Impact of heat stress on oxidative stress markers in Red Kandhari cattle during different seasons

M.J. Sanap[1*], P.V. Yeotikar[1], P.B. Ghorpade[1], V.B. Dongre[2], S.A. Amrutkar[1], MFMF Siddiqui[1]

[1]College of Veterinary and Animal Sciences, Parbhani, [2]College of Veterinary and Animal Sciences Udgir, MAFSU, Maharashtra India
[*]Corresponding author: maharudrasanap@mafsu.ac.in

Journal of Livestock Science (ISSN online 2277-6214) 16: 315-321
Received on 18/1/25; Accepted on 20/4/25; Published on 26/4/25
doi. 10.33259/JLivestSci.2025.315-321

Abstract

Present study was conducted on Red Kandhari cattle to determine the impact of heat stress on levels of oxidative stress markers viz. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), malondialdehyde (MDA) and total antioxidant capacity (TAC) during peak winter, summer and rainy seasons. For the study, total twenty-four (24) apparently healthy, Red Kandhari male F(n=12) and female animals (n=12) irrespective of physiological status with average age (1-4 years) were selected from Livestock Farm Complex, College of Veterinary and Animal Sciences, Parbhani. The metrological variables viz. dry bulb temperature and relative humidity was recorded for each season and THI was calculated for peak winter (month of January), summer (month of May), and rainy (month of September) seasons. The blood samples were aseptically collected by jugular venipuncture with K_3EDTA vacutainer at peak winter, summer and rainy seasons. The parameters of MDA, SOD, GPx, GSH, CAT and TAC were estimated using standard methods. Significantly higher levels SOD, catalase and MDA; significant ($p<0.01$) decrease in GPx activity, GSH concentration and TAC was observed in the Red Kandhari cattle during peak summer season as compared to winter and rainy seasons. It was concluded that the elevated activity of SOD, catalase, increased MDA concentration accompanied by decrease in the Gpx activity along with the drop in the GSH concentration may results into oxidative stress at cellular level. However, decrease in TAC implies that an antioxidant potential of animals to scavenge the free radicals has been utilized to combat oxidative stress produced during peak summer season in Red Kandhari cattle.

Key words: Red Kandhari cattle; temperature humidity index; oxidative stress markers; Summer season; oxidative status;antioxidants.

Introduction

Livestock is a backbone of Indian economy, as it provides livelihood for rural community. About 20.5 million farmer's livelihood depends on livestock production. Amongst the indigenous breeds of cattle, Red Kandhari is one of the most important indigenous breed of Marathwada region of Maharashtra State, it is well known as Lal Kandhari locally. The purest form this breed found in various breeding tracts i.e. Kandhar, Mukhed, Nanded, Biloli and Naigaon tehsils of Nanded district; adjoining pockets of Ahmadpur, Chakur, Shirur-Anantpal, Ausa; Udgir tehsils of Latur district; Parli tehsil of Beed and Hingoli tehsil of Hingoli district of Marathwada region (Chauhan *et al*., 2008). Red Kandhari cows are low producers of milk but have high breeding efficiency. They are compact in body size, the bullocks of this breed are attractive and very powerful animals due to which they are greatly valued by the farmers. However, the state Government has been implementing the various schemes for the developing the Red Kandhari cattle breeds. The preservation of germplasm of this breed has been undertaken by the Government as a developmental activity in the livestock sector.

Climate change, in particular global warming affecting the health and welfare of farm animals (IPCC 2007). Especially, heat stress in tropical countries (India) is a problem of great concern among farmers and livestock producers. Thermo neutral zone (TNZ) is defined schematically the interrelationships between an animal and its environment, the ambient temperature (comfortable zone) for the cow ranges from 5 to 25°C (Roenfeldt 1998). Although, Ruminants having well-developed thermoregulatory mechanisms, they are unable to maintain adequate homeothermy under severe HS. However, they regulate their core body temperature through physiological and metabolic adaptation such as hyperthermia, panting and reducing feed intake (Constable *et al*., 2017).

Heat stress is defined as biological response to thermal stimuli in the environments (Constable *et al*., 2017) which leads to increase in pro-oxidants exceeding the antioxidant state, oxidative stress (OS) develops (Sies *et al*., 2017; Nachare *et al.*, 2024)) as a result of excessive production of reactive oxygen species (free radicals), which causes oxidative damage to cell components, such as fat, protein, and DNA (Dekany *et al.,* 2008). Under normal metabolic processes, reactive oxygen species (ROS) are constantly produced (Indo *et al*., 2007) which are neutralized by the antioxidant system (Omidi *et al.,* 2017).

Antioxidant enzymes such as catalase detoxifies H2O2 produced during different metabolic processes and also in stressful conditions by reducing it to H2O and O2, glutathione peroxidase catalyse the detoxification of hydrogen peroxides, organic hydroperoxides and lipid peroxides by utilizing glutathione as reductive substance (Halliwell and Gutteridge, 1999) and Superoxide dismutase catalyzing the conversion of two superoxide radicals to hydrogen peroxide along with molecular oxygen, thereby reducing the toxicity of ROS (Marreiro *et al.,* 2017).

Malondialdehyde (MDA) is one of the several low-molecular-weight end products i.e. aldehyde formed by reactive oxygen species induced (ROS) radicals due to the decomposition of polyunsaturated fatty acid (Janero 1990). The glutathione is a principal non-protein thiol, chemically, known as γ-glutamyl-cysteinyl-glycine (Satyanarayana 1999), which act as a component of antioxidant defence systems to scavenge reactive oxygen species (ROS). However, the total antioxidant capacity (TAC) is defined as the measure of sum of all antioxidants potential (Erel 2005).

Therefore, taking into consideration the significance of antioxidants, present study was undertaken to determine the effect of heat stress on the levels oxidative stress markers in Red Kandhari cattle during peak winter summer and rainy seasons.

Materials and Methods

The experiment was conducted on total twenty-four (24) apparently healthy, Red Kandhari male (n=12) and female animals (n=12) irrespective of physiological status with average age (1-4 years) at Livestock Farm Complex, College of Veterinary and Animal Sciences, Parbhani. The farm is situated at 347 meters above sea level at approximately 19.27° North latitude and 76.78° East longitude. They were kept under similar managemental and nutritional regimen throughout the duration (January to September 2023) of the experiment. The whole blood samples (14 mL) at once collected by using freshly prepared 10% EDTA solution during peak winter (month of January), peak summer (month of May), rainy (month of September) seasons. The cold chain was maintained while collecting the blood samples. Plasma was separated out, stored at -20°C until analysis.

The hemolysate was prepared as per method given by Kumar *et al.* (2011a). It was stored by making aliquots at -20°C for the determination of haemoglobin and oxidative stress markers.

Temperature humidity Index

The metrological variables viz. dry bulb/wet bulb temperature and relative humidity was recorded during peak summer, winter and rainy seasons. The observations were used to calculate the temperature humidity index (THI) as per the formula given by Mader *et al*. (2006). THI = (0.8 x Tdb) + ([RH/100) x (Tdb-14.4)] + 46.4.

Where, Tdb= Dry bulb temperature and RH= Relative humidity.

Estimation of hemoglobin

The estimation of haemoglobin from hemolysates was done by employing modified cyanide method (Dacie and Lewis, 1975**)**. The optical density was recorded at 540 nm against a blank and the concentration of haemoglobin was determined and expressed as g/dL.

Superoxide dismutase (SOD)

Superoxide dismutase enzyme activity was determined from hemolysate by measuring the increase in absorbance at 560 nm based on amount of enzyme concentration required to inhibit the chromogen production by 50% in min under assay conditions (Nishikimi *et al.,* 1972). The SOD activity was expressed as U/mg Hb.

Glutathione Peroxidase (GPx)

The protocol was standardized with different concentration of NADPH for test samples (hemolysates) against blank. The equipment UV-VIS spectrophotometer (Systronics model no. 117) was used to record absorbance depletion of NADPH at 340 nm wavelength for 3 min (at an interval of 30 sec) against blank. The enzyme activity was calculated as µM NADPH oxidized/min/g Hb with the molar extinction coefficient of 6.22 x $^{3}M^{-1}CM^{-1}$. The glutathione peroxidase activity was expressed as U/g Hb (Paglia and Valentine, 1967).

Catalase (CAT)

Catalase activity was determined by recording the changes in absorbance at 570 nm against the blank as per method described by Sinha 1972 (cited by Hadwan 2016). The catalase activity was expressed as kU/L.

Reduced glutathione (GSH)

The reduced form of glutathione from whole blood sample was estimated as per method proposed by Ellman (1959). The standard curve was prepared with known concentrations viz. 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM and 1.0 mM of glutathione against their absorbances. The standard curve was used to calculate GSH concentration of test samples. The concentration of GSH was expressedas mM/L.

Lipid peroxidation

The malondialdehyde (MDA) was estimated from hemolysate samples as per standard protocol elucidated by Satoh (1978). The concentration of MDA expressed as nmol/g Hb.

Total antioxidant capacity (TAC)

Based on the reaction of specific redox chromogen (2,6-dichlorophenolindophenol) with glutathione present in the test samples, a changes in the colour of the reagent was determined photometrically at 630 nm as per the method by Ciuti and Liguri (2017). The concentration of total antioxidant capacity was expressed as mM/L. The reduced glutathione was used as the calibrator. The calibrator for the assay was prepared using reduced glutathione (2 mM), phosphate buffer (50 mM) and and Na_2EDTA (1 mM; pH 6.5). Absorbance against blank was recorded by using UV-Visible Spectrophotometer at 630 nm wavelength at T_0, T_1 and T_2 after mixing the sample (1:10) or calibrator with the reagent.

Statistical Analysis

Statistical analysis of data was done by using SPSS statistical software for windows (version 24). One way ANOVA was applied to analyse the variance about mean values for oxidative stress markers and total antioxidant capacity parameters amongst seasons.

Results and discussion

Temperature Humidity Index (THI)

The mean values of THI recorded at peak months during different seasons viz. summer (May), winter (January) and rainy (September) were 83.36 ± 2.74, 67.84 ± 2.57, 78.24 ± 1.44 respectively.

In the present study, mean values of THI in peak summer, winter and rainy was found to be 83.36, 67.84, and 78.24 respectively. The highest THI (83.36± 2.74) was found during the peak month (May) of the summer season. In agreement with the present results, the combination of ambient temperature and humidity act as primary factor that causes the heat stress in livestock which is affecting the reproductive performances (Sinha *et al*., 2017a), reducing the fertility of dairy cattle through its deleterious impact on oocyte maturation and early embryo development (Chandrabhan *et al*., 2012).

However, in the crossbred cows during summer season, mean values of THI in control group (shed with sprinkler), treatment group (fogger plus fans and mosquito net) and open paddock group were 78.28±1.98, 74.24±1.50 and 82.20±2.17, respectively (Sinha *et al*., 2017), which are in line with the present results. The Harithalekshmi and Ajithkumar, (2021), who reported that the THI can be used to quantify impact of heat stress on cattle.

The present observations of average maximum temperatures (°C) were 38.96±0.06, 30.01±0.04 and 31.18±0.05 and minimum temperatures (°C) were 23.81±0.05, 12.17±0.05 and 22.23±0.03 for the peak summer (month of May), winter and rainy seasons, respectively; the average relative humidity were 57.39±0.13, 87.23 ±0.07 and 89.97±0.07 for the peak summer (month of May), peak winter (month of January) and peak rainy (month of September) seasons respectively. In contrary to our results, the maximum temperature was in August at 29.5 °C, high temperature stress affecting the productivity of dairy cows accompanied by increase in the temperature of rumen at 39.15 °C in the Korea (Nam *et al*., 2024).

In agreement with the present results, Sinha *et al*. (2017), who reported that the maximum temperatures (°C) were 35.44±0.44, 33.50±0.53 and 37.16±0.64 and minimum temperature (°C) were 21.53±1.02, 20.72±0.94 and 20.62±1.24 for control, treatment and open paddock, respectively.

Oxidative stress markers

The activity of superoxide dismutase in hemolysate was found significantly ($p<0.01$) higher during summer as compared to rainy & winter seasons, the activity of glutathione peroxidase in hemolysate was shown to be significantly higher ($p<0.01$) during winter as compared to summer & rainy seasons and the activity of catalase in plasma was recorded significantly higher ($p<0.01$) during summer as compared to winter and rainy seasons as shown in Table 1.

In the present results, the increase in the catalase activity indicating that the presence of free radicals leading to the development of oxidative stress in Red kandhari cattle during summer seasons (Roy 2013). The Chetia *et al*., (2017), who reported that the Superoxide dismutase, glutathione peroxidase and catalase were the important components of intracellular antioxidant defence system. They also reported similar results with present findings that the mean values of SOD and catalase was found to be significantly ($p<0.05$) higher during hot/humid summer as compared to winter season in zebu cattle. In the present results, the concentrations of glutathione peroxidase was significantly increased during winter as compared to summer season, which might be due to the fact that high ambient temperature reduces the glutathione peroxidase activity (Sakatani 2012).

The increase in the activity of SOD result into generation of hydrogen peroxide during heat stress conditions (Ganaie *et al*., 2013), which causes reduction in plasma antioxidant activity, increased production of free radicals and decrease in the synthesis of endogenous antioxidants (Colakoglu *et al*., 2017). In agreement with the present results, Uttarani *et al.* (2017), who reported that there was significant ($p<0.05$) increase in SOD during summer; however, in contrast to present study, they observed significantly increase in GPx activities during winter seasons in both Tharparkar and Karan fries cattle.

Our results were in agreement with the Chandra and Aggarwal (2009), who reported that levels of erythrocytic antioxidant enzymes were significantly higher in prepartum cross bred cows during summer than winter seasons. Normally, cells able to detoxify superoxide radicals using enzymatic antioxidants such as SOD, GPx and CAT (Sunil *et al*., 2011).

The concentration of the reduced glutathione was significantly higher ($p<0.01$) during rainy as compared to winter and summer seasons in Red Kandhari cattle, however, the level of reduced glutathione was observed to be lower during summer as compared to winter season and its level was higher during rainy when compared to summer seasons as shown in Table 1.

In present study, concentration of reduced glutathione was significantly ($p<0.01$) decreased; reduction in the levels of GSH makes the cells more prone to oxidative injuries (Kumar and Padhy, 2013). Our findings were in agreement with Perumal *et al*. (2022), who reported significantly higher ($p<0.05$) levels of GSH during winter and spring seasons as compared to summer season in Mithun bulls, which is attributed to the glutathione which protects cysteine-rich proteins from binding with free metal ions (Jozefczak 2012). In contrary to our results, Kumar *et al*. (2011), who reported that level of reduced glutathione was significantly ($p<0.05$) increased after exposure heat stress in both hot/dry as well as hot/humid conditions.

The concentration of MDA in hemolysate was significantly ($p<0.01$) higher during summer as compared to rainy & winter seasons in Red Kandhari cattle. The MDA concentration was 5-6 folds higher during summer than winter seasons as shown in Table 2. Present findings were in agreement with Perumal *et al*. (2022), who reported that the levels of MDA were significantly ($p<0.05$) higher during summer as compared to winter and spring seasons in Mithun bulls. Yehia *et al*. (2021) also, reported the similar observations collinear with the present study that, the MDA level was significantly higher during the summer as compared to winter season in Holstein dairy cows.

In the present results, significantly ($p<0.05$) higher levels of catalase, SOD and MDA were found as reported by Yatoo *et al*., (2014) in both the lactating and non-lactating cattle during summer as compared to the spring season, the higher temperature causes the formation of malondialdehyde (MDA) due to lipid peroxidation (Horvath and Babinszky, 2019) in cattle, the MDA concentration during summer was 133.4% versus autumn (Guo *et al*., 2018).

Table 1. Oxidative stress profile (Mean ± SEM) in Red Kandhari cattle during different seasons

S. N.	Oxidative stress profile parameters	Summer (n=24)	Winter (n=24)	Rainy (n=24)	p-value
1.	Superoxide dismutase (U/mg Hb)	$10.36^{a}\pm$ 0.83	$1.17^{c} \pm 0.03$	$6.89^{b} \pm 0.36$	0.00
2.	Glutathione peroxidase (U/g Hb)	$41.30^{b}\pm 1.81$	$169.05^{a} \pm 2.32$	$4.09^{c} \pm 0.39$	0.00
3.	Catalase (kU/L)	$47.82^{a} \pm 1.21$	$42.40^{b} \pm 1.86$	$30.64^{c} \pm 1.02$	0.00
4.	Reduced glutathione (mM/L)	$0.19^{c} \pm 0.01$	$0.39^{b} \pm 0.01$	$0.76^{a} \pm 0.01$	0.00
5.	MDA (nmol/g Hb)	$6.25^{a} \pm 0.50$	$0.43^{c} \pm 0.04$	$3.3^{b} \pm 0.17$	0.00

^{abc}Means in a row with different superscripts differ significantly ($p<0.01$)

Total antioxidant capacity (TAC)

The concentration of TAC in plasma was significantly ($p<0.01$) higher during winter as compared to summer and rainy seasons in Red Kandhari cattle as shown in Table 2. In the present study, the concentration TAC was significantly reduced during peak summer season which is in line with the Li *et al*. (2021), who found that the total anti-oxidizing capability (T-AOC) was reduced during heat stressed cows. The total oxidant capacity is the measure of overall oxidative status of the body i.e. TOC represents the sum of all the oxidant substances present in blood (Kurt *et al*., 2021).

Table 2. Total antioxidant capacity (Mean ± SEM) (mM/L) in Red Kandhari cattle during different seasons

Particulars	Summer (n=24)	Winter (n=24)	Rainy (n=24)	p-value
Total antioxidant capacity (mM/L)	41.08^{b} ± 1.99	71.58^{a} ± 2.65	23.92^{c} ± 0.61	0.00

^{abc}Means in a row with different superscripts differ significantly ($p<0.01$)

Therefore, it can be concluded that the Red kandhari cattle exposed to severe heat stress resulted into variation in the levels of oxidative stress markers to combat heat stress during peak summer as compared to winter and rainy seasons. However, decrease in the level of plasma TAC antioxidant capacity (TAC) is attributed to combat heat stress during peak summer season. This implies that animals being exposed to severe dry heat stress are adapted to thermal climatic conditions during peak summer season.

Ethical statement

In this study, the experimental measures using Red Kandhari cattle have been conducted after approval from the Institutional Animal Ethical Committee (IAEC) of the College of Veterinary and Animal Sciences, Parbhani (Maharashtra Animal and Fishery Sciences University, Nagpur) vide no. IAEC/100/2022. All the research investigations with Red Kandhari cattle were carried out according to the IAEC guidelines.

References

1) Chandra B, Singh SV, Hooda OK, Upadhyay RC, Baliyan B., Vaidya M. 2012. Influence of temperature variability on physiological, hematological and biochemical profile of growing and adult Sahiwal cattle. Journal of Environmental Research and Development **7(2A)**: 986-994.
2) Chandra G, Aggarwal A. 2009. Effect of DL-α-Tocopherol acetate on calving induced oxidative stress in periparturient crossbred cows during summer and winter seasons. Indian Journal of Animal Nutrition **26**(3): 204-210.
3) Chauhan DS, Thombre BM, Mitkari KR, Yadav KN and Bainwad DV. 2008. Origin and Historical Background of Red Kandhari Cattle of Maharashtra. National Symposium - SOCDAB, Veterinary College, Bangalore, 266.
4) Chetia M, Sharma S, Nath R and Tamuly S. 2017. Studies on the seasonal variations in the antioxidant enzymes in Indian zebu cattle. Journal of Entomology and Zoology Studies **5(**2): 1362-1365.
5) Colakoglu HE, Yazlik MO, Kaya U, Colakoglu EC, Kurt S, Oz B, Bayramoglu R, Rifat MV and Kuplulu S. 2017. MDA and GSH-Px activity in transition dairy cows under seasonal variations and their relationship with reproductive performance. Journal of Veterinary Research **61**: 497-502. DOI:10.1515/jvetres-2017-0067.
6) Constable PD, Hinchcliff KW, Done SH and Grunberg W. 2017. Veterinary Medicine. A Textbook of the Diseases of Cattle, Horse, Sheep, Pigs and Goats. 11th Edition, Saunders Elsevier, Missouri.
7) Ciuti R and Liguri G. 2017. A Novel Assay for Measuring Total Antioxidant Capacity in Whole Blood and Other Biological Samples. Journal of Biomedical Science and Engineering **10**: 60-76. https://doi.org/10.4236/jbise.2017.102007
8) Dacie JV, Lewis SM. 1975. Clinical haematology. 5th ed. Churchill Livingstone, p. 32.
9) Dekany M, Nemeskeri V, Gyore I, Ekes E, Golg A, Szots G, Petrekanits M, Taylor AW, Berkes J and Pucsok J. 2008. Physical performance and antioxidants effects in triathletes. Biology of Sport **25**(2): 101-114.
10) Ellman GL. 1959. Tissue sulfhydryl groups. In: Archives of Biochemistry and Biophysics **82**(1) :70-7. DOI: 10.1016/0003-9861(59)90090-6
11) Erel O. 2005. A new automated colorimetric method for measuring total oxidant status. In: Clinical Biochemistry. Journal of the Canadian Society of Clinical Chemists **38 (12)**:103-1111. https://doi.org/10.1016/j.clinbiochem.2005.08.008
12) Ganaie AH, Shanker G, Bumla NA, Ghasura RS, Mir NA, Sa W and Gb D. 2013. Biochemical and Physiological Changes during Thermal Stress in Bovines. Journal of Veterinary Science Technology **4**(1): 1–6. https://doi.org/10.4172/2157-7579.1000126

13) Guo J, Gao S, Quan S, Zhang Y, Bu D, and Wang J. 2018. Blood amino acids profile responding to heat stress in dairy cows. Asian–Australasian Journal of Animal Science **31**(1): 47–53. https://doi.org/10.5713/ajas.16.0428
14) Hadwan MH. 2016. New Method for Assessment of Serum Catalase Activity. Indian Journal of Science and Technology **9**(4): 1-5. https://doi.org/10.17485/ijst/2016/v9i4/80499
15) Halliwell B. and Gutteridge JMC.1999. Free radicals in biology and medicine. In: Halliwell, B. and Gutteridge, J.M.C., Eds., 3rd Edition, Oxford University Press, Oxford, 1-25.
16) Harithalekshmi V and Ajithkumar B. 2021. Intercomparison of seven temperature-humidity index (THI) equations for assessing the impact of heat stress in relation to milk production to find out best THI for central zone of Kerala. MAUSAM **72** (3): 669-672. https://doi.org/10.54302/mausam.v72i3.1318
17) Horvath M, and Babinszky L. 2019. Impact of selected antioxidant vitamins (Vitamin A, E and C) and micro minerals (Zn, Se) on the antioxidant status and performance under high environmental temperature in poultry. A review. Acta Agriculturae Scandinavica, Section A. Animal Science 0(0): 1–9. https://doi.org/10.1080/09064702.2019.16119 13.
18) Indo HP, Davidson M, Yen HC, Suenaga S, Tomita K, Nishii T, Higuchi M, Koga Y, Ozawa T, Majima HJ. 2007. Evidence of ROS generation by mitochondria in cells with impaired electron transport chain and mitochondrial DNA damage. Mitochondrion **7**:106–118. DOI: 10.1016/j.mito.2006.11.026
19) IPCC (Intergovernmental Panel on Climate Change). 2007. The intergovernmental panel on climate change, 4th Assessment report. Jackson Institute, University College, London.www.ipcc.ch/.
20) Janero DR. 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidase tissue injury. Free Radical Biology and Medicine **9**(6):515-540. DOI: 10.1016/0891-5849(90)90131-2
21) Jozefczak M, Remans T, Vangronsveld J and Cuypers A. 2012. Glutathione is a key player in metal-induced oxidative stress defences. International Journal of Molecular Sciences **13**(3): 3145–3175. DOI: 10.3390/ijms13033145.
22) Kumar C, Igbaria AC, D'Autreaux AB, Planson AG, Junot C, Godat E, Bachhawat AK, Agnès DS and Michel BT. 2011. Glutathione revisited: a vital function in iron metabolism and ancillary role in thiol redox control. The EMBO Journal **30** (10): 2044-2056. https://doi.org/10.1038/emboj.2011.105
23) Kumar G, Karthik L and Bhaskara Rao KV. 2011a. Haemolytic activity of Indian medicinal plants toward human erythrocytes: An in vitro study. Elixir Applied Botany **40**: 5534-5537. https://www.researchgate.net/publication/216174268
24) Kumar M and Padhy PK. 2013. Oxidative Stress and Heavy Metals: An appraisal with reference to environmental biology. International Research Journal of Biological Sciences **2**(10): 91-101. www.isca.in, www.isca.me
25) Kurt S, Eski F, Salar S, Sel T and Bastan A. 2021. The effect of heat stress on total oxidant capacity in hair goats. Journal of the Turkish Veterinary Medical Society **92**(1): 24-30. DOI: 10.33188/vetheder.771696
26) Li H, Zhang Y, Li R, Wu Y, Zhang D, Xu H and Qi Z. 2021. Effect of seasonal thermal stress on oxidative status, immune response and stress hormones of lactating dairy cows. Animal Nutrition **7**(1): 216-223. https://doi.org/10.1016/j.aninu.2020.07.006
27) Mader TL, Davis, MS and Brown-brandl T. 2006. Environmental factor influencing heat stress in feedlot cattle. Journal of Animal Science **84**: 712–719. https://digitalcommons.unl.edu/animalscifacpub/608.
28) Marreiro DDN, Cruz KYC, Morais JBS, Beserra JB, Severo JS and Oliveira ARS de. 2017. Zinc and oxidative stress: Current mechanism. Antioxidants **6 (2):** 1-9. https://doi.org/10.3390/antiox6020024.
29) Nachare VV, Sonawane GS, Jadhav SN, Doiphode AY, Shende TC, Shelar RR, Nimbalkar VG, Bhokre SM 2024. Oxidative stress and NRF2 gene expression in blood of indigenous pigs. Journal of Livestock Science 15: 111-119 doi. 10.33259/JLivestSci.2024.111-119
30) Nam KT, Choi N, Na Y and Choi Y. 2024. Effect of the temperature– humidity index on the productivity of dairy cows and the correlation between the temperature–humidity index and rumen temperature using a rumen sensor. Animals **14**(19): 2848. https://doi.org/10.3390/ ani14192848.
31) Nishikimi M, Appaji Rao N and Yagi K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. In: Biochemical and Biophysical Research Communications **46**(2): 849-854. https://doi.org/10.1016/S0006-291X(72)80218-3
32) Omidi A, Fathi MH and Parker MO. 2017. Alterations of antioxidant status in dairy cows during lactation and in the dry period. Journal of Dairy Research **84**(1): 49-53. doi: 10.1017/S0022029916000753.
33) Paglia DE, Valentine WN. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine **70**(1):158–169.
34) Perumal P, Khate K and Vupru P. 2022. Effect of season and age on biochemical, antioxidants and oxidative profiles in mithun bull. Indian Journal of Animal Sciences: **92** (1): 44 - 51. https://doi.org/10.56093/ijans.v92i1.120916
35) Roenfeldt S. 1998. You can't afford to ignore heat stress. Dairy Management **35**: 6–12.

36) Roy S, Hazra B, Mandal N and Chaudhuri TA 2013. Assessment of the antioxidant and free radical scavenging activities of methanolic extract of Diplazium esculentum. International Journal of Food Properties **16**(6): 1351-1370. https://doi.org/10.1080/10942912.2011.587382
37) Sakatani M, Balboula AZ, Yamanaka K and Takahashi M. 2012. Effect of summer heat environment on body temperature, estrous cycles, and blood antioxidant levels in Japanese Black cow. Animal Science Journal **83**: 394–402. DOI: 10.1111/j.1740-0929.2011.00967.x
38) Satoh K. 1978. Serum lipid peroxide in cerebrovascular disorder determined by a new calorimetric method. Clinica Chimica Acta **90**(1): 37-43. DOI: 10.1016/0009-8981(78)90081-5
39) Satyanarayana U. 1999. Proteins and amino acids. Biochemistry: Edn. 2nd, Arunabha Sen Books and Allied (p) Ltd. 8/1 Chintamoni Das Lane, Kolkata, pp. 69.
40) Sies H, Berndt C, and Jones DP. 2017. Oxidative stress. Annual Review of Biochemistry **86**:715–48. https://doi.org/10.1146/annurev-biochem-061516-045037
41) Sinha KA. 1972. Colorimetric assay of catalase. In: Analytical Biochemistry **47**(2): 389-394. doi: 10.1016/0003-2697(72)90132-7.
42) Sinha R, Kamboj ML, Ranjan A and Devi I. 2017. Effect of microclimatic variables on physiological and hematological parameters of crossbred cows in summer season. Indian Journal of Animal research **53**(2): 173-177. DOI: 10.18805/ijar.B-3480
43) Sinha R, Ranjan A, Lone S, Rahim A, Devi I and Tiwari S. 2017a. The impact of climate change on livestock production and reproduction: Ameliorative management. International Journal of Livestock Research **7**(6): 1-8. DOI: 10.5455/ijlr.20170417042102
44) Sunil Kumar BV, Kumar A. and Kataria M. 2011. Effect of heat stress in tropical livestock and different strategies for its amelioration. Journal of Stress Physiology & Biochemistry **7**(1): 45–54. https://www.researchgate.net/publication/50315383
45) Uttarani M, Hooda OK, Sharma PS, Singh SV and Upadhyay RC. 2017. Seasonal change in oxidative stress markers in blood plasma of Tharparkar (Bosindicus) and Karan fries (Bosindicusx Bostaurus) cattle under tropical climate International Journal of Current Microbiology and Applied Sciences **6**(2): 172. DOI: 10.1080/09291016.2017.1307905
46) Yatoo MI, Dimri U and Sharma MC. 2014. Seasonal changes in certain blood antioxidants in cattle and buffaloes. Indian Journal of Animal Sciences **84**(2): 173–176. https://doi.org/10.56093/ijans.v84i2.37845
47) Yehia SG, Ramadan ES, Megahed EA and Salem NY. 2021. Influence of season and lactation stage on oxidative, haematological, and metabolic profile parameters in Holstein dairy cows. Advances in Animal and Veterinary Sciences **9**(12): 2095-2102. DOIhttp:// dx.doi.org/10.17582/journal.aavs/ 2021/ 9.12.2095.2102