



## Differential level of oxidative stress markers in skin tissue of zebu and crossbred cattle during thermal stress



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### ABSTRACT

The study aimed to evaluate oxidative stress markers (ROS, GPx, GRx) in skin tissue of cattle with different coat colors during different seasons. Ten each of Tharparkar (zebu) and Karan Fries (crossbred) heifers were selected from NDRI herd, Karnal. Animals were maintained under standard managemental practices followed at the farm. Skin biopsies were aseptically collected from each animal during winter, spring and summer. ROS, GPx, GRx were determined by ELISA. Real time PCR was performed to examine the expression of skin color genes (*MC1R* and *PMEL*) in skin tissue. In both the breeds, significantly higher ( $P < 0.05$ ) levels of ROS, GPx, GRx were observed during summer followed by winter and spring. Expression of *MC1R* and *PMEL* was increased during summer and winter than spring, but magnitude of expression was lower during summer than winter. ROS, GPx, GRx levels during summer were higher ( $P < 0.05$ ) in Karan Fries than Tharparkar, whereas *MC1R* and *PMEL* expression levels were higher in Tharparkar than Karan Fries. The study concluded that oxidative stress in skin tissue increased with heat stress in cattle, but the generation of oxidative stress due to heat stress was higher in lighter pigmented skin of Karan Fries than darker pigmented skin of Tharparkar. Higher expression of skin color genes in skin tissue of Tharparkar (*Bos indicus*) might have induced greater pigmentation in them compared to crossbred (*Bos indicus* × *Bos taurus*) cattle, thus increasing the skin protective capacity and reducing oxidative stress in them under heat stress. This might be a critically important factor for superior heat tolerance of zebu than crossbred cattle.

### 1. Introduction

Oxidation is an integral part of normal cellular metabolism 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). Antioxidant defense systems have also co-evolved with aerobic metabolism to counteract the destructive effects of ROS to minimize their potential to cause tissue damage. When reactive forms of oxygen are produced faster than they can be safely neutralized by antioxidant mechanisms, oxidative stress results (Sies, 1991).

Skin, the largest body organ, provides a major interface between the environment and the body and is constantly exposed to an array of chemical and physical environmental pollutants (Athar, 2002). These environmental toxicants or their metabolites are inherent oxidants and/or directly or indirectly drive the production of a variety of reactive oxidants also known as reactive oxygen species (ROS). Skin exposure to

ionizing and UV radiation and/or xenobiotics/drugs is reported to generate ROS in excessive quantities that quickly overwhelm tissue antioxidants and other oxidant-degrading pathways (Bickers and Athar, 2006). Several studies reported that heat stress can also induce oxidative stress in living organisms. Frei (1994) observed higher catalase (CAT) concentrations in the blood of dairy cow during summer. Banerjee and Ashutosh (2011) also reported an increase in super oxide dismutase (SOD) concentrations in heat stressed dairy cattle.

At the same time, climate is changing throughout the world. Most of these changes are in the direction expected with warming temperature (Rosenzweig et al., 2008). The Intergovernmental Panel 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 and 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 (Ganaie et al., 2013). Animal with

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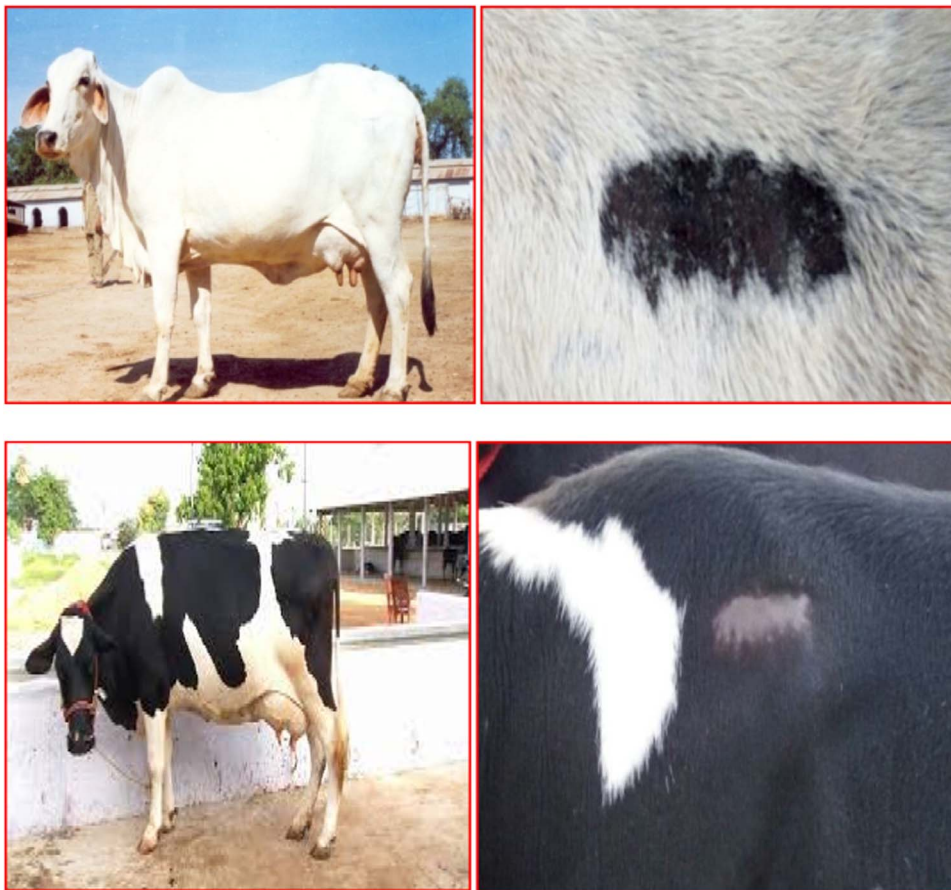


Fig. 1. Coat colors of two breeds of cattle a) Tharparkar b) Karan Fries.

different coat colors is a highly visible trait. The significance of such large diversified trait in relation to thermal stress is least understood in livestock. Animals with light colored hair coat and darkly pigmented skin are reported to be better adapted under tropical climatic conditions with high levels of solar radiation (Finch and Western, 1977; Finch et al., 1984). The indigenous cattle breed (Tharparkar and Sahiwal) have been reported to exhibit better heat tolerance than exotic breed (Hansen, 2004). Similar findings were also reported by Singh et al. (2014) in crossbred (Karan Fries) and zebu cattle (Tharparkar) when exposed to heat stress. With the above facts in mind, the study was conducted to observe the oxidative stress markers (reactive oxygen species, glutathione peroxidase and glutathione reductase) in skin tissue of Tharparkar (*Bos indicus*) and Karan Fries (*Bos indicus* × *Bos taurus*) cattle. Tharparkar has lighter hair but darker skin, whereas Karan Fries has darker hair and lighter skin (Fig. 1). Expression of skin color genes (*MC1R* and *PMEL*) were also observed in them during different seasons.

## 2. Materials and methods

### 2.1. Chemicals and reagents

The chemicals, reagents and plasticwares were procured chiefly from Thermo Scientific (Rockford, IL, USA), Fermentas (St. Leon-Rot, Germany), Qiagen (Germany), MyBioSource (San Diego, California, USA) etc. which is indicated wherever needed.

### 2.2. Ethical permission

The animals were treated following the compliance of the institute's norms for ethical treatment. 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.

### 2.3. Location, animals and experimental design

Experiment was conducted in the cattle yard of the National Dairy Research Institute (NDRI), Karnal, Haryana. It is situated at an altitude of 250 m above mean sea level, latitude of 29°42" N and longitude of 79°54" E. Ambient temperature of the place ranges from a maximum temperature of 45 °C during summer and minimum temperature of 0 °C during winter with a diurnal variation of 15–20 °C. Average annual rainfall is 700 mm, most of which is received from early July to mid-September.

Ten each of healthy and non-pregnant Tharparkar and Karan Fries heifers of approximately 2 years of age were selected from NDRI herd for the experiment. Same animals were used throughout the experiment during the three seasons viz. spring/thermoneutral (mid Feb–mid Mar), peak summer (mid May–mid June) and peak winter (mid Dec–mid Jan). All the animals were maintained under general management practices followed for heifers at the institute. The animals were clinically healthy and free from any physical or anatomical abnormalities. Skin samples were collected during the extremes of winter (mid Dec. to mid Jan.), summer (mid May to mid Jun.) and spring (mid Feb. to mid Mar.) season. Samples were collected twice per each season from each animal after the animals have been exposed to the extreme conditions of the particular season for a certain period of time i.e. during the mid and the end of said period of the particular season. Data on meteorological variables was collected from meteorological station, CSSRI, Karnal (Table 1) and THI was calculated using the formula  $0.72 (T_{ab}^{\circ}C + T_{wb}^{\circ}C) + 40.6^{\circ}$  (Johnson et al., 1963).

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