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Expression of Mn-SOD and Cu,Zn-SOD of Sahiwal and Karan-Fries in response to acute heat exposure

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ABSTRACT

The present study aimed to investigate the expression of Mn-SOD and Cu,Zn-SOD mRNA in lymphocytes of Sahiwal and Karan-Fries during acute thermal exposure. Six numbers each of Sahiwal and Karan-Fries heifers in the age group of 2–2.5 years were exposed at 40±1°C and 50% relative humidity inside a psychrometric chamber for 4 h in a day. Respiration rate, heart rate and rectal temperatures were recorded to calculate heat tolerance index by calculating Dairy Search Index (DSI). Blood samples were collected at 0, 1, 2, 3 and 4 h of heat exposure for estimation of Mn-SOD and Cu,Zn-SOD mRNA expression by RT-PCR. DSI of Sahiwal was not influenced by heat exposure even after 4 h of heat exposure. After 1 h of heat exposure, DSI of Karan-Fries increased which further escalated after 3 h of exposure in Karan-Fries. Mn-SOD expression increased after 4 h and 2 h of heat exposure in Sahiwal and Karan-Fries respectively, however the expression declined after 3 h of exposure in Karan-Fries. Expression of Cu,Zn-SOD mRNA of Sahiwal and Karan-Fries was not influenced by heat exposure. The study revealed better equipped antioxidant system of Sahiwal than Karan-Fries in response to acute thermal exposure.

Key words: Cu, Zn-SOD, Heat stress, Karan-Fries, Mn-SOD, Sahiwal

Thermal stress occurs due to any single or a combination of environmental factors when the effective temperature of the environment is higher than the animal's thermoneutral zone. The heat stress impinging on the animal causes a chain of physiological, anatomical and behavioural changes leading to a reduction in productive functions. The reduction of productivity with devastating economic consequences to the global dairy industry due to warm environment has been documented (Bernabucci *et al.* 2010).

Thermal stress can also lead to oxidative stress in a living organism as a result of imbalance between the production of reactive oxygen metabolites and the capacity of the antioxidant mechanism to neutralize these reactive oxygen species (Sies 1997, Duanghaklang *et al.* 2015). Heat stress induced increase in SOD enzyme activity had been well documented both *in vitro* (Yadav *et al.* 2011) and *in vivo* (Yatoo *et al.* 2014, Yadav *et al.* 2015, Maibam *et al.* 2017). There was increase in expression of Mn-SOD and Cu,Zn-SOD mRNA in response to oxidative stress due to immobilization (Oishi and Machida 2002), heat stress in skeletal muscle of pig (Montilla *et al.* 2014), sheep (Chauhan *et al.* 2014) and expression of Cu,Zn-SOD mRNA had been found to correlate the degree of thermotolerance of bovine embryos (Lazzari *et al.* 2002).

In the present study, attempts had been made in order to study the *in-vivo* expression of Mn-SOD mRNA and Cu,Zn-SOD mRNA of a Zebu cattle, Sahiwal and crossbred cattle, Karan-Fries (KF) in relation to their heat tolerance indices when subjected to acute heat exposure. The study will add in better understanding of the cellular responses adopted by Zebu and crossbred cattle during thermal exposures which will thereby help in management of livestock to ameliorate the impact of global climate change.

MATERIALS AND METHODS

Experimental animals: Sahiwal and Karan-Fries (Tharparkar × Holstein-Friesian) heifers numbering 6 each in the age group of 2 to 2.5 years maintained at National Dairy Research Institute (NDRI), Karnal, India were selected. The average body weight of animals was 301.3±6.91 kg. The animals were given a maintenance concentrate mixture @1 kg/animal in addition to *ad lib.* roughages and water as per Kearl's standard (Kearl 1982). Concentrate mixture consisted of mustard cake, maize, wheat bran, rice bran, mineral mixture and salt. The CP and TDN in diet was 12% and 60% respectively.

Thermal exposure: The study was carried out during period when Tmax/Tmin was 30.80°C/13.60°C. Sahiwal and Karan Fries heifers were placed in the psychrometric

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chamber (temperature and humidity controlled) with a dimension of 22'6''×10'10''×8' for 4 h (from 9 AM to 1 PM) maintained at 40±1°C and 50% relative humidity (RH) for inducing acute heat stress. Heat exposure was carried out for 4 h in one day. Prior to the experiment, all the animals were kept in the psychrometric chamber at the prevailing temperature for 4 h every day for 10 days to acclimatize to the chamber environment.

Physiological indices and dairy search index (DSI): Respiration rate (RR breaths/min) was recorded by observing the abdominal movement. Heart rate (HR beats/min) of individual animals was calculated from the electrocardiograms (ECG) of individual animals. Rectal temperature (°C) was recorded with a digital thermometer by keeping the thermometer in contact of rectal mucosa for about 2 min. DSI was calculated by following the formula given by Thomas *et al.* (1973)

$$DSI = 0.5\frac{x1}{x} + 0.2\frac{y1}{y} + 0.3\frac{z1}{z}$$

where x1, y1 and z1 were the observed RT, RR and HR, respectively while x, y and z were normal/expected RT, RR and HR rate, i.e. 38.33°C, 23 breaths/min and 78 beats/min, respectively.

Blood sampling and total RNA separation: Blood sample (2 ml each) were collected at 0, 1, 2, 3, and 4 h of heat exposure for separation of peripheral blood mononuclear cells (PBMC) and subsequent isolation of total RNA. PBMC were separated by gradient centrifugation with Histopaque (Sigma). The PBMC were cultured by incubation at 38°C for 3 h in lymphocyte selective media, RPMI-1640 supplemented with penicillin @ 100 mg/ml, streptomycin @ 100 mg/ml and bovine serum albumin @ 0.5% to obtain the lymphocytes. The lymphocytes were collected by centrifugation and dried to make pellets. The cell pellets were stored at –20°C until RNA isolation. Total RNA was isolated by using Tri reagent (Sigma) as per the guidelines with slight modifications. After determining the purity and quality of RNA, the RNA samples were reversed transcribed to cDNA by using RevertAid First Strand cDNA synthesis kit (Fermentas). From the reversed transcribed cDNA, PCR was carried out for Mn-SOD and Cu,Zn-SOD genes by using specific primers. The GAPDH gene was used as a house keeping gene for relative measure of expression of desired genes. The details of the primers used in the experiment are presented in Table 1.

RT-PCR of Mn-SOD and Cu,Zn-SOD: The PCR of Mn-SOD cDNA and Cu,Zn-SOD cDNA and subsequent record of amplified products by SDS-PAGE were carried out by following the methods as described by Sambrook *et. al.* (1989) with slight modifications. Semi quantitative measure of expression of mRNA of genes of interest was obtained from the digital pictures recorded from the electrophoresed products. The integrated density value (IDV) of each of the bands was measured in GelDoc (Image AIDE 10990* Syn1234*mpcs5870337c Spectronics, Gel Doc Software). Expression of mRNA of gene of interest was obtained from the ratio of IDV of specific gene to IDV of GAPDH.

The data were analyzed by using SYSTAT Version 6.0.1, SPSS Inc., Chicago, IL. The one way analysis of variance (ANOVA) was carried to find out the effect of periods and breed. Fisher's Least-Significant-Difference test was applied to find out Matrix of pair wise comparison probabilities between different groups.

RESULTS AND DISCUSSION

The variation in Mean±SE of RR, HR, RT, DSI, and expression of Mn-SOD and Cu,Zn-SOD of Sahiwal and Karan-Fries during the period of heat exposure are presented in Tables 2 and 3 respectively.

Heat exposure for 3 h caused no significant change in RR of Sahiwal which increased after 4 h of exposure while RR of Karan-Fries increased ($P<0.05$) after 3 h of heat exposure (Table 2). RR during heat exposure is known to increase more rapidly than other responses and often occurs at a lesser critical ambient temperature than other responses such as RT or changes in feed intake (Hahn 1999). In addition, RR (an indicator of respiratory evaporative heat loss) is one of several effecter responses, including sweat rate and peripheral vasodilation that determine the internal body temperature in response to heat stress. In theory, it is only when the avenues for heat loss are compromised, or limits of effectiveness are reached, that there would be an increase in internal body temperature (Scharf *et al.* 2010). Prior to heat exposure and even after heat exposure for 3 h, the two breeds showed statistically similar RT (Table 2). However, after heat exposure for 4 h RT of KF was significantly higher than of Sahiwal (Table 2). The present

Table 1. Details of primers used for amplification of Mn-SOD, Cu,Zn-SOD and GAPDH genes

Gene	Primer sequence	T^A (°C)	Amplicon size (bp)	Gene bank accession number (Reference)
Mn-SOD	5'-CCCATGAAGCCTTTCTAATCCTG-3' 5'-TTCAGAGGCGCTACTATTTCCTTC-3'	64	307	L22092.1 (Lonergan *et al.* 2003)
Cu,Zn-SOD	5'-AAGGCCGTGTGCGTGCTGAA-3' 5'-CAGGTCTCCAACATGCCTCT-3'	60	246	Y00404 (Lazzari *et al.* 2002)
GAPDH	5'-CCCATCACCATCTTCCAGG-3' 5'-AGTGAGCTTCCCGTTCAGC-3'	54	471	(Correa *et al.* 2007)

T^A, Annealing temperature.

Table 2. Effect of heat exposure on RR, HR, RT and DSI of Sahiwal and Karan-Fries (Mean±SE)

Parameter	Breed	Period of heat exposure				
		0 h	1 h	2 h	3 h	4 h
RR (breaths/min)	Sahiwal	30 ± 1^{b}	34 ± 3^{ab}	34 ± 2^{ab}	32 ± 2^{ab}	38 ± 2^{a}
	KF	$38\pm2^{b*}$	45 ± 9^{ab}	49 ± 6^{ab}	$57\pm4^{a*}$	$54\pm4^{a*}$
HR (beats/min)	Sahiwal	92±5	94±4	95±4	93±5	93±5
	KF	78±6	79±8	76±7	78±7	77±8
RT (°C)	Sahiwal	38.44 ± 0.06^{c}	39.00 ± 0.06^{b}	39.22 ± 0.03^{a}	39.28 ± 0.03^{a}	39.32 ± 0.02^{a}
	KF	38.72 ± 0.12^{b}	39.13 ± 0.05^{ab}	39.26 ± 0.04^{ab}	39.33 ± 0.06^{a}	$39.44\pm0.03^{a*}$
DSI	Sahiwal	1.12±0.01	1.17±0.03	1.17±0.03	1.15±0.03	1.20±0.03
	KF	1.14 ± 0.01^{c}	1.21 ± 0.06^{b}	1.23 ± 0.03^{b}	1.30 ± 0.03^{a}	1.28 ± 0.02^{a}

Values in the same row with different superscripts differ significantly (P<0.05). *Indicates significant difference between the breeds (P<0.05).

finding agrees to the previous reports that crossbred cattle had higher RT than Zebu when exposed to extreme heat stress (Gaughan *et al.* 1999, Yadav *et al.* 2016).

Another most commonly used variable to assess heat tolerance is RT because RT is easy to measure, well documented and it makes a reliable index. Above a threshold environmental condition, RT begins to increase as a result of the animal's inability to adequately dissipate the excess heat load by increased respiratory vaporization (Scharf *et al.* 2010). In the present investigation, RT of Sahiwal increased (P<0.05) after heat exposure of 1 h which further increased after 2 h (P<0.05) and then remained stable till 4 h of exposure (Table 2). There was no significant change in RT of Karan-Fries till 2 h of exposure as compared to 0 h of exposure while RT of Karan-Fries recorded after 3 and 4 h of exposure was higher (P<0.05) than that recorded at 0 h of exposure (Table 2). It was evident that Sahiwal increased RT without affecting the RR and HR during heat exposure while Karan-Fries was found to increase RT with increase in RR (Table 2).

HR is another instantaneous measure of sympathetic and parasympathetic activity in animal body. Record of HR had been indicated to be a non-invasive means for detection of presence of stress in cattle (Lefcourt *et al.* 1999). Normal heart rate of Sahiwal and KF varied in a wide range with no significant effects of heat exposure indicating that the changes in the heart rates were within the normal physiological ranges in both the breeds. Further thermotolerance of the two breeds were investigated by estimating DSI. DSI of Sahiwal was not affected by heat exposure while DSI of Karan-Fries increased from 0 h to 1 h of exposure (P<0.05) and further increase (P<0.05) was recorded after 3 h of exposure (Table 2). The present finding indicated better thermotolerance of Sahiwal than Karan-Fries during acute heat exposure unlike similar levels of thermotolerance between Sahiwal and Karan-Fries in the natural climatic conditions (Mayengbam *et al.* 2015). It was most likely that in cases of drastic climatic changes the crossbred cattle could be more affected than the native Zebu breeds.

In order to detect presence of heat stress induced oxidative stress in Sahiwal and Karan-Fries, further investigation was carried out to estimate the expression of Mn-SOD and Cu,Zn-SOD mRNA. The effects of heat exposure on expression of Mn-SOD mRNA were evident (P<0.05) in both the breeds (Fig. 1). Similar to the reports of heat exposure at 40±1°C and 50% RH caused different effects on expression of Mn-SOD of Sahiwal and Karan-Fries. Sahiwal was found to increase expression of Mn-SOD mRNA gradually which became significant (P<0.05) after exposure of 4 h (Fig. 1). Apart from involvement of SOD in mechanisms against oxidative stress (Ellah *et al.* 2009, Zlatkoviæ and Filipoviæ 2011), expression of SOD had been detected to associate degree of thermotolerance *in vitro* (Loven *et al.* 1985). Sheep supplemented with Vit. E and Selenium showed higher Mn-SOD expression when subjected to heat stress as compared to control (Chauhan *et al.* 2014).

The present finding of gradual increase in expression of Mn-SOD was indicative of activation of antioxidative mechanism in Sahiwal. Karan-Fries was able to increase (P<0.05) expression of Mn-SOD initially during heat exposure of 2 h which declined (P<0.05) after exposure of 3 and 4 h (Fig. 1) similar to the reports of Montilla *et al.* (2014) that expression of Mn-SOD of pig skeletal muscle increased after 1 h and declined after 3 h and 6 h of acute heat stress. After 4 h of heat exposure, Sahiwal was found to have higher Mn-SOD expression than Karan-Fries. The decline in Mn-SOD expression of Karan-Fries after 3 h and 4 h of heat exposure and lower level of Mn-SOD expression

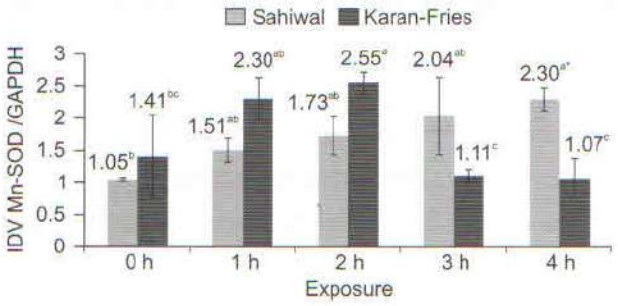

Fig. 1. Expression of Mn-SOD mRNA of Sahiwal and Karan-Fries during heat exposure. Means of same breed with different superscripts differ significantly from each other (P<0.05). *Indicates significant difference between breeds (P<0.05).

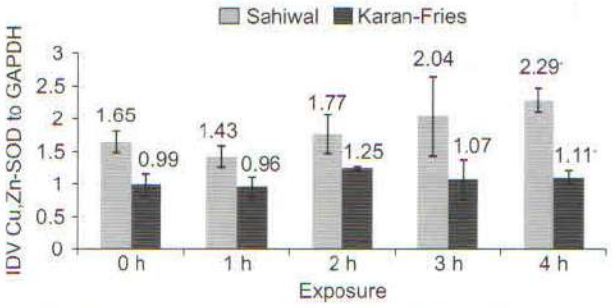

Fig. 2. Expression of Cu,Zn-SOD mRNA of Sahiwal and Karan-Fries during heat exposure. Means of same breed with different superscripts differ significantly from each other (P<0.05). *Indicates significant difference between breeds (P<0.05).

in Karan-Fries than in Sahiwal could be due to depletion of antioxidative mechanism as previous reports revealed lower expression of Mn-SOD by heat stressed sheep skeletal muscle than sheep reared under thermoneutral condition (Chauhan *et al.* 2014). Significant increase in Mn-SOD expression of Sahiwal after 4 h could be one adaptive mechanism of the breed with increased RR without affecting DSI indicating the thermotolerance of the breed.

Heat exposure at 40±1°C and 50% RH caused no significant change in expression of Cu,Zn-SOD of Sahiwal and Karan-Fries indicating that the variation in expression of Cu,Zn-SOD was within the physiological limits. The reports of Volodina *et al.* (2017) indicated that short term heat stress caused significant increase in antioxidant enzyme activity of pig skeletal muscle while the expression of Cu,Zn-SOD mRNA was not affected. In previous reports, temperature humidity index (THI) ranging from 53.63 to 80.26 caused no significant change in expression of Cu,Zn-SOD in Sahiwal while an average THI of 80.3 caused significant increase in Cu,Zn-SOD of Karan-Fries Mayengbam *et al.* 2015). In the present study, Sahiwal had higher expression of Cu,Zn-SOD than Karan after 4 h of heat exposure. It could be possible that expression of Cu,Zn-SOD was dependent on duration and intensity of heat stress as sheep reared under heat stress had lower Cu,Zn-SOD expression than sheep reared under thermoneutral condition (Chauhan *et al.* 2014).

The present study revealed changes in physiological responses of Sahiwal and Karan-Fries during acute heat exposure. When subjected to acute heat exposure, Sahiwal had better equipped antioxidant system than Karan-Fries with better physiological indices. The study indicated the most likely impacts of global climate change to crossbred cattle than to Zebu cattle.

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REFERENCES

Bernabucci U, Lacetera N, Baumgard L H, Rhoads R P, Ronchi B and Nardone A. 2010. Metabolic and hormonal adaptations to heat stress in domesticated ruminants. *Animal* **4**: 1167–83.

Bernabucci U, Ronchi B, Lacetera N and Nardone A. 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *Journal of Dairy Science* **85**: 2173–79.

Chauhan S S, Celi P, Fahri F T, Leury B J and Dunshea F R. 2014. Dietary antioxidants at supranutritional doses modulate skeletal muscle heat shock protein and inflammatory gene expression in sheep exposed to heat stress. *Journal of Animal Science* **92**: 4897–908.

Corr^ea G A, Rumpf R, Mundim T C D, Franco M M and Dode M A N. 2007. Oxygen tension during *in vitro* culture of bovine embryos: effect in production and expression of genes related to oxidative stress. *Animal Reproduction Science* **67**: 767–75.

Duanghaklang N, Duangjinda M and Sujikara K. 2015. Expression of antioxidant enzyme and heat stress genes in Thai native chicken (Chee KKU12) and Thai synthetic chicken (Khai Mook Esarn KKU50). *Khon Kaen Agriculture Journal* **43** Suppl. (2): 178–82.

Ellah A M R, Okada K, Goryo M, Oishi A and Yasuda J. 2009. Superoxide dismutase activity as a measure of hepatic oxidative stress in cattle following ethionine administration. *Veterinary Journal* **182**(2): 336–41.

Gaughan J B, Mader T L, Holt S M, Josey M J and Rowan K J. 1999. Heat tolerance of Boran and Tuli crossbred Steers. *Journal of Animal Science* **77**: 2398–405.

Hahn G L, Gaughan J B, Mader T L and Eigenberg R A. 2009. Thermal indices and their applications for livestock environments. Livestock Energetics and Thermal Environmental Management. (Eds) Deshazer J A and Joseph MSt. ASABE, pp. 113–30.

Hahn G L. 1999. Dynamic responses of cattle to thermal heat loads. *Journal of Animal Science* **77**(Suppl. 2): 10–20.

Hansen P J. 2004. Physiological and cellular adaptations of Zebu cattle to thermal stress. *Animal Reproduction Science* **82–83**: 349–60.

Kearl L C. 1982. Nutrient Requirements of Ruminants in Developing Countries. International Feedstuffs Institute, Utah Agricultural Experiment Station, Utah State University, Logan, Utah, USA.

Lazzari G, Wrenzycki C, Herrmann D, Duchi R, Kruip T, Niemann H and Galli C. 2002. Cellular and molecular deviations in bovine *in vitro*-produced embryos are related to the large offspring syndrome. *Biology of Reproduction* **67**: 767–75.

Lefcourt A M, Erez B, Varner M A, Barfield R and Tasch U. 1999. A non-invasive radiotelemetry system to monitor heart rate for assessing stress responses of bovines. *Journal of Dairy Science* **82**: 1179–87.

Lonergan P, Rizos D, Gutie´rrez-Ada´n A, Moreira P M, Pintado B, de la Fuente J and Boland M P. 2003. Temporal divergence in the pattern of messenger RNA expression in bovine embryos cultured from the zygote to blastocyst stage *in vitro* or in *vivo*. *Biology of Reproduction* **69**: 1424–31.

Loven D P, Leeper D B and Oberley L W. 1985. Superoxide dismutase levels in Chinese hamster ovary cells and ovary carcinoma cells after hyperthermia of exposure to cycloheximide. *Cancer Research* **45**: 3029–33.

Mayengbam P, Tolenkhomba T C and Upadhyay R C. 2015. Mn-SOD and Cu,Zn-SOD mRNA expression in relation to physiological indices of Sahiwal and Karan-Fries heifers under different temperature humidity indices. *Journal of Agrometrology* **17**(2): 172–78.

Montilla S I R, Johnson T P, Pearce S C, Gardan-Salmon D, Gabler

N K, Ross J W, Rhoads R P, Baumgard L H, Lonergan S M, and Selsby J T. 2014. Heat stress causes oxidative stress but not inflammatory signaling in porcine skeletal muscle. *Temperature* **1**(1): 42–50.

Oishi K and Machida K. 2002. Different effects of immobilization stress on the mRNA expression of antioxidant enzymes in rat peripheral organs. *Scandinavian Journal of Clinical and Laboratory Investigation* **62**(2): 115–21.

Sambrook J, Fristsch E F and Maniatis T. 1989. Molecular cloning: A Laboratory Manual. Vols 1, 2, and 3. 3rd edn. Cold Spring Harbor, New York.

Scharf B, Carroll J A, Riley D G, Chase Jr C C, Coleman S W, Keisler D H, Weaber R L and Spiers D E. 2010. Evaluation of physiological and blood serum differences in heat-tolerant (Romosinuano) and heat-susceptible (Angus) *Bos taurus* cattle during controlled heat challenge. *Journal of Animal Science* **88**: 2321–36.

Sies H. 1997. Oxidative stress: oxidants and antioxidants. *Experimental Physiology* **82**: 291–95.

Thomas C K, Sharma K N S, Georgie GC and Razdan M N. 1973. A new heat tolerance index for cattle. *Indian Journal of Animal Sciences* **43**(6): 505–10.

Volodina O, Ganesan S, Pearce S C, Gabler N K, Baumgard L H, Rhoads R P and Selsby J T. 2017. Short-term heat stress alters redox balance in porcine skeletal muscle. *Physiological Reports* **5**(8): 1–10.

Yadav B, Singh G and Wankar A. 2015. Adaptive capability as indicated by redox status and endocrine responses in crossbred cattle exposed to thermal stress. *Journal of Animal Research* **5**(1): 67–73.

Yadav B, Singh G, Wankar A, Dutta N, Chaturvedi V B and Verma M R. 2016. Effect of simulated heat stress on digestibility, methane emission and metabolic adaptability in crossbred cattle. *Asian Australasian Journal of Animal Sciences* **29**(11): 1585–92.

Yadav Y C, Srivastava D N, Saini V, Singhal S, Seth A K and Kumar S. 2011. *In-vitro* antioxidant activity of methanolic extraction of *Ficus Benghalensis* L. latex. *Pharmacologyonline* **1**: 140–48.

Yatoo M I, Dimri U and Sharma M C. 2014. Seasonal changes in certain blood antioxidants in cattle and buffaloes. *Indian Journal of Animal Sciences* **84**(2): 173–76.

Zlatkoviæ J and Filipoviæ D. 2011. Stress-induced alternations in CuZnSOD and MnSOD activity in cellular compartments of rat liver. *Molecular Cell Biochemistry* **357**(1–2): 143–50.