress during summer than other seasons [17]. Also significantly (P<0.01) higher THI value during hot humid summer season (81.60 – 85.66) than winter season (THI = 61.9 – 71.9) was reported by [18]. The study was conducted to evaluate the effect of different temperature humidity indices and recorded THI for group I (hot dry), group II (hot humid) and group III (comfortable) which were 81.70, 80.30 and 70.00, respectively [19]. The upper critical temperature of caprine species is maintained between 25 ^{0}C and 30 ^{0}C and heat stress occurs when goats are exposed to ambient temperatures above 30 ^{0}C indicating significantly (P<0.05) higher THI values during summer (75.7 ± 2.8) compared to spring season (56.2 ± 2.8) [20]. However, [21] recorded a significantly (P<0.05) higher THI in hot humid (83.01) than hot dry (80.31) seasons.

3.2.1 Plasma Copper: The overall mean value in the present study to plasma copper in Deccani sheep was 1.07 ± 0.08 ppm which is closely (± 0.25) corroborated with the findings of [22, 23] in sheep. The levels of plasma copper reported in the present investigation in Deccani sheep were lower than in sheep, Sangamneri goat and Pandharpuri buffaloe reported by [24, 25, 26], respectively. However, the values of plasma copper recorded in the present study were higher than those reported by [27, 28] in sheep and goats, respectively.

3.2.2 Seasonal variation of plasma copper: The Mean ± SE values of plasma copper during summer and rainy seasons are depicted in Table 1. Statistical analysis of the fortnightly data of plasma copper during summer and rainy season indicated non-significant results with numerically higher values in summer (1.20 ± 0.154 ppm) than in the rainy season (0.947 ± 0.067 ppm). The present findings are corroborated with other

researchers [29, 30, 31] in goats whereas, non-significant difference in blood copper concentration during the dry and wet season in grazing goats was observed by [32]. The accelerated growth rate in wet season increases mineral requirement of animals and so animals mobilizes the mineral from body tissue to meet the increased demands whereas, during the dry period reverse effect is observed by [33]. However, significantly (P<0.05) higher levels of plasma copper during summer than in rainy and winter seasons were observed in Java thin tailed sheep [34], and lactating Kankrej cows which may be attributed to greater assimilation of copper to counteract oxidative stress occurring due to heat stress [35]. On the contrary, significantly (P<0.05) higher levels of plasma copper during winter than summer season was observed in nonlactating goats and sheep which might be due to its rapid distribution and inhibitory effect of Fe^{+} along with the active process of its excretion through faeces and urine in summer [36, 37, 38, 39]. The significantly (P<0.05) higher levels of blood copper during Autumn than Spring and Summer season was reported in sheep and Raeini Cashmere goats [40,41]. The copper concentration was found to be significantly (P<0.01) lower in the spring than fall season in sheep of South Bohemia [42].

3.3.1 Plasma Zinc: The overall mean value in the present study with respect to plasma zinc in Deccani sheep was 1.46 ± 0.08 ppm which is closely (± 0.25) corroborated with the findings of other researchers in sheep, Sangamneri goat and Pandharpuri buffaloes[22,25,26] whereas, the higher levels of blood zinc in Wumen semi-fine wool sheep was reported by[43] than the present study. On the contrary, the plasma zinc value evaluated in the present study was lower than the values observed in sheep [23, 24].

3.3.2 Seasonal variation of plasma zinc: The Mean ± SE values of plasma zinc during the summer and rainy seasons are depicted in Table 1. Statistical analysis of the fortnightly data indicated significantly (P<0.01) higher levels of plasma zinc during summer (1.81 ± 0.13 ppm) than rainy (1.10 ± 0.07 ppm) season. The findings of the present study corroborated with the others in crossbred goats, sheep, ruminant animals and pregnant and non-pregnant Changthangi ewes [29, 33, 44, 45]. The non-significant variation among the season with the numerically higher values of zinc in rainy season than other seasons was observed in sheep and grazing goats by [46, 31] whereas, significantly (P<0.01) higher values of zinc was observed in autumn than spring season in sheep by [47]. On the contrary, significantly (P<0.05) higher serum zinc concentration during spring lactation was than summer lactation was observed by[48]. However, significantly (P<0.05) higher values in winter than other seasons were reported in lactating Kankrej cows and healthy sheep [35, 36, 39].

3.4 Correlation of THI with plasma copper and zinc levels: From the present investigation, the relationship of THI was found to be positively correlated with the levels of plasma copper (r = 0.256) and plasma zinc (r =0.362) at 5% level of significance.

Table 1: Season wise Mean ± S. E. values of Plasma copper and zinc (ppm) in Deccani Sheep (n = 08)

Fortnight	Copper (ppm)		Zinc (ppm)	
	Summer	Rainy	Summer	Rainy
I	0.9	0.86	1.70	0.93
II	1.00	0.99	1.71	1.00
III	1.63	1.11	2.25	1.21
IV	1.44	1.12	2.12	1.24
V	1.02	0.75	1.51	1.12
VI	1.25	0.81	1.58	1.09
Mean ± SE	1.20^{a} ± 0.15	0.94^{a} ± 0.07	1.81^{a} ± 0.13	1.10^{b} ± 0.07

The mean values with different superscripts differ significantly at 1% and 5% level of significance

4. Conclusion: The mean values of plasma copper and zinc during summer and rainy season recorded in the present study were above the critical level (0.6 ppm for copper and 0.8 ppm for zinc) suggested by [49]. The plasma copper levels showed non-significant difference among both season whereas, the plasma zinc levels were significantly (P<0.01) higher during summer than rainy season in Deccani sheep. The lower levels of plasma copper and zinc in rainy season samples might be due to leaching of soils during monsoon [50].

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