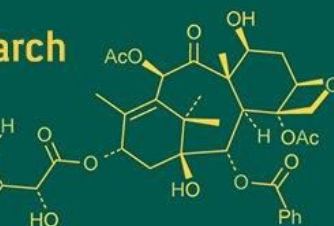


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(7): 688-693
www.biochemjournal.com
Received: 10-05-2025
Accepted: 13-06-2025

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Study of oxidative stress biomarkers and physio-haematological conditions during different seasons in *Sonadi corriedale* sheep

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DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i7Si.4973>

Abstract

The experiments were performed on twelve sheep (*Sonadi corriedale*). The mean body weights of animals were 45.0 Kgs (approx.). Deworming was done for both ecto and endoparasites on regular basis. The animals were maintained in the Sheep shed of Livestock Farm Complex, BASU, Patna. The experiment was carried out for three different seasons from December, 2019 to June, 2020 includes winter, spring and summer season. The mean significant value of RBC ($10^6/\text{mm}^3$) blood hemoglobin (g/dl) and Packed Cell volume (%) did not observed any significant different was observed in blood hemoglobin (g/dl) of animal during season's winter, spring and summer. The markers of oxidative profile such as Total antioxidant capacity, SOD, MDA, Catalase and Cortisol in this study were found to increase during the summer season as compared to the rainy season, indicating that *Sonadi corriedale* sheep have experienced elevated oxidative stress in the summer season than in the winter season. Hence, the activities of these oxidative stress markers, which are influenced by THI of the seasons and its up-regulation was maintained during elevated THI in summer than in the winter and spring season, could also be used as a sensitive biomarker of oxidative stress caused due to heat stress in sheep.

Keywords: Oxidative stress biomarkers, THI, Seasons, *Sonadi Corriedale* sheep

1. Introduction

In the last decade, the evaluation of oxidative stress has become increasingly important in ruminant health and animal production as complementary tools in the evaluation of the nutritional and metabolic status of the animals (Choudhary *et al.*, 2018) [8]. Climate change induced heat stress is thus one of the complex factors making sheep management and husbandry challenging in many geographical locations in the world. Within the sheep industry, reproductive wastage is a major challenge throughout the varying breeding landscapes. Reproductive wastage is defined as the early losses of embryos undergoing natural and/or artificial breeding programs. Heat stress affects the maternal reproductive performance in the Merino sheep and also impacts on the wool industry. Sheep provide meat and wool, and contribute to the total gross domestic product (GDP) of the country (Department of Livestock Services, 2014). Relative humidity is high during winter season than summer season, whereas ambient temperature is very high during summer season as compare to winter season. THI is highest during the summer season. Thus, it is considered that the high ambient temperature causes higher THI during summer season which is more stressful as compared to winter. Das *et al.*, (2016) [10] showed a negative correlation between reproduction traits, temperature humidity index (THI) with animals experiencing adverse effects of heat stress when the THI crosses a threshold level greater than 75. The heat stress (THI > 75) and elevated glucocorticoid levels are linked to embryo loss in Merino ewes. The hematological and biochemical reference values are vital to provide baseline screening and diagnosis of diseases (Work, 1996) [42]. Clinical interpretation of laboratory data of hematology and serum biochemistry is easiest and authentic. Data on hematological, physiological parameters and Oxidative stress biomarkers of indigenous sheep in the Indian subcontinent are limited and only few studies were done in domestic animals such as cattle (Mamun *et al.*, 2014) [27], goat (Hossan *et al.*, 2013) [17], and small ruminants (Bhatta *et al.*, 2014 [5] and Choudhary *et al.*, 2018) [8].

However, there is lack of data related to the reference value for hematology and serum chemistry profiles of indigenous sheep of the Indian subcontinent. Hematological and biochemical tests are done regularly for sheep but lack of reference value for these parameters make it complicated to interpret the results.

The changes in the biological functions of sheep due to exposure to heat stress include the depression in feed intake and utilization, disturbance in the metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites (Habeeb *et al.*, 1992^[16] and Marai *et al.*, 2000)^[28]. The thermal environment is a major factor that negatively affects sheep performance. Increased body temperature and respiration rate are the most important signs for heat stress in sheep. The increase in body temperature is associated with marked reduction in feed intake, redistribution in blood flow and changes in endocrine functions that will affect negatively the productive and reproductive performance of the sheep (Eltawill and Narendran, 1990)^[15]. These physiological adjustments are essential to maintain normal body temperature and to prevent hyperthermia (Al-Haidary, 2012; Lowe *et al.*, 2001)^[24].

It is well recognized that Oxidative stress biomarkers and Physio-hematochemical parameters are influenced by several factors including breed, age, reproductive status, housing, starvation, environmental factors, stress, and transportation. These differences suggest the need to establish appropriate physiological baseline values for livestock which could be used in the realistic evaluation of the management practice, nutrition and diagnosis of health condition as well as in determining the physiological status of animals. Therefore, there is urgent demand to address the effect of seasonal variation on Oxidative stress biomarkers, Physio-hemato-logical parameters in sheep.

2. Materials and Methods

2.1 Experimental animals

The experiments were performed on twelve sheep (*Sonadi corriedale*). The mean body weights of animals were 45.0 Kgs (approx.). Deworming was done for both ecto and endoparasites on regular basis. The animals were maintained in the Sheep shed of Livestock Farm Complex, BASU, Patna. All the animals were reared under strict management and proper hygienic condition throughout the period of study. The animals were provided stall feeding along with measured quantity of supplements, and they were allowed for grazing in the University farm land. All the animals were provided ad libitum clean and fresh water. The experiment was carried out for three different seasons from December, 2019 to June, 2020 includes winter, spring and summer season.

2.2 Meteorological parameters

The data on meteorological variables like temperature and relative humidity were obtained from meteorological station, Patna. Temperature Humidity Index (THI) was calculated from dry and wet bulb temperature using following formula (Johnson *et al.* 1963)^[21].

$$THI = 0.72 (Dbt + Wbt) + 40.6$$

Whereas

Dbt = dry bulb temperature in °C

Wbt = wet bulb temperature in °C

2.3 Physiological responses

Rectal temperature (°F) was recorded with a digital thermometer by keeping the thermometer in contact of rectal mucosa for about 2 min. Respiration rate per minute of each animal was recorded by visual observations of inward and outward abdominal movement. Pulse rate per minute was recorded from femoral artery.

2.3.1 Schedule of Blood collection

Blood sample was collected two times in month at 15 days' interval in each season from jugular vein in tube containing anticoagulant EDTA and tube without anticoagulant. Blood with anticoagulant was used for hematological study and blood without anticoagulant was centrifuge at 3000 RPM for 15 minutes to separate the serum. The separated serum was stored at -20 °C till further use.

2.3.2 Haematological estimation in blood

All hematological study was performed within 24 hours of blood collection. The haematological parameters like Haemoglobin, Total erythrocyte count (TEC), Total leucocyte count (TLC) and Packed cell volume (PCV) were recorded. Haemoglobin (Hb) was estimated in gm% by using method as described by Schalm *et al.* (1975)^[34]. Packed Cell Volume was determined by using Wintrobe haematocrit tube as per the standard method described by Jain (1993)^[18] and expressed in terms of percentage (%). Total Erythrocyte Count (TEC) and Total Leucocyte Count (TLC) were enumerated by haemocytometer method as described by Jain (1993)^[18]. TEC was expressed in millions per cubic millimeter (10⁶/cmm) and TLC expressed in thousand per cubic millimeter (10³/cmm).

2.3.4 Hormone estimation: Plasma cortisol level will be determined by EIA method (ELISA reader).

2.4 Oxidative Stress Markers

Blood samples for estimation of oxidative stress parameters were obtained between 08.00 am and 09.00 am to avoid diurnal influences by jugular venipuncture into vacutainers containing EDTA as an anticoagulant. After centrifugation at 3000 RPM for 15 minutes, the supernatant plasma was separated and stored at -20°C to estimate different oxidative stress biomarkers.

2.4.1 Total Antioxidant Capacity (TAC)

Total antioxidant capacity was estimated by OCPC method using commercial kit coral clinical system as per manufacturer's protocol.

2.4.2 Reduced glutathione (GSH)

Reduced glutathione level in serum was estimated using the method given by Prins and Loos (1969)^[31]. Serum sample (0.2 ml) was added to 4 ml of H₂SO₄ and mixed carefully. After 10 min of standing at room temperature, 0.5 ml of tungstate solution was added. The tube was vortexed and then waits for 5 min in order to avoid crust formation on top of the supernatant. The mixture was then centrifuged for 15 min at 2000 rpm at room temperature. After centrifugation, 2 ml of the supernatant was added to 2.5 ml of Tris-buffer;

0.2 ml of DDNB reagent was added and mixed well. Within a minute, absorbance was measured at 412 nm against blank in which 2 ml of distilled water was substituted for the supernatant.

GSH levels were conveniently calculated by using the extinction co-efficient (EC) $13100 \text{ M}^{-1} \text{ cm}^{-3}$ and results are expressed as mM GSH per ml of serum (mM/ml), using the following formula.

$$\text{GSH (mM/ml of serum)} = \frac{\text{OD of test}}{\text{EC}} \times \frac{\text{Total volume of reaction mixture}}{\text{Volume of supernatant added}} \times \text{dilution factor}$$

2.4.3 Superoxide dismutase (SOD) activity

Serum superoxide dismutase activity was measured using the method as described by Madesh and Balasubramanian (1997) [25] with some modifications. In the microliter plate method, the assay mixture in a total volume of 300 μl per well consisted of 120 μl PBS, 10 μl serum sample, 5 μl of 1.25 mM MTT and 15 μl of freshly prepared 1mM pyragallol solution to be added at the end. Sample was replaced with PBS in the blank. After an incubation period of 15 minutes, 150 μl DMSO was added and absorbance was taken in ELISA reader at 570 nm.

The percent inhibition by the presence of SOD was calculated from the reduction of the MTT colour formation as compared to the MTT formazan formed in the absence of SOD which was taken as 100%. One unit of SOD was defined as the amount of protein required to inhibit the MTT reduction by 50%.

$$\text{SOD activity (units/ml)} = 2 \times 100 \times A_T/A_B$$

Where, A_T = Absorbance for test.

And A_B = Absorbance for blank.

2.4.4 Catalase (CAT)

Serum catalase activity was estimated spectrophotometrically as per the method given by Cohen *et al.* (1970) [9]. The reaction commences with the addition of 50 μl of serum to 2.95ml of phosphate buffer- H_2O_2 solution. In the blank, sample was substituted by same amount of PBS. Phosphate buffer- H_2O_2 gives an absorbance of 0.5-0.6 at 240 nm. The decrease in absorbance was measured for every 20 seconds up to 1 minute. Since, a decrease in absorbance of 0.05 at 240 nm corresponds to the disappearance of 3.45 μmoles of H_2O_2 , the units of catalase activity per ml serum was calculated as below. 0.05 change in absorbance = 3.45 μmoles of H_2O_2 disappeared 'A' change in absorbance in 50 μl sample = $3.45 \times A/0.05$. So, change in absorbance in 1 ml sample = $3.45 \times A/(0.05 \times 0.05) = 1380 A$

2.4.5 Lipid peroxide (LPO)

Lipid peroxidation was measured by determining the malondialdehyde (MDA) production using thiobarbituric acid (TBA) as per modified method of Stock and Dormancy (1971) [37] as described by Jain (1988) [19]. 0.2 ml of RBC suspension was suspended in 0.8 ml of phosphate buffer saline (PBS) followed by addition of 0.5 ml of Tri-choloro acetic acid. Mixture was vortexed and incubated for 2 hours at 37 °C. Now suspension was centrifuged at 2000rpm for 15 minutes. 1ml of each supernatant was transferred in to another tube and to this 0.075 ml EDTA and 0.025 ml of

thiobarbituric acid was added. The mixture was heated in a boiling water bath for 15 minutes and then allows cooling at room temperature. Absorbance was measured at 535nm.

Calculation was done using the molar extinction coefficient (EC) of MDA-TBA complex at 535 nm, i.e., $1.56 \times 10^5 \text{ mmol}^{-1} \text{ cm}$. The amount of LPO was expressed as nano-mol of MDA formed per ml of whole blood by employing following formula.

$$\text{MDA produced (n moles/g Hb)} = \frac{\text{OD of test}}{\text{EC}} \times \frac{\text{Total volume of reaction mixture}}{\text{Volume of supernatant added} \times \text{Hb}} \times \text{dilution factor}$$

3. Results and Discussions

3.1 Temperature Humidity Index

The mean value of THI_{max} , THI_{min} and THI avg. of different seasons; summer, spring, and winter were presented in table 1. The mean value of THI_{max} revealed in summer (100.71 ± 0.31) was higher ($p < 0.05$) than spring (76.01 ± 0.32) and winter (72.28 ± 0.39). The mean value of THI_{min} revealed in summer (77.47 ± 0.30) was higher ($p < 0.05$) than spring (63.03 ± 0.31) and winter (52.52 ± 0.32). The mean value for THI avg. in summer (89.09 ± 0.30) was higher ($p < 0.05$) than spring (69.52 ± 0.42) and winter (62.40 ± 1.19).

Temperature humidity index (THI) allows an objective comparison of environmental conditions (EI-Tarabany, 2016) [14]. During experimental period, the values of THI were more than 72 in summer and monsoon seasons, indicating that sheep were environmental stress, while during post-monsoon winter season the THI value was closer to or below 72. In earlier reports THI of 70 or less is considered comfortable, 75-78 is stressful, while greater than 78 shows that animals are under stress and are unable to maintain homeothermy (Banerjee *et al.*, 2015 and Ocheja *et al.*, 2020) [30].

3.2 Physiological Variables

The mean $\pm$ SE of Rectal Temperature (RT), Respiration Rate (RR) and Pulse Rate (PR) in sheep Sonadi corriedale during the different extreme seasons has been presented in table 2. The mean value of the rectal temperature of sheep Sonadi corriedale observed in winter (103.12 ± 0.91) was non-significantly higher than spring (101.74 ± 0.15) and summer (102.05 ± 0.61). The mean values of RR (beat/min) sheep for observed/found presented higher ($p < 0.05$) in summer (37.20 ± 0.14) than winter (26.76 ± 0.63) and spring (24.72 ± 0.27). The mean value of pulse rate of sheep Sonadi corriedale revealed in summer (87.24 ± 0.20) was higher ($p < 0.05$) than spring (85.49 ± 0.28) and winter (84.83 ± 0.44). Physiological responses like Rectal Temperature (RT), Respiration Rate (RR) and Pulse Rate (PR) showed a significant variation between different seasons and found maximum during summer and minimum during winter in this study. In several studies sheep RT was elevated as rise in environmental temperature and they cope up with this by increasing the evaporative cooling mechanism and enhancing RR which ultimately reduce the extra heat load in them (Singh *et al.*, 2016 [36] and Kumar, *et al.*, 2017). According to Devendra (1987) [12] change in metabolism and muscle activity of goats also changes pulsation and respiration rates, increase in heart rate (HR) and PR is attributed to two causes. One is the increase in muscular activity controlling the rate of respiration, at the same time with elevated RR. The second is the reduction in resistance

of peripheral vascular beds Banerjee *et. al.*, (2015) [3]. Similar kind of result was also reported by Rathwa *et. al.*, (2017) [32] in goat during different seasons.

3.3 Hematological Parameters

The mean value for white blood corpuscles (WBC), Red Blood Corpuscles (RBC), Hemoglobin (Hb) and Packed Cell volume (PCV) for winter, spring and summer has been presented in table 3. The mean values of white blood corpuscles observed were higher ($p < 0.05$) in summer (10376.15 ± 21.00) than winter (8759.58 ± 61.00) and spring (8383.33 ± 36.00) respectively. The mean value of red blood corpuscles was observed in summer (3.59 ± 0.12), winter (3.80 ± 0.11) and spring (3.82 ± 0.13). There was no significant difference observed in RBC $10^6/\text{mm}$ of sheep. The mean value of haemoglobin was observed in summer (10.77 ± 0.35), winter (11.63 ± 0.32) and spring (11.48 ± 0.39). No significant difference was observed in blood hemoglobin. The mean value of packed cell volume was observed in summer (32.30 ± 1.06), winter (34.19 ± 1.01) and spring (34.44 ± 1.18) and no significant values were observed in Packed cell volume of animals.

Blood cells could experience sequence of adaptive transformation on exposure to different season's environment and sensitive to temperature variation and acts as key indicator of physiological responses to stressors (Casella *et. al.*, 2013) [6]. The results for hematological parameters in this study were within the reported ranges. The increased Hb and PCV could be due to severe dehydration during heat stress in ewes (Sejian *et. al.*, 2010) [35]. Singh *et. al.*, (2016) [36] reported significant decrease in Hb and PCV in all the three sheep (Chokla, Marwari and Magra) breeds due to heat stress. Our result in this study is in agreement with the findings of Sejian *et. al.*, (2010) [35] and Singh *et. al.*, (2016) [36]. In hot and humid environment decrease in hematological parameter is associated with enhanced water intake due to heat stress Habibu *et. al.* (2017b). Decline in erythrocyte count during summer is associated with decrease of thyroid hormones secretion which is related to declining the process of erythropoiesis Bhatta *et. al.*, (2014) [5].

3.4 Oxidative Stress Markers

The mean value of cortisol, Total Antioxidant Capacity (TCA), Superoxide dismutase, Catalase, Lipid Peroxidation and Glutathione for Sheep Sonadi Corriedale in different seasons is presented in table 4. The value of cortisol (ng/ml) depicted in summer (18.66 ± 0.13) was higher ($p < 0.05$) than spring (15.61 ± 0.32) and winter (14.56 ± 0.10). The value of total antioxidant capacity (mM) was observed in summer (2.47 ± 0.07) was higher ($p < 0.05$) than winter (0.99 ± 0.01) and spring (1.10 ± 0.01). The value of SOD (U/ml) of sheep in summer (86.65 ± 0.86) was higher ($p < 0.05$) than winter (57.7 ± 0.41) and spring (60.18 ± 0.55). The mean value of catalase (U/ml) in summer (177.46 ± 0.24) was higher ($p < 0.05$) than winter (94.78 ± 0.16) and spring (101.57 ± 1.17). The mean value of Lipid Peroxidation (nmol/ml) in summer (3.93 ± 0.05) was higher ($p < 0.05$) than winter (2.81 ± 0.02) and Spring (2.98 ± 0.04). The mean value of Glutathione ($\mu\text{m}/\text{ml}$) revealed in summer (0.71 ± 0.02) was higher ($p < 0.05$) than winter (0.19 ± 0.00) and spring (0.58 ± 0.02).

Elevated environmental temperatures increase the production of reactive oxygen species (ROS) are with subsequent induction of oxidative damage to lipids (Maibam *et. al.*, 2018 [26] and Abdelnour *et. al.*, 2019) [1]. The

unsaturated lipids are more prone to free radicals damage and hence, lipid peroxidation is considered as the biomarker of free radical load (Wagner *et. al.*, 1994) [40]. Malondialdehyde (MDA) is a low molecular weight and product which is generated as the free radical damage the lipids (Wong-Ekkabut *et. al.*, 2007) [41]. MDA is an important product of lipid peroxidation and acts as good indicator of oxidative damage of erythrocyte (Banerjee *et. al.*, 2020). Increase in MDA concentration during summer season may be due to higher ROS mediated lipid peroxidation. In our study, also MDA concentration was maximum in summer during which environmental temperature and minimum in winter environmental temperature is low.

Elevated Superoxide dismutase (SOD) and catalase (CAT) activities found during summer as compare to other season indicating that more ROS is produce during summer (Maibam *et. al.*, 2018 [26]; Abdelnour *et. al.*, 2019) [1].

Higher concentration of GSH was found during summer season and it may be due to decrease in activity of enzyme glutathione-S-transferase (John *et. al.*, 2001 [20]; Bernabucci *et. al.*, 2002) [4]. GSH is keystone to the cellular antioxidant defenses as it acts as an essential cofactor for antioxidant enzyme such as glutathione peroxidase, glutathione reductase and glutathione-S-transferase (Dhanasree *et. al.*, 2020) [13]. The elevated GSH concentration during summer season as compare to other season indicating that erythrocyte in under oxidative stress and this increase may be due to the decrease in activity of enzyme glutathione-S-transferase in erythrocytes. (John *et. al.*, 2001 [20]; Bernabucci *et. al.*, 2002). GSH is an essential cofactor for glutathione-S-transferase (Dhanasree *et. al.*, 2020) [13]. Chaudhary *et. al.*, (2015) reported that elevated serum total antioxidant capacity (TAC) during summer and monsoon season in surti buffalo.

Erythrocytes are carrier of oxygen and they are more prone to oxidative stress caused by ROS (Mohanty *et. al.*, 2017) [29]. Activities of SOD and CAT in erythrocyte were higher during summer season in cattle (Chetia *et. al.*, 2017 [7]; Verma *et. al.*, 2020 [39]; Kalmath and Swamy, 2020) [22]. In our study also activities of SOD and CAT in erythrocyte was higher during summer season as compare to other seasons. SOD along with CAT neutralizes ROS produce to stress and elevated temperature increases ROS production (Maibam *et. al.*, 2018 [26]; Abdelnour *et. al.*, 2019) [1].

Table 1: Mean ($\pm$ S.E.) temperature humidity index during the experimental period

Season	THI max. ($^{\circ}\text{C}$)	THI min. ($^{\circ}\text{C}$)	THI avg.
Winter	72.28 ± 0.39^a	52.52 ± 0.32^a	62.40 ± 1.19^a
Spring	76.01 ± 0.32^a	63.03 ± 0.31^a	69.52 ± 0.42^a
summer	100.71 ± 0.31^b	77.47 ± 0.30^b	89.09 ± 0.30^b

Values with different superscripts a-b within a column differ significantly ($p \leq 0.05$)

The values of mean $\pm$ SE are observations on twelve animals

Table 2: Mean ($\pm$ SE) rectal temperature ($^{\circ}\text{F}$), respiration rate (beat/min) and pulse rate (beat/min) of sheep (Sonadi Corriedale) during different season.

Season	Temp ($^{\circ}\text{F}$)	RR (beat/min)	PR (beat/min)
winter	101.74 ± 0.15^a	26.76 ± 0.63^b	84.83 ± 0.44^a
spring	102.05 ± 0.61^b	24.72 ± 0.27^a	85.49 ± 0.28^b
summer	103.12 ± 0.91^c	37.20 ± 0.14^c	87.24 ± 0.20^c

Values with different superscripts a-c within a column differ significantly ($p \leq 0.05$)

The values of mean $\pm$ SE are observations on twelve animals

Table 3: Mean ($\pm$ SE) Hematological Parameters of sheep (*Sonadi Corridale*) during different season.

Season	Hemoglobin (g/dl)	PCV (%)	RBC ($10^6/\text{mm}^3$)	WBC ($10^3/\text{mm}^3$)
winter	11.63 $\pm$ 0.32	34.19 $\pm$ 1.01	3.80 $\pm$ 0.11	8759.58 $\pm$ 61.00 ^a
spring	11.48 $\pm$ 0.39	34.44 $\pm$ 1.18	3.82 $\pm$ 0.13	8383.33 $\pm$ 36.00 ^a
summer	10.77 $\pm$ 0.35	32.30 $\pm$ 1.06	3.59 $\pm$ 0.12	10376.15 $\pm$ 21.00 ^b

Values with different superscripts a-b within a column differ significantly ($p \leq 0.05$)

The values of mean $\pm$ SE are observations on twelve animals

Table 4: Mean ($\pm$ SE) cortisol (ng/ml) and total antioxidant capacity (mM) of sheep (*Sonadi Corridale*) during different season.

Season	Cortisol (ng/ml)	Total Antioxidant Capacity (mM)	Superoxide dismutase (U/ml)	Catalase (U/ml)	Lipid peroxidation (nmol/ml)	Glutathione (Mm/ml)
Winter	14.56 $\pm$ 0.10 ^b	0.99 $\pm$ 0.01 ^a	57.71 $\pm$ 0.41 ^a	94.78 $\pm$ 0.16 ^a	2.81 $\pm$ 0.02 ^a	0.19 $\pm$ 0.00 ^a
spring	15.61 $\pm$ 0.32 ^a	1.10 $\pm$ 0.01 ^a	60.18 $\pm$ 0.55 ^a	101.57 $\pm$ 1.17 ^b	2.98 $\pm$ 0.04 ^b	0.58 $\pm$ 0.02 ^b
summer	18.66 $\pm$ 0.13 ^c	2.47 $\pm$ 0.07 ^b	86.65 $\pm$ 0.86 ^b	177.46 $\pm$ 0.24 ^c	3.93 $\pm$ 0.05 ^c	0.71 $\pm$ 0.02 ^c

Values with different superscripts a-b within a column differ significantly ($p \leq 0.05$)

The values of mean $\pm$ SE are observations on twelve animals

Conclusion

The mean significant value of RBC ($10^6/\text{mm}^3$) blood hemoglobin (g/dl) and Packed Cell volume (%) did not observed any significant different was observed in blood hemoglobin (g/dl) of animal during season's winter, spring and summer. The markers of oxidative profile such as Total antioxidant capacity, SOD, MDA, Catalase and Cortisol in this study were found to increase during the summer season as compared to the rainy season, indicating that *Sonadi corriedale* sheep have experienced elevated oxidative stress in the summer season than in the winter season. Hence, the activities of these oxidative stress markers, which are influenced by THI of the seasons and its up-regulation was maintained during elevated THI in summer than in the winter and spring season, could also be used as a sensitive biomarker of oxidative stress caused due to heat stress in sheep.

Acknowledgement

The authors are thankful to the Bihar Animal Sciences University, Patna-14 (Bihar), for providing the necessary facilities for research work

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