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Studies on the seasonal variations in the antioxidant enzymes in Indian zebu cattle

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Abstract
One of the main reasons for oxidative stress in animals during summer season is heat stress. The present experiment was aimed to study the effect of summer and winter season on the stress related antioxidant enzymatic profile of zebu cattle in Assam. The different enzyme activities such as SOD (Superoxide Dismutase), GSH-Px (Glutathione Peroxidase) and CAT (Catalase) were analysed by different standard methods. It was observed that all the erythrocytic antioxidant enzyme activities were increased in summer compared to winter season and the difference was highly significant ($P<0.01$). The increase in antioxidant enzyme activities might be attributed to the adaptation and the acclimatization of the animals towards the thermal stress of the summer season.

Keywords: Season, antioxidants enzymes, zebu cattle

1. Introduction

Heat stress is one of the major influencing factors to reduce animal reproduction and productivity. Excessive heat load caused by a combination of air temperature, relative humidity, low wind velocity and solar radiation increases body physiological characters and can reduce feed intake and milk production [1, 2]. The variation in climatic variables like temperature, humidity and radiations were recognized as the potential hazards in the growth and production of all domestic livestock species. The degree of environmental impact is modified by stage of the animal's life cycle and adaptation of the breed and species. A high environmental temperature clearly reduces the milk yield of a cow due to reduced feed intake and extra energy being utilized to combat heat stress. A decline of 8 percent in milk yield was found in summer season when the mean afternoon temperature was 39.8 °C compared with winter season [3]. One of the recent economic studies has suggested severe losses if the current management systems are not changed as a result of climate change [4]. Therefore, there is great need of attention in understanding how domestic animals respond to climate stressors. With thermal discomfort, the animal seeks ways to lose heat by means of a sequence of adaptation devices involving respiratory, circulatory and excretory mechanisms. Compositely, the adaptation characteristics can determine the tolerance of each breed to their environment, which can be estimated from the enzymatic traits [5]. Coordination of all these systems is required in order to maintain the productive potential under thermal stress irrespective of species, breeds and individuals. Changes that induce thermal imbalance include a combination of several environmental factors namely, sunlight, thermal radiation, humidity, air temperature and thermo-regulatory mechanisms [6]. It is established fact that the heat stress is one of the factors causing Reactive Oxygen Species (ROS) to mediate oxidative stress in farm animals. As a consequence, the body employs antioxidants to quench these free radicals [7]. Further, oxidation is essential to nearly all cells in the body to provide energy for vital functions [8]. Approximately, 95 to 98% of the oxygen consumed is reduced to water during aerobic metabolism, but the remaining fraction may be converted to oxidative by-products-reactive oxygen species, that may damage the DNA of genes and contribute to degenerative changes. Superoxide dismutase (SOD), glutathione peroxidase and catalase are the main constituents of intracellular antioxidant defense system [9]. Hence, these antioxidants help to maintain the bodily homeostasis and are having prime importance to counteract heat stress. Therefore, the present study was formulated to examine the alterations in the different stress related antioxidant enzymes due to seasonal changes in indigenous zebu cattle.

2. Materials and methods

2.1 Experimental period
The experiment was conducted in the two seasons i.e. summer (June, July and August of 2015) and winter (December 2015, January and February of 2016).

2.2. Experimental animals and collection of samples
The study was conducted on twelve numbers of female Indian zebu cattle between 2-3 years of age, free from any anatomical and reproductive disabilities and diseases. They were maintained under semi-intensive system in the Experimental Animal Shed, Department of Veterinary Physiology, C.V.Sc. Khanapara, Assam Agricultural University, Guwahati-22, Assam, India with latitude and longitude position being 26° 10' N and 91° 44' E respectively. The animals were supplied with both green fodder and concentrate as per the standard feeding practices of the farm. The venous blood was collected aseptically from each of the experimental animal fortnightly during the winter (December to February) and the hot humid summer (June to August) season. Blood samples (3-5 mL) were collected aseptically in EDTA vacutainers (BD Biosciences) after puncturing the jugular vein of animals and transferred to the laboratory in ice-box immediately.

2.3 Ethical permission
The protocol of this experiment was approved by Institutional Animal Ethics Committee of Assam Agricultural University, Khanapara, Guwahati, Assam, India.

2.4 Temperature Humidity Index
Temperature-Humidity Index was calculated out from the data of ambient temperature and relative humidity [10]. The aforesaid necessary meteorological data i.e. dry bulb temperature and relative humidity were recorded daily for winter season (December to February) and summer season (June to August) from the Automatic Weather Station (AWS) installed at the Experimental Animal Shed, Department of Veterinary Physiology, C.V.Sc., Khanapara, AAU, Guwahati-22, Assam, India. The temperature-humidity index was calculated for the entire period of study using the below mentioned formula:

$$THI = (0.8 \times T_{db}) + [(RH/100) \times (T_{db} - 14.4)] + 46.4.$$

2.5 Preparation of haemolysate (%)
1 % haemolysate was prepared for the estimation of different enzyme concentration. The collected blood sample was centrifuged at 2000 g for 15 minutes and the plasma was discarded. The sediment remaining in the test tube was washed with normal saline solution (NSS) thrice. For washing equal volume of NSS was added to the sediment, mixed properly and was centrifuged for 10 minutes. The supernatant was discarded along with the buffy coat and again equal volume of NSS was added to the sediment, mixed gently and centrifuged. The process was repeated thrice. The sediment was resuspended in 100 µl of NSS. One percent haemolysate (100 µl washed RBC+ 9.9ml of 0.05M PBS, PH 7.4) for determination of enzymes associated with erythrocyte membrane was made.

2.6 Estimation of antioxidants
The erythrocytic superoxide dismutase activity was estimated following the NBT reduction method [11]. The erythrocytic glutathione peroxidase enzyme was assayed by Rotruck method [12] and the activity of erythrocytic catalase was assayed by Sinha method [13].

2.7 Statistical analysis
The analysis of variance was performed to determine the presence or absence of significant differences in the different antioxidants in the different seasons using GraphPad PRISM version 5.0 statistical software package and differences between the seasons were tested by Tukey's multiple comparison test. P values less than 0.05 were considered significant.

3. Results and Discussion
The THI values in the three different hours and the erythrocytic antioxidant concentration in the two different seasons were presented in figure 1 and 2. The mean THI were recorded as 61.9 and 81.60 at 6:00 hours; 71.9 and 85.66 at 14:00 hours and 69.78 and 83.85 at 19:00 hours during the winter and hot humid summer seasons respectively. The mean erythrocytic superoxide dismutase (SOD), glutathione peroxides (GSH-Px) and catalase (CAT) concentration of Indian zebu cattle was recorded as 3.28 ±0.08 and 5.91 ±0.06, 44.48±0.65 and 72.17±0.75 and 23.60 ±0.40 and 44.86±0.52 unit/mg of protein for the winter and summer season respectively. The erythrocytic antioxidant enzyme concentrations in the zebu cattle were increased during the hot humid summer season as compared to winter seasons. There was a highly significant ($P<0.01$) difference in all the erythrocytic antioxidant enzymes between the two seasons.

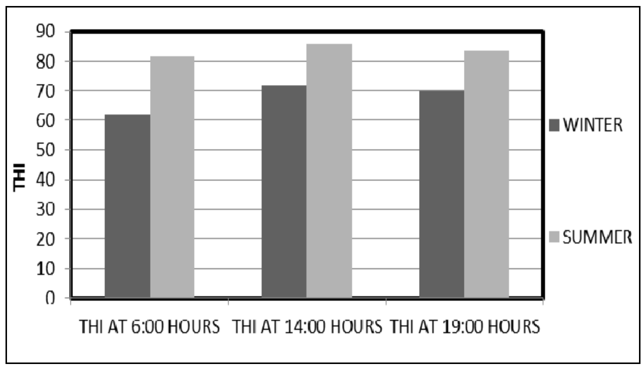

Fig 1: Temperature humidity index (mean± se) during the different seasons

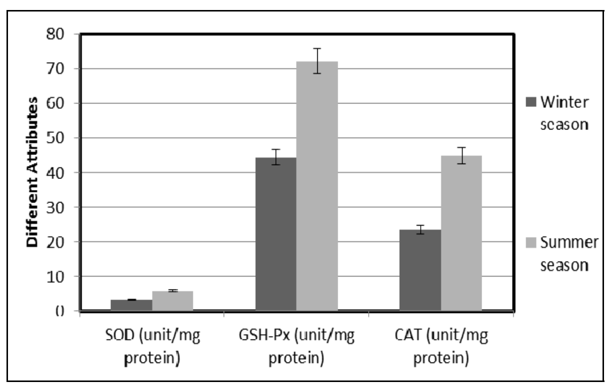

Fig 2: Enzyme concentration (Mean±SE) in zebu cattle in hot humid summer and cold winter season in Assam.

The Temperature Humidity Index (THI) has been widely used as an indicator of thermal stress in livestock and it involves both ambient wet/dry bulb temperature and relative humidity, and is universally used as a heat stress index for livestock production [14, 15]. A THI value of 70 or less are considered comfortable, 75-78 stressful and values greater than 78 cause extreme distress and the animals are unable to maintain the thermoregulatory mechanism or the normal body temperature [16]. Therefore, in the present study, the obtained values indicated that the animals were in severe stress in the summer season and no stress in the winter during 6:00 and 19:00 hours. However, THI in the month of winter season during 14:00 hours gives a warning of thermal stress [17]. In the present study, the summer season proved to be more stressful for the cattle compared to the winter season as the THI was found to be above 80 [18]. The negative production effects of heat stress on high producing dairy cows begin at THI values 65 and 68 [19]. However, the daily average THI values in excess of 72 leads to heat stress in dairy cattle [20]. The thermal stress stimulates excessive production of reactive oxygen species (ROS), such as superoxide anion (O_2^-), hydroxyl ion (OH) and hydrogen peroxide (H_2O_2), which are continuously produced in the course of normal aerobic metabolism and these free radicals can damage healthy cells, and if they are not being eliminated and can cause some disturbances which are reflected as disturbed physiology and altered biochemical profile of the animal [21]. There might be an increase in the production of hydrogen peroxide in the body during summer season which resulted in increase SOD activity. In order to neutralize that hydrogen peroxide, the glutathione peroxidase and catalase activity are also increased [22, 23]. Thus, a positive and significant correlation exists between GSH-Px, SOD and CAT activities**.** Therefore, the increase in the antioxidant enzymes during the summer season might be due to the thermal stress. These antioxidant enzyme activities are, thus, sensitive markers of oxidative stress as their level may increase or decrease in response to reactive oxygen species. SOD that catalizes dismutation of superoxide becomes important in the defense mechanisms against oxidative stress [24]. The role of intracellular SOD is to scavenge the superoxide ($\bullet O^{2-}$) that is produced by a number of reaction mechanisms, including several enzyme systems, as a part of normal cellular functions [25]. An increase in SOD activity with the advancement of pregnancy during summer season was reported [26]. Our findings corroborated with previous workers who stated that higher SOD levels in prepartum crossbred cattle during summer (3.83±13.59 mM/l) compared to winter (3.39±16.50 mM/l) and thereby indicating effect of hot summer season on the oxidative status of the transient dairy cows. Therefore, lower SOD activity observed in winter season compared to summer in zebu cattle, in the present study indicated a reduced oxidative stress in animals. The effect of winter and summer seasons on antioxidant status of growing, heifer and lactating Murrah buffaloes and reported significantly higher SOD levels during summer compared to winter [27]. Likewise, the oxidative parameters such as lipid peroxides, SOD, Catalase and Glutathione peroxidases were significantly higher during peak summer as compared to winter and moderately cold season in Barbari goats (tropical) [28]. Our results agree with previous studies [21, 29, 30] which supported the view that SOD, GPx and CAT increased during stressful conditions.

4. Conclusion
During hot and humid summer season in Assam, India, there is an increase in the production of different antioxidants enzymes in zebu cattle. All the erythrocytic antioxidant enzymes were found to be significantly ($P<0.01$) higher in the summer compared to the winter season. This increase concentration might help the cattle to adapt and acclimatize themselves to the changing environmental condition of this region.

5. Acknowledgment
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