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Modulations in antioxidant defence system and expression of genes involved in antioxidant and apoptosis pathway helps ward-off heat stress in cows reared in hot-arid ambience of Thar desert

GAYATRI GUJAR[1], VIJAY KUMAR[1], MONIKA SODHI[2], PRINCE VIVEK[2], MANISH TIWARI[2] and MANISHI MUKESH[2]✉

Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan 334 001 India

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ABSTRACT

The present study was designed to assess the seasonal perturbations in key antioxidant parameters: ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) scavenging activity, lipid per oxidation (LPO), uric acid and reduced glutathione (GSH) assay along with expression profile of genes that regulate the antioxidant pathway: manganese superoxide dismutase (*SOD2*), Glutathione Peroxidase 1 (*GPX1*) and apoptosis pathway: B-cell lymphoma 2 (*BCL2*) and Bcl-2-associated X protein (*BAX*) in the two crucial indicine cattle breeds-Sahiwal and Kankrej. The study was carried out in the hot-arid region of Rajasthan across four seasons, wherein, the meteorological parameters were recorded across seasons to calculate the prevailing temperature-humidity index (THI) to assess the extent of heat load on cows. The study revealed huge diurnal variations in the temperature across four seasons, while the THI during summer and hot-humid seasons was well in excess of 80, highlighting significant heat stress on cows. All the key antioxidant parameters, except GSH registered an increase with the incremental THI. The transcriptional profile of *SOD2* and *GPX1* mRNA expression revealed significantly higher expression during the higher THI months of both summer and hot-humid seasons. The expression pattern of *BAX* and *BCL2* mRNA in PBMCs of Sahiwal cows revealed an initial induction during the month of summer season with significantly higher expression, followed by a decline by the hot-humid season. The findings highlight a crucial role played by antioxidant defence and genes regulating apoptosis pathway in thermotolerance of indicine cattle breeds.

Keywords: Antioxidants, Apoptosis, *Bos indicus*, Gene expression, Seasonal study, Temperature humidity index, Thermotolerance

Heat stress is a key abiotic stressor that negatively impacts animal productivity and welfare, globally (Collier *et al.* 2017). As the surface temperature in India has recorded an increasing trend, it presents a formidable challenge for the animal sector. The increase in intensity and frequency of heat waves in India will put dairy animals under substantial thermal stress (IPCC 2022). The already stretched resources of agriculture and livestock in the country are threatened due to unpredictable climate patterns, further worsening thermal stress on animals, especially dairy cows. Better understanding of the effects of climate on the animals and their subsequent response, will help in better management decisions, at the same time enhancing animal welfare (Polsky and von Keyserlingk 2017).

Heat stress in animals have been associated with increased free radicals, especially, reactive oxygen species (ROS) production. Antioxidants are the very first line of defence against the deleterious effects of ROS, and related free radicals that are main culprits of cellular damage. The role of antioxidants in protecting the cells against various shocks can't be stressed enough, especially when it comes to the attack of reactive oxygen species (ROS) and free radicals (Lallawmkimi 2010, Yatoo *et al.* 2014). The changes in antioxidant defence parameters of vertebrates in relation to the season indicates a composite disclosure of impact of various biotic and abiotic factors on the physiological state of animals (Chainy *et al.* 2016). The cows reared in the hot arid ambience of western Rajasthan are perpetually exposed to huge diurnal temperature fluctuations and stressful environment round the year with scorching summer, highly hot-humid and short monsoon, and very cold winters (Gujar *et al*. 2022). Hence, this study was an attempt to understand the key adaptations of two important indicine cattle breeds, the Sahiwal and Kankrej cows at cellular levels that aids in thermotolerance in harsh desert environment in a comprehensive manner.

Present address: [1]Department of Livestock Production Management, Rajasthan University of Veterinary and Animal Sciences, Rajasthan. [2]ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana. ✉Corresponding author email: mmukesh_26@hotmail.com

MATERIALS AND METHODS

Location and climatic conditions: This study was conducted over a period of one year (2019-20) spanning across four seasons (winter, spring, hot summer, hot-humid) in Sahiwal and Kankrej cattle, the two major native cattle breeds of India maintained in the cattle yard of livestock research station (LRS), Kodamdesar, about 32 km away from the city of Bikaner, Western Rajasthan, India. The LRS is situated at an altitude of 201 meters above the mean sea level in the Thar Desert. Since the place is located in the arid region, it is subjected to extremes climate with scorching summer having temperatures as high as 45°C and beyond; and a very chilly winter where temperature drops to around 5ºC or below, with very little precipitation.

Experimental animals and management: A total of 30 apparently healthy animals of Sahiwal (n=15) and Kankrej (n=15) breeds were randomly selected and kept loose in an open paddock throughout the day and night. There were provisions for shelter along one side of the paddocks that served the need of animal to rest during day or night time. The animals were housed in tie-barn with individual tying system in a row at the time of milking, feeding, and during blood sample collection.

Sampling procedure: At each sampling day, every season, the temperature and humidity values of farm microenvironment was recorded twice, morning (8:00-10:00 AM) and afternoon (1:30-3:30 PM) time points for three consecutive days using digital thermo-hygrometer and temperature humidity index (THI) was calculated using the formula:

$$THI = (1.8 \times Tdb + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times Tdb - 26), \text{(NRC, 1971)}$$

Where, Tdb, dry bulb temperature in °C and RH, relative humidity (%).

The blood samples were collected during the afternoon period every season, from each animal under study. For serum isolation, 7-8 ml blood was collected from both Sahiwal (n=15) and Kankrej (n=15) cows in plain vacutainer tubes (without any anticoagulant), after proper restraining of the animals. The samples were carefully drawn by puncturing the jugular vein of the animals. All the sampling and restraining of animals was done as per the guidance and approval of Institute Animal Ethical Committee (IAEC). The tubes were centrifuged at 3000 rpm for 15 min at 4˚C for serum and stored at -40°C until further analysis was done.

Estimation of antioxidant parameters: The serum samples of Sahiwal and Kankrej cows representing four seasons were processed in duplicates to assess the different antioxidant parameters. The absorbance in different protocols in this study was recorded in Microplate reader (Tecan-i-control Infinite 200 PRO, Männedorf, Switzerland).

Parameters studied

Ferric reducing/antioxidant power (FRAP) assay for total antioxidant capacity (TAC): The protocol originally described by Benzie and Strain (1999) was used with little customization.

DPPH (2,2-di(4-tert-octylphenyl)-1- icrylhydrazyl) assay for measurement of scavenging activity: It was determined as the percentage of DPPH• scavenged by serum as per the procedure originally described by Chrzczanowicz *et al.* (2008) with minor modifications.

Lipid peroxidation assay (LPO): The LPO assay was carried out according to the protocol of Buege and Aust (1978) with few modifications.

Reduced glutathione (GSH) assay: Serum GSH concentration was measured as per Moron *et al.* (1979) with some minor modifications.

Uric acid assay: For estimation of uric acid, a kit based on phosphotungstate method (M/s Span diagnostic) was used.

Transcriptional analysis of heat shock protein and apoptotic genes

Blood collection: Blood samples (7-8 ml) were collected from Sahiwal (n=5) and Kankrej (n=5) cows in each season in EDTA-vacutainer tubes to isolate RNA from peripheral blood mononuclear cells (PBMCs).

Isolation of peripheral blood mononuclear cells (PBMCs): A total of 40 PBMCs (5 samples × 4 seasons × 2

Table 1. Primer sequences and other details for reference genes (RGs)

Gene		Sequence	Region	Product length	Accession No.
ACTB	F	GCGTGGCTACAGCTTCACC	677-730	54	NM_173979.3
	R	TTGATGTCACGGACGATTTC			
B2M	F	CTGCTATGTGTATGGGTTCC	169-309	141	NM_173893.3
	R	GGAGTGAACTCAGCGTG			
GAPDH	F	TGGAAAGGCCATCACCATT	275-327	53	NM_001034034.2
	R	CCCACTTGATGTTGGCAG			
RPS9	F	CCTCGACCAAGAGCTGAAG	128-191	64	NM_001101152.2
	R	CCTCCAGACCTCACGTTTGT			
RPS15	F	GAATGGTGCGCATGAATGT	29-129	101	NM_001037443.2
	R	GACTTTGGAGCACGGCCTA			
RPS23	F	CCCAATGATGGTTGCTTGAA	287-387	101	NM_001034690.2
	R	CGGACTCCAGGAATGTCAC			

Table 2. Primer sequences and other details for target genes

Gene		Primer Sequence	Region	Product Size	Accession No.
Gpx	F	TTCGAGAAGTTCCTGGTG	587-688	102	NM_174076.3
	R	GGACAGCAGGGTTTCAAT			
SOD2	F	CGCTGGAGAAGGGTGATC	284-383	100	NM_201527.2
	R	AGATTTGTCCAGAAGATGCTGTGA			
Bax	F	TTTGCTTCAGGGTTTCATCC	75-320	246	NM_173894.1
	R	CAGTTGAAGTTGCCGTCAGA			
Bcl2	F	ATGTGTGTGGAGAGCGTCAA	439-639	201	NM_001166486.1
	R	CAGACTGAGCAGTGCCTTCA			

breeds) were isolated from whole blood for gene expression profiling. PBMCs was extracted using Hisep LSM 1084 reagent following manufacturer's protocol.

Total RNA extraction and primers: Total RNA extraction was done using Trizol reagent (RDP Trio™, Himedia) as per the manufacture's protocol. The details of primers for reference genes and target genes are provided in Tables 1 and 2.

Complementary DNA (cDNA) synthesis: RNA of high purity, with mean optical density (O.D) ~ 2.084±0.014, were used for synthesis of cDNA. Briefly, 200 ng of total RNA was reverse transcribed into cDNA using RevertAid™ First Strand cDNA Synthesis Kit (Thermo Scientific™, USA) at 25°C for 5 min, followed by 50°C for 60 min and finally 70°C for 15 min following manufacturer's method.

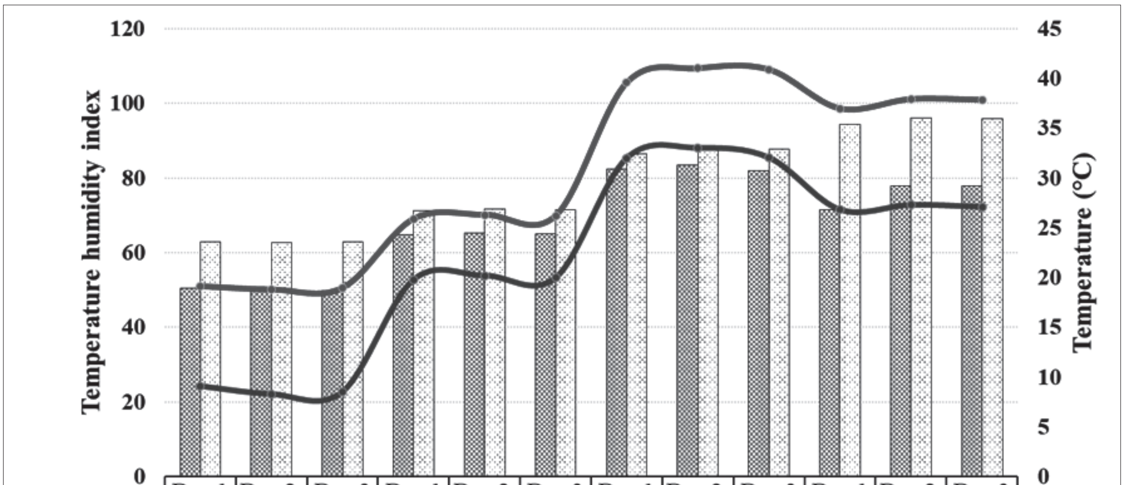
Real time quantitative PCR: Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, Massachusetts, United States) was used in Applied Biosystem Step One Plus instrument (ABI, Thermo Fischer Scientific, California, USA) and procedure was carried out as per manufacturer's method.

Statistical analysis: The raw data pertaining to antioxidant parameters was subjected to one way ANOVA and Tukey's post-hoc comparison test with $p<0.05$ value considered as significant in SPSS version 24. For analysis of qPCR data, the most stable RGs for normalization of data was selected based on output of geNorm, Normfinder, Bestkeeper, and refFinder software. The expression data was normalized using the best stable reference genes as per the relative quantification $2^{-\Delta\Delta CT}$ method described by Livak and Schmittgen (2001). Finally, the fold change in expression of individual genes was calculated to determine whether a particular gene was up-regulated or down-regulated in PBMCs samples of two cattle breeds across seasons.

RESULTS AND DISCUSSION

The prevailing meteorological parameters and THI are presented in Fig. 1. Data pertaining to the microclimatic attributes revealed significant diurnal variation in temperature across all four seasons. The THI was highest (95.39±0.56) during the afternoon of hot-humid season, followed by summer noon (87.23±0.37). The THI across both summer and hot-humid season was well beyond the thermal comfort zone established for dairy cows.

The role of antioxidants in protecting the cells against



	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
	Winter			Spring			Summer			Hot-humid		
THI Min	50.53	49.53	49.84	64.79	65.22	64.98	82.46	83.37	81.86	71.341	77.879	77.965
THI Max	62.98	62.60	62.80	71.26	71.57	71.51	86.52	87.47	87.71	94.28	96.11	95.77
Temp Min	9.10	8.30	8.55	19.80	20.20	20.00	32.00	33.01	32.05	26.82	27.3	27.1
Temp Max	19.10	18.78	18.95	25.89	26.30	26.20	39.58	41.02	40.90	36.98	37.93	37.85

Fig. 1. Prevailing climatic conditions across four seasons at Livestock research station (LRS), Kodamdesar.

Table 3. Mean±SEM of various antioxidant parameters of Sahiwal and Kankrej cows across four seasons

Season	Breed	Winter	Spring	Summer	Humid
DPPH Scavenging activity (%)	Sahiwal	73.58±0.67 ^{b}	66.22±2.16^{abx}	57.65±1.29^{ax}	73.15±0.60^{b}
	Kankrej	73.56±0.59^{b}	73.08±0.74^{by}	63.55±1.66^{ay}	73.49±0.32^{b}
FRAP (µM/L)	Sahiwal	370.04±9.89^{b}	385.87±9.12^{b}	437.18±22.47^{ax}	458.09±19.55^{a}
	Kankrej	392.15±11.79^{b}	422.31±13.74^{a}	459.08±16.20^{ay}	434.53±11.87^{a}
MDA	Sahiwal	9.98±0.55^{b}	9.45±0.66^{b}	14.98±0.96^{a}	14.52±0.79^{a}
	Kankrej	10.0±0.40^{b}	9.95±0.82^{b}	13.29±0.68^{a}	14.02±0.58^{a}
GSH (µM/L)	Sahiwal	158.63±15.99^{ax}	927.18±27.79^{bx}	240.43±13.86^{a}	196.68±22.25^{ax}
	Kankrej	138.03±16.85^{ay}	659.47±15.25^{by}	188.15±16.19^{a}	129.31±12.17^{ay}
Uric acid (mg/dl)	Sahiwal	3.10±0.05 ^{a}	3.36±0.06 ^{a}	3.32±0.12^{ax}	5.39±0.16^{b}
	Kankrej	3.61±0.09^{a}	3.43±0.11^{a}	3.27±0.09^{ay}	5.16±0.09^{b}

Mean bearing different superscripts (a,b,c,d) in a row vary significantly p<0.05. Mean bearing different superscripts (x,y,z) in a column vary significantly p<0.05.

various shocks can't be stressed enough, especially when it comes to the attack of reactive oxygen species (ROS) and free radicals. The changes in antioxidant defence parameters of vertebrates in relation to the season indicates a composite disclosure of impacts of various biotic and abiotic factors on the physiological state of animals (Chainy *et al.* 2016). The data pertaining to various antioxidant parameters recorded in Sahiwal and Kankrej cows during winter, spring, summer and hot-humid seasons have been presented in Table 3. Statistical analysis revealed significantly higher DPPH scavenging activity ($p<0.05$) during the spring, winter, and hot-humid seasons in both Sahiwal and Kankrej cows (Fig. 2a). DPPH scavenging activity of biological fluids has been used as a crucial parameter for evaluation of the antioxidant capacity in terms of their radical scavenging capability. In Sahiwal cattle, the DPPH scavenging activity reduced with increase in the temperature (spring and hot summer), however, its value increased during hot-humid season. Overall, the DPPH scavenging activity was significantly lower ($p<0.05$) during spring and summer seasons compared to winter and hot-humid seasons. The DPPH scavenging activity in Kankrej cows followed a similar trend, however, the induction began in summer season, and recovery was evident by hot-humid season. Similar trend in DPPH activity was reported by Cecchini and Fazio (2020) who found an initial decrease in the DPPH scavenging activity when stress was encountered, followed by recovery as the body acclimatized. Similarly,

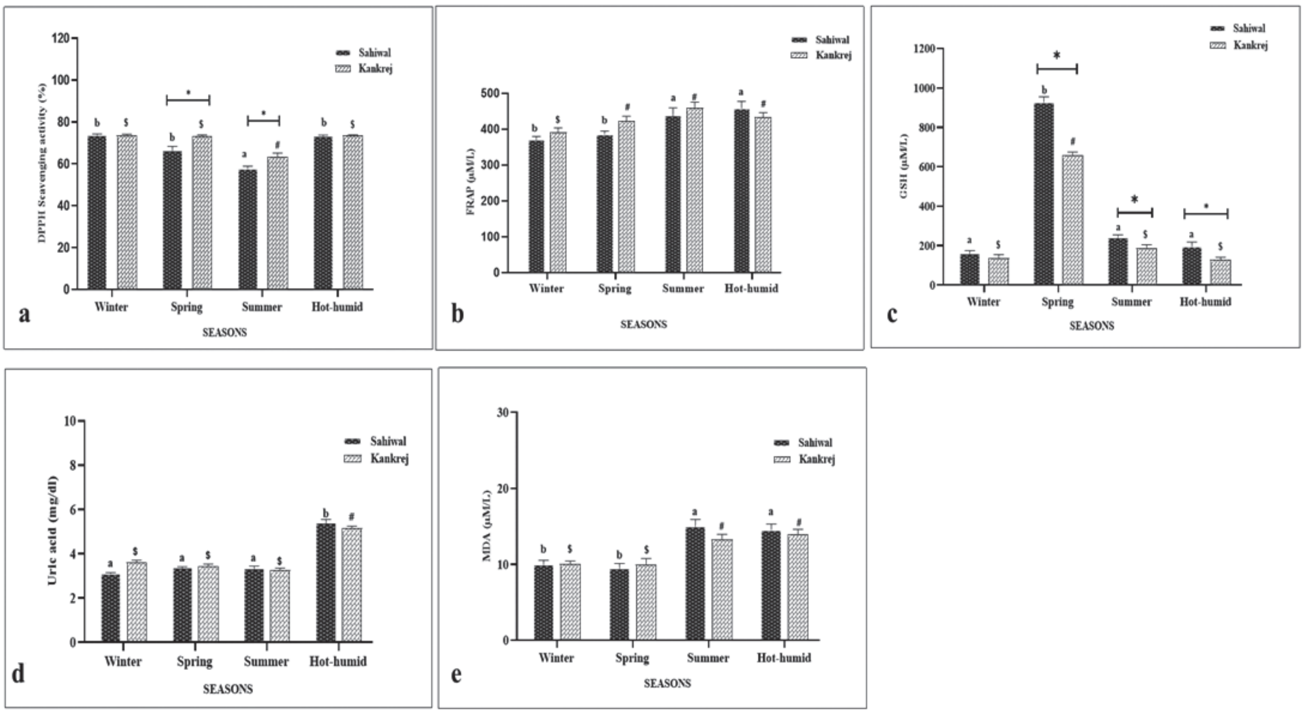


Fig. 2. Graphs showing different antioxidant parameters: (a) Serum DPPH scavenging activity of Sahiwal and Kankrej cows across four seasons; (b) Serum FRAP of Sahiwal and Kankrej cows across four seasons; (c) Serum GSH levels of Sahiwal and Kankrej cows across four seasons; (d) Serum uric acid concentration of Sahiwal and Kankrej cows across four seasons; (e) Serum MDA concentration of Sahiwal and Kankrej cows across four seasons. [Note-bars bearing different alphabets (a,b,c) shows significant difference in Sahiwal cows while bars bearing different symbols ($,#) shows significant difference in Kankrej cows for serum uric acid levels between seasons].

Giri *et al.* (2018) also reported a decreased serum DPPH scavenging activity in serum of Jersey cows when they were exposed to substantial cold stress. However, data pertaining to seasonal modulations in DPPH scavenging activity of serum in livestock species is still lacking. Earlier studies on DPPH scavenging activity were mostly centred on food items including animal products. Recently there have been few studies that employed the evaluation of DPPH scavenging activity in blood plasma/serum to assess TAC in animals (Lamo *et al.* 2020). The FRAP assessment is also a widely used method employed to evaluate the TAC in animals. The FRAP values were significantly higher ($p<0.05$) during the hot-humid and summer seasons in both Sahiwal and Kankrej breeds, compared to Spring season (Fig. 2b). Between breed analysis revealed a statistically higher ($p<0.05$) FRAP in Kankrej cows during summer season, but other seasons did not reveal any breed influence. The variation during summer season might be due to difference in thermal adaptability. The TAC of Sahiwal cows in terms of their serum FRAP was found to be significantly higher ($p<0.05$) during the hotter months of summer and hot-humid seasons, compared to winter and spring seasons. Similarly, the FRAP values in Kankrej cows were significantly higher ($p<0.05$) during the hotter months of summer and hot-humid seasons, compared to winter and spring seasons. On breed-wise comparison, the FRAP values were significantly higher in Kankrej cows during summer season compared to Sahiwal cows. However, no significant breed interaction was evident in FRAP values of Sahiwal and Kankrej cows across hot-humid, winter, and spring seasons. The trends evident in FRAP values in our study concurs with the earlier reports of Kumar *et al.* (2019) who reported an increased FRAP in Hariana Bos indicus cows as the THI increased to 84. Similar to our findings, Cecchini and Fazio (2020) also reported increased FRAP in stressed hen. The FRAP is mainly determined by endogenous antioxidants like ascorbic acid (vitamin C), α-tocopherol (vitamin E), uric acid, bilirubin, and polyphenolic compounds while disregarding thiol compounds like GSH (an important cellular antioxidant) and high molecular mass compounds like albumin (Benzie and Devaki 2018). The increase in FRAP can be attributed to the response of animal body to an increased oxidative challenge as the THI increases. The animals cope with increased attack of pro-oxidants by increasing the various antioxidant molecules and enzymes (Mirzad *et al.* 2017), that translates into an increased TAC. Contradictory to present findings, there have been reports of a decreased FRAP in cows when they were subjected to thermal stress conditions (Aengwanich *et al.* 2011, Giri *et al.* 2018). However, the disagreement in FRAP values may be on the account of difference in antioxidant capability of the cow breeds under study, as animals that are able to ward of oxidative challenge on account of a better evolved antioxidant system will in turn have higher TAC/FRAP.

The two important endogenous antioxidants, GSH and Uric acid (Fig. 2c and 2d) in serum samples of the two cattle breeds investigated were also evaluated. The GSH levels were evaluated in both Sahiwal and Kankrej cows, across four seasons. The GSH levels revealed a decreasing trend with the increase in THI in both Sahiwal and Kankrej cows. This concurs with the findings of Sakatani *et al.* (2012) who reported significantly lower GSH levels in Japanese black cows during summer season, compared to thermoneutral season. Interestingly, the GSH values were significantly higher during the spring season in both Sahiwal and Kankrej cows, compared to all the three seasons of winter, summer and hot-humid ambience. The lowered values of GSH during stressful periods have been reported in several studies in animals (Bozuklukhan *et al.* 2017, El-Mandrawy and Alam 2018, Yeotikar *et al.* 2019). The understanding of GSH metabolism suggest a shift towards oxidation, wherein GSH gets oxidized to produce GSSG, as a response of animal body to deal with free radical attack (Lakritz *et al.* 2002). It is a well-established fact that GSH is a potent free radical scavenger. However, the GSH level is under the control of numerous factors that works in tandem, the feed quality and availability being one of them (Nečasová *et al.* 2019). The availability of quality green feed during spring season also translated into higher GSH levels in both Sahiwal and Kankrej cows. The present study noted significant breed interaction in GSH levels across winter, spring and hot-humid seasons, with significantly higher levels in Sahiwal cows across all three seasons. This concurs with the reports of Kumar *et al.* (2019) who found variation in GSH levels according to breed, while working with different Indian goat breeds. Uric acid, another potent endogenous radical scavenger was also evaluated in both Sahiwal and Kankrej cows, across all four seasons. The uric acid levels were significantly higher during hot-humid season in both Sahiwal and Kankrej cows. While no significant difference was evident in uric acid levels during summer season. This is in line with the findings of Giri *et al.* (2018) and Sakatani *et al.* (2012) who found higher uric acid levels when cows were under thermal stress. Recent metabolomic insights into candidate markers of HS revealed significant fold increase in uric acid of heat stressed Holstein cows (Fan *et al.* 2018). Rathwa *et al.* (2017) also reported a higher Uric acid level during summer season in indicine sheep breed. Similarly, higher uric acid levels were reported in the urine of Xuanhan yellow cattle compared to crossbred cows and yaks, concomitant with lower allantoin levels (Liao *et al.* 2018). The higher level of uric acid under stressful conditions is believed to be associated with higher purine metabolism, as uric acid is a by-product of purine metabolism the uric acid values increases concomitantly. Whenever the animal body encounters oxidative stress that manifests into higher ROS, uric acid gets converted to allantoin, however animals capable of coping with OS challenge register higher uric acid that helps in warding off the OS by scavenging the free radicals.

The extent of lipid peroxidation in terms of MDA was also evaluated in both Sahiwal and Kankrej cattle

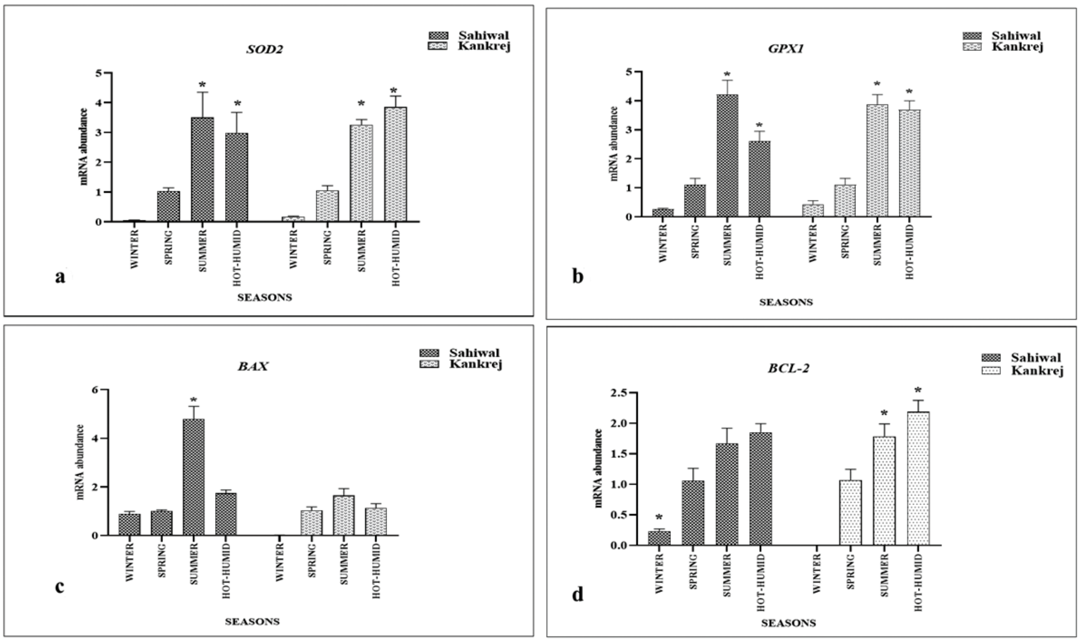


Fig. 3. Graphs showing (a) Expression profile of SOD2 mRNA across four seasons in Sahiwal and Kankrej PBMCs; (b) Expression profile of GPX1 mRNA across four seasons in Sahiwal and Kankrej PBMCs; (c) Expression profile of BAX mRNA across four in Sahiwal and Kankrej PBMCs; (d) Expression profile of Bcl-2 mRNA across four seasons in Sahiwal and Kankrej PBMCs.

breeds, across all four seasons. The susceptibility of lipids, particularly the unsaturated fatty acids to free radical damage makes the assessment of lipid peroxidation in terms of their biomarkers like MDA, and related TBARS a crucial indicator of oxidative stress in various living systems (Georgieva 2005). The MDA values in our study revealed a significant increase in both Sahiwal and Kankrej cows (Fig. 2e), as the animal body transitioned from thermoneutral season to hotter months of summer and hot-humid seasons. This is in line with reports of Sengar *et al.* (2017), Aengwanich *et al.* (2011) and Bernabucci *et al.* (2002) who found significantly higher MDA levels in heat stressed cows. Furthermore, studies centred around the seasonal modulations in lipid peroxidation has found a concomitant increase in MDA levels as the THI increased (Sakatani *et al.* 2012, Chaudhary *et al.* 2015, Lakhani *et al.* 2018), indicating a positive correlation between OS and lipid peroxidation. The increased attack of free radicals that increases the extent of lipid peroxidation explains the higher MDA values during the higher THI months, that is associated with OS during summer and hot-humid months. However, no significant breed interaction was noticed in the extent of lipid peroxidation in this study.

Among the antioxidant defence mechanisms evolved in animals, Superoxide dismutase enzyme is the first line of defence in fighting of the oxidative challenge posed by free radicals during stress. In this study, the expression of *SOD2* that translates into the mitochondrial Mn-SOD was significantly ($p<0.05$) higher during the summer and hot-humid months in the PBMCs of both Sahiwal and Kankrej cows (Fig. 3a). Concurring with our findings, ~3-fold and ~2-fold increase in *SOD2* mRNA expression was seen in heat stressed dermal fibroblasts of Tharparkar and Karan Fries cows, respectively (Singh *et al.* 2020). Similar induction of *SOD2* mRNA following heat stress challenge was observed in heat stressed bovine granulosa cells that persisted with increased temperature of heat stress treatment (Khan *et al.* 2020). Furthermore, Mustafi *et al*. (2009) also reported overexpression of MnSOD during chronic heat stress in hamster lung. Along the same line, both CuSOD (*SOD1*) and MnSOD (*SOD2*) expression increased several folds in the heat stressed skeletal muscles of crossbred gilts (Ganesan *et al.* 2017). As increased activity of SOD is associated with heat stress (Gill *et al.* 2017, Abbas *et al.* 2020), this translates into higher induction of gene encoding SOD to fight the oxidative challenge posed by free radicals, that is one of the consequences of heat stress in animals. Contradictory to these, Madhusoodan *et al.* (2020) reported a significant decrease in SOD mRNA expression in the hepatic tissue of Salem black goats, which indicated the lack of heat shock response on the account of low HS. Similar to *SOD2*, the mRNA expression of *GPX1* was also significantly ($p<0.05$) higher during the higher THI months of summer and hot-humid seasons (Fig. 3b). GPx is one of the key members of antioxidant defence triad and especially crucial for protection of mitochondria against peroxide free radicals. Higher activity of GPx has been associated with heat stress in Indian crossbred cows (Sengar *et al.* 2017, Jeelani *et al.* 2019). Interestingly, both *SOD2* and *GPX1* showed a significant downregulation post heat stress in the skeletal muscles of Merino × Poll Dorset crossbred ewes following chronic heat stress, which indicates the difference in response to HS (Chauhan *et al.* 2014). The trends in *GPX1* mRNA expression in this study coincides with the significant decrease in GSH levels with the increased *GPX1* mRNA abundance, indicating the oxidation of GSH to GSSG by GPX1 in order to neutralize the peroxide molecules.

The expression pattern of genes involved in apoptosis pathway helps in assessing the sensitivity of cells to programmed cell death. The members of Bcl-2 protein family are key mediator of caspase-mediated cell death, which includes the pro-apoptotic Bax and anti-apoptotic Bcl-2 as key members (Donovan and Cotter 2004, Sharpe *et al.* 2004, Li *et al.* 2011). In this study, the *BAX* mRNA expression registered a significant increase during summer season in Sahiwal PBMCs, however, the expression of *BAX* though higher, was statistically not significant in Kankrej PBMCs (Fig. 3c). Following induction in summer season, there was a downward trend in *BAX* mRNA expression in hot-humid season. Interestingly, the *BCL2* mRNA expression also revealed an upward trend during summer, however, the induction was non-significant in the PBMCs of Sahiwal cows. On the other hand, the *BCL2* mRNA expression was significantly higher during summer and hot-humid seasons in the PBMCs of Kankrej cows (Fig. 3d). This is in agreement with the findings of Xi *et al.* (2017) who reported concomitant increase in both *BAX* and *BCL2* expression in boar testes exposed to heat stress of 42°C for three hours. Similarly, the *BAX* and *BCL2* expression was significantly higher in the PBMCs of Sahiwal cows during summer season (Somal *et al.* 2015). On the same line, both *BAX* and *BCL2* expression registered significant increase in the heat stressed bovine granulosa cells (Li *et al.* 2016). The Bax/Bcl ratio acts as a fine trigger involved in stimulation of apoptosis through caspases (Yuan *et al.* 2012) and their relative concentration determines the fate of cells during stressful periods (Setroikromo *et al.* 2007). BCL2 is a pro-survival protein and prevents apoptosis (Hardwick and Soane 2013), under physiological harmony it also binds to pro-apoptotic counterparts like BAX. Stressful situation disturbs this fine balance and BAX is released which is known to alter the mitochondrial membrane permeability in the favour of apoptosis (Korsmeyer 1999).

In conclusion, the present study highlights the crucial role played by antioxidant defence system in heat stress tolerance of cows reared in harsh tropical desert environment. The transcription profile of crucial genes implicated in antioxidant pathway and apoptosis pathway revealed key modulations based on outside environment, that highlights molecular adaptation in the indicine breeds.

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