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Seasonal Change in Oxidative Stress Markers in Blood Plasma of Tharparkar (*Bos indicus*) and Karan Fries (*Bos indicus x Bos taurus*) Cattle under Tropical Climate

Uttarani Maibam^{1*}, Om K. Hooda¹, Phurailatpam S. Sharma²,
Sohan V. Singh¹ and Ramesh C. Upadhyay¹

¹Animal Physiology Division, ICAR-National Dairy Research Institute, 132001-Karnal, Haryana, India

²Animal Nutrition Division, ICAR-National Dairy Research Institute, 132001-Karnal, Haryana, India

*Corresponding author

ABSTRACT

Keywords

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The study aimed to evaluate oxidative stress status in cattle breeds of different thermotolerance during different seasons. Ten each of Tharparkar (*Bos indicus*) and Karan Fries (*Bos indicus x Bos taurus*) heifers were selected from NDRI herd, Karnal. Animals were maintained under standard managerial practices followed at the farm. Blood samples were aseptically collected from each animal during winter, spring and summer. Oxidative stress markers (ROS, GPx and SOD) and heat stress markers (HSP70.1 and HSP70.2) were determined by ELISA. In both the breeds, higher ($P<0.05$) levels of ROS, GPx, SOD and HSP70s were observed during summer followed by winter and spring. Breeds showed no significant difference during winter and spring. During summer, ROS, antioxidant enzymes and HSP70s were higher ($P<0.05$) in Karan Fries than Tharparkar. The study concludes that plasma levels of ROS, antioxidant enzymes and HSP70 were strongly affected by seasonal change in temperature. Plasma stress markers can be used for evaluation of the effect of thermal stress on oxidative status of cattle. Summer conditions capable of producing heat stress cause oxidative stress in cattle. Higher generation of oxidative stress in Karan Fries might be an important factor for their greater sensitivity to heat stress than Tharparkar.

Introduction

Free radical reactions are the integral part of normal metabolism. Normally, oxidation is necessary to provide energy for maintenance of cellular integrity and functions (Miller *et al.*, 1993). In normal cellular metabolism, low to moderate ROS is generated as part of the signaling pathways, and in innate and adaptive immune response against danger signals (Martins Chaves *et al.*, 2000). When

reactive forms of oxygen are produced faster than they can be safely neutralized by antioxidant mechanisms, oxidative stress results (Sies, 1991). OxS is considered as a part of the stress response to heat exposure (Lin *et al.*, 2006). OxS can be induced by both exogenous and endogenous factors in living organisms (Cross *et al.*, 1998; Lesser, 2006). Animals are exposed to a wide variety

of environmental conditions during different seasons of the year. Physiological, biochemical, and morphological responses vary with environmental conditions and animal's genetic makeup (Maibam *et al.*, 2014).

At the same time, the climate is changing. Significant changes in physical and biological systems have already occurred in all continents and in most oceans. Most of these changes are in them direction expected with warming temperature (Rosenzweig *et al.*, 2008). It is also very likely that heat waves will occur with a higher frequency and duration. The Intergovernmental Panal on Climate Change (IPCC) projected that there will be an increase in temperature by 0.2°C per decade, and predicted that the increase in global average surface temperature would be between 1.8°C to 4.0°C by 2100 (IPCC, 2007). High environmental temperature challenges the homeostatic system and stimulates excessive production of free radicals (Bernabucci *et al.*, 2002). Production of superoxide radicals increases from threshold concentration due to thermal stress (Ganie *et al.*, 2013). A well organized system of both chemical and enzymatic antioxidants which work in a synergistic manner to protect it against the overload of oxidant species (Briganti and Picardo, 2003). However, continuous rise in air temperature does not protect cellular damage due to disequilibrium between diverse physiological and cellular functions (Patir and Upadhyay, 2010). These conditions can contribute or lead to the onset of health disorders in cattle (Miller *et al.*, 1993). ROS induced damage of cells and molecules is reported to be one of the reasons responsible for the decline in an animal's performance during heat stress (Mujahid *et al.*, 2006).

Studies on the effects of heat stress on oxidative status in cattle are lacking, and only

preliminary reports are available. Oxidative stress can be monitored with several biomarkers (antioxidants and pro-oxidants) which can be assessed in plasma and/or erythrocytes (Passi *et al.*, 2001). Zebu cattle are reported to be more heat tolerant than crossbred cattle. We assumed that the oxidative status of zebu and crossbred cattle might be impaired differentially by heat stress. Environmental conditions vary significantly during different seasons in tropical climate (Maibam *et al.*, 2017). Therefore, we analyzed changes in several markers of oxidative status and heat stress in plasma of Tharparker and Karan Fries cattle during different seasons.

Materials and Methods

The experiment was conducted in the cattle yard of the National Dairy Research Institute (NDRI), Karnal, Haryana, altitude 250 m, 29° 42" N and 79° 54" E. Ambient temperature ranges from near 0°C in winter to 45°C in summer, and annual rainfall averages 700 mm.

Animals and ethical permission

Ten each of Tharparkar and Karan Fries heifers of approximately 2 years of age were selected from NDRI herd for experiments during winter, spring and summer season. All these animals were maintained under general management practices followed for heifers at the institute. At the time of the actual experiment, all the animals were clinically healthy and free from any physical or anatomical abnormalities. The experiment was approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the article number 13 of the CPCSEA–rules, laid down by Government of India. Norms regarding the ethical treatment of animals during the whole operation were strictly followed.

Blood sampling and clinical parameters

From each animal, blood samples were collected twice per season during winter (Dec-Jan), spring (Feb-Mar) and summer (May-June) season. Blood samples (10 ml) were collected from animals by jugular vein puncture in Lithium Heparin coated Vacutainer® tubes (BD Biosciences, Franklin Lakes, NJ, USA) under aseptic conditions and were transported to the laboratory under refrigeration (4°C). The collected blood samples were centrifuged at 2300 rpm for 40 minutes to separate the plasma. Plasma samples were analysed for the levels of oxidative stress markers (ROS, GPx, SOD) and heat stress markers (HSP70.1, HSP70.2). On the day of blood sampling, skin and rectal temperature of each animal were recorded. The peripheral skin temperature at different anatomical sites viz. forehead, dorsal, and flank regions of each animal were recorded using infrared thermometer (Raytek, Model Raynger ST2L, M/s. Surrey Scientific, Surrey, U.K.) by keeping it 2–3 in. away from the skin surface site and average of different sites were taken. Rectal temperature was recorded using digital thermometer by keeping the thermometer in contact with the rectal mucosa of animal for about 2 min. Data on meteorological variables was collected from CSSRI, Karnal and THI was calculated using the formula $0.72 (T_{db}^{\circ}C + T_{wb}^{\circ}C) + 40.6^{\circ}$ (Johnson *et al.*, 1963).

Estimation of oxidative stress markers

Reactive oxygen species (ROS), glutathione peroxidase (GPx) and superoxide dismutase (SOD) were determined using Bovine Reactive Oxygen Species, Bovine Glutathione Peroxidase and Bovine Superoxide Dismutase ELISA kit supplied by MyBioSource, San Diego, California, US according to the manufacturer's instructions. The sensitivity for ROS and GPx assay was 1ng/ml and that for SOD was 0.1µg/ml.

Estimation of heat stress markers

Similarly, heat shock proteins viz. HSP70.1 and HSP70.2 were determined using bovine specific HSP70.1 and HSP70.2 ELISA kit, respectively supplied by MyBioSource, San Diego, California, US. The sensitivity for HSP70.1 and HSP70.2 assay kits was 1ng/ml and 0.1ng/ml, respectively.

Statistical analysis

Data of present study were normally distributed as checked by Shapiro-Wilk test in SAS system. Data were analyzed by analysis of variance using SAS software, version (9.1) of the SAS system for Window, Copyright© (2011) SAS Institute Inc., Cary, NC, USA. Results were expressed as the means ± SEM. A difference with value $P < 0.05$ was considered statistically significant.

Results and Discussion

Environmental conditions and clinical parameters

During spring, mean daily values of THI was 51.9. In winter, mean daily values of THI was equal to 64.5 and it increased to 80.7 during summer (Table 1). THI during summer was above 80 representing severe heat stress. Rectal and skin temperatures differed significantly ($P < 0.05$) between seasons in both Tharparkar and Karan Fries. RT and ST were higher ($P < 0.05$) in Karan Fries than Tharparkar during summer (Table 2).

Oxidative stress markers

ROS levels in blood plasma of zebu (Tharparkar) and crossbred (Karan Fries) cattle during different seasons are presented in Figure 1. Plasma ROS production was significantly ($P < 0.05$) higher during winter and summer than spring season in both

Tharparkar and Karan Fries cattle. Highest concentration of ROS was observed during summer in both the breeds. Breed differences were non-significant during spring and winter season. However, plasma ROS level was significantly ($P<0.05$) higher in Karan Fries than Tharparkar cattle during summer season.

Similarly, plasma levels of antioxidant enzymes (GPx and SOD) were also significantly ($P<0.05$) higher during winter and summer compared to spring season in both Tharparkar and Karan Fries. The two breeds showed highest concentrations of GPx and SOD during summer (Figure 2 and 3). The levels of antioxidant enzymes during winter and spring season showed no significant differences between Tharparkar and Karan Fries. But, GPx and SOD levels during summer were significantly higher in crossbred (Karan Fries) than zebu (Tharparkar) cattle.

Heat stress markers

Plasma levels of HSP70.1 and HSP70.2 levels increased significantly ($P<0.05$) during winter and summer compared to spring season in both Tharparkar and Karan Fries (Figure 4 and 5). The magnitude of increase was significantly ($P<0.05$) higher during summer than winter in both the breeds. HSP70.1 and HSP70.2 levels showed no significant differences between the breeds during winter season. However, plasma levels of HSP70i were significantly ($P<0.05$) higher in crossbred (Karan Fries) than zebu (Tharparkar) cattle during summer.

The present findings indicated that there were marked variations in the plasma levels of oxidative stress markers (ROS, GPx and SOD) and heat stress markers (HSP70.1 and HSP70.2) of Tharparkar and Karan Fries cattle during different seasons. The parameters under study were significantly

higher during winter and summer compared to spring in both the breeds. These changes might be due to thermal (hypothermic or heat) stress induced in animals during winter and summer season. The weather conditions (Table 1) prevailed during the study was sufficient to elicit stress response to the animals. Skin and rectal temperature of Tharparkar and Karan Fries cattle also showed marked variations with seasonal change in temperature (Table 2). Bello-Klein *et al.*, (2000) also indicated that seasonal alterations of the oxidative status depend more on weather conditions such as temperature and humidity.

The magnitude of increase in plasma levels ROS, GPx, SOD and HSP70s was higher during summer than winter in both Tharparkar and Karan Fries. Among the stressors, heat stress has been of major concern in reducing animal's productivity in tropical, sub-tropical and arid areas (Silanikove *et al.*, 1997). Lovell *et al.*, (2007) also reported that heat greatly accelerates the synthesis of the HSP70 and this HSP70 was found to have both cellular and systemic protective role (Kregel, 2002) Banerjee *et al.*, (2014) reported higher expression of HSP70 during summer than winter season in goat breeds. Heat stress was also suggested to be responsible of inducing oxidative stress during summer in livestock animals (Ganaie *et al.*, 2013; Nizar *et al.*, 2013). Normally, oxidative metabolic process is essential to provide energy for maintenance of cellular integrity and functions (Miller *et al.*, 1993). But, when cells are exposed to heat stress there is acceleration of mitochondrial respiration (Keller *et al.*, 2004) and promotion of cellular oxidation. Similar findings were reported by Yang *et al.*, (2010) who observed a significant increase in ROS and antioxidant enzymes on exposure to heat stress. Zuo *et al.*, (2000) and Mujahid *et al.*, (2005) demonstrated the heat-induced increase of

ROS production. Kaldur *et al.*, (2014) reported that heat acclimation increases OxS level in human. Kataria *et al.*, (2010) also observed that hot environmental conditions can produce oxidative stress in cows by modulation of physiological mechanisms in response to heat stress. Bernabucci *et al.*, (2002) demonstrated increase in level of antioxidant enzymes to neutralize the adverse effect of free radicals produced. Therefore, we speculate that the coordinate increase in plasma GPx and SOD observed in the present study is an indirect compensatory response of cells to increased oxidant challenge during heat stress. Chandra and Aggarwal (2009) also reported higher erythrocyte antioxidant enzyme levels in prepartum crossbred cows during summer than winter season. A study on the effect of winter and summer seasons on antioxidant status of growing heifers and lactating Murrah buffaloes reported significantly higher erythrocyte SOD and CAT levels during summer than winter

(Lallawmkimi, 2009). The coordinate upregulation of HSP70 observed in the present study may also potentiate the protective effect of antioxidant enzyme through its chaperone functions. It was reported that HSP upregulation reduces oxidative stress (Kalmar and Greensmith, 2009), improves antioxidant capacity and inhibits the lipid peroxidation production (Gu *et al.*, 2012), thus protects the animal from harmful effects of heat stress. Similar findings were reported by Droge (2002) who observed that body releases HSPs in addition to antioxidant enzymes on exposure to heat stress to protect itself from the deleterious cellular effects of ROS.

The present investigation observed that there was no significant difference between zebu (Tharparkar) and crossbred (Karan Fries) cattle in plasma levels of ROS, GPx, SOD and HSP70s during spring and winter season.

Table.1 Mean environmental variables during different seasons of experimental period

Season	Max. temp (°C)	Min. temp (°C)	RH %	T _{db} (°C)	T _{wb} (°C)	THI
Thermoneutral	23.4	11.4	75.0	18.0	15.2	64.5
Winter	14.8	6.6	85.5	11.2	10.0	51.9
Summer	40.3	23.6	43.0	33.1	22.6	80.7

Table.2 Skin and rectal temperature of Tharparkar and Karan Fries cattle during different seasons

Parameters	Breeds	Season		
		Thermoneutral	Winter	Summer
Skin temperature (°C)	Tharparkar	33.83±0.21 ^{bA}	24.77±0.63 ^{cB}	39.07±0.23 ^{aB}
	Karan Fries	34.32±0.78 ^{bA}	28.97±0.55 ^{cA}	43.01±1.16 ^{aA}
Rectal Temperature (°C)	Tharparkar	38.41±0.06 ^{bA}	37.84± 0.06 ^{cB}	38.88±0.05 ^{aB}
	Karan Fries	38.58±0.03 ^{bA}	38.13 ^c ± 0.07 ^{cA}	39.47 ^d ± 0.06 ^{aA}

*Values are means ± S.E of twenty observations of ten animals. Columns with different superscripts a, b, c and rows with different superscripts A,B differed significantly (P<0.05).

Fig.1 ROS levels (ng/ml) in blood plasma of Tharparkar and Karan Fries cattle during different seasons. Bars with different superscripts a,b,c and A,B differed significantly ($P<0.05$) between seasons and breeds, respectively.

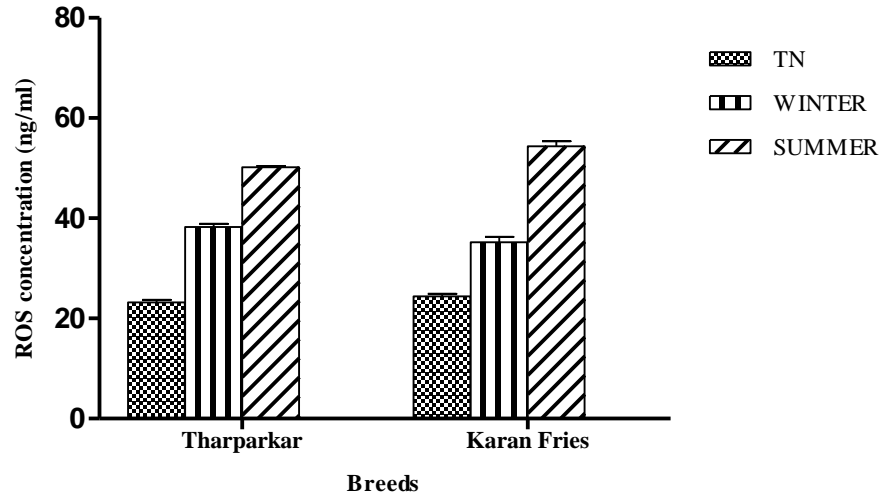


Fig.2 GPx levels (ng/ml) in blood plasma of Tharparkar and Karan Fries cattle during different seasons. Bars with different superscripts a,b,c and A,B differed significantly ($P<0.05$) between seasons and breeds, respectively.

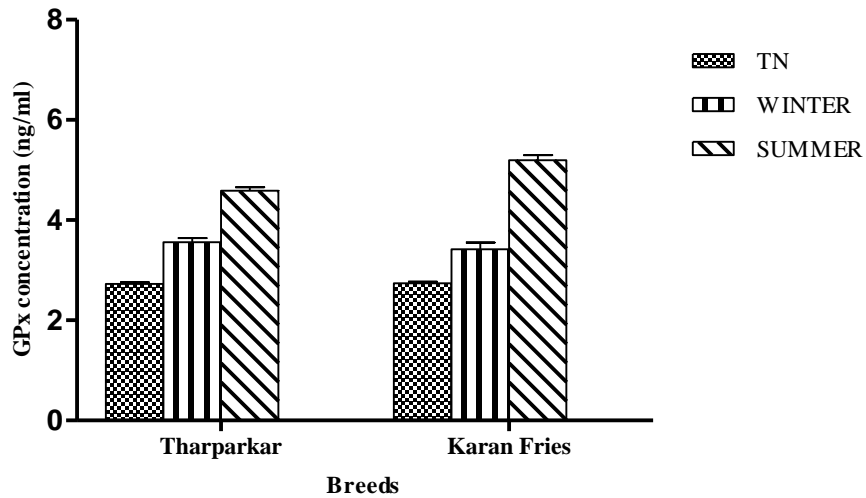


Fig.3 SOD levels (ng/ml) in blood plasma of Tharparkar and Karan Fries cattle during different seasons. Bars with different superscripts a,b,c and A,B differed significantly ($P<0.05$) between seasons and breeds, respectively.

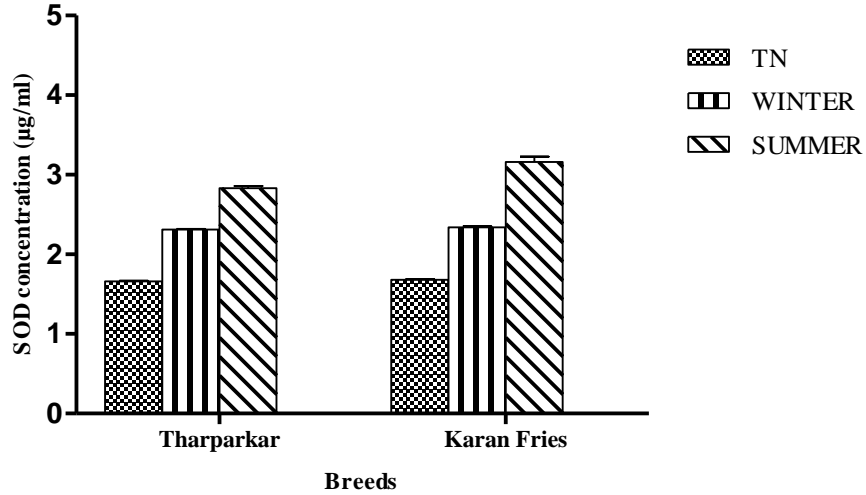


Fig.4 HSP70.1 (ng/ml) in blood plasma of Tharparkar and Karan Fries cattle during different seasons. Bars with different superscripts a,b,c and A,B differed significantly ($P<0.05$) between seasons and breeds, respectively.

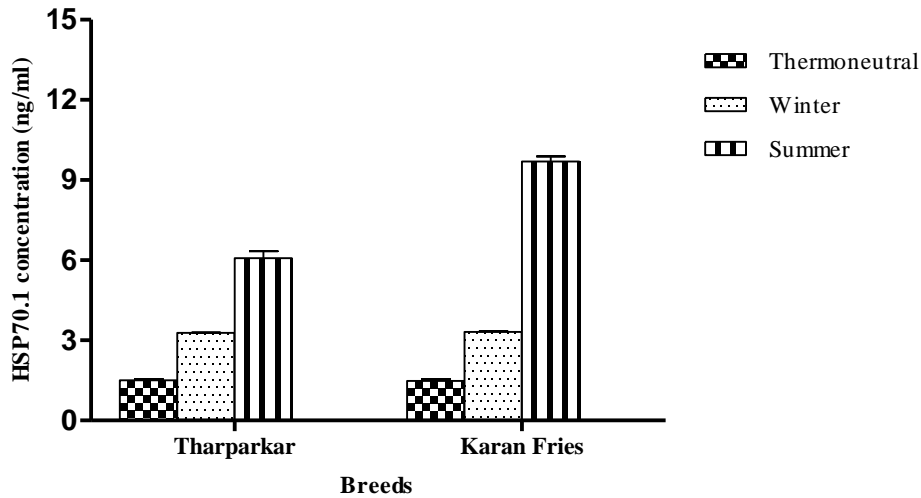
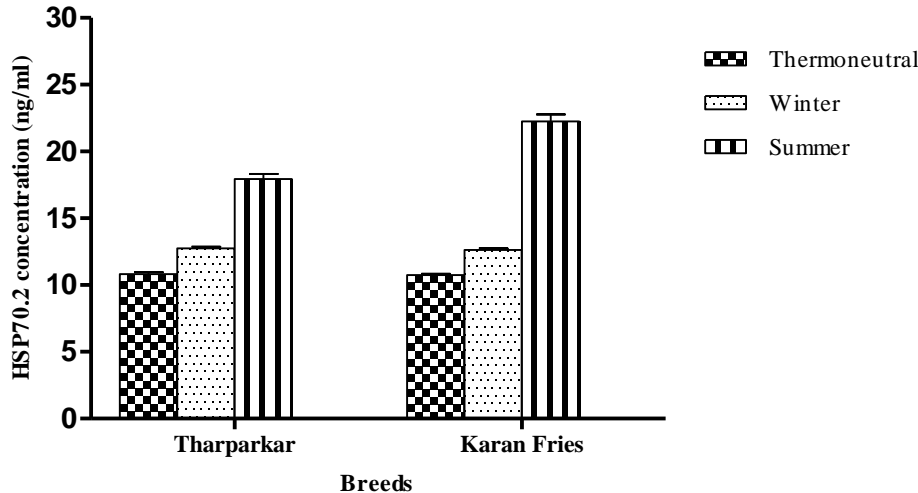


Fig.5 HSP70.2 (ng/ml) in blood plasma of Tharparkar and Karan Fries cattle during different seasons. Bars with different superscripts a,b,c and A,B differed significantly ($P<0.05$) between seasons and breeds, respectively.



However, all these parameters were significantly higher in Karan Fries than Tharparkar during summer season. These differences might be attributed to the differences in thermotolerance of the two breeds to heat stress. The higher abundance of oxidative stress markers (ROS, GPx, SOD) and heat stress markers (HSP70.1 and HSP70.2) in Karan Fries during summer indicated that they are under more stress during summer heat than Tharparkar. Lacetera *et al.*, (2006) reported that HSP and oxidative stress levels are one of the most important stress markers to know the animal's comfort level and adaptability. Gihan *et al.*, (2009) reported that breeds have variations in physiological and antioxidant enzyme activities. Therefore, in the present study, higher levels of ROS, GPx, SOD and HSP70s levels in Karan Fries than Tharparkar during summer season indicated higher status of oxidative stress with greater sensitivity to heat stress.

In conclusion, increase in plasma levels of HSPs, GPx and SOD during winter and summer seasons are likely to represent adaptive changes of cattle in response to

thermal stress. The difference in plasma levels of stress markers with seasonal change in environmental temperature indicates that plasma markers can be used for the evaluation of the effect of thermal stress on cattle. Summer conditions capable of producing heat stress cause oxidative stress in cattle. The generation of higher amount of reactive oxygen species and oxidative stress status in Karan Fries might be an important factor for their greater sensitivity to heat stress than Tharparkar.

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