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Daily and seasonal rhythmic secretary pattern of antioxidant and oxidative stress biomarkers in mithun bull

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ABSTRACT

A study was conducted to assess the rhythmic changes of antioxidant and oxidative stress profiles (antioxidant and oxidative stressors together is called as oxidative stress biomarkers: OSBs) to understand their flow pattern in 24 h during different seasons (winter, spring, summer and autumn) in intact adult mithun bulls (n=6; 5–6 years). Antioxidants [total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione (GSH), glutathione reductase (GSHR) and catalase (CAT)] and oxidative stressor [malondialdehyde (MDA)] were estimated at 4 h interval for a whole day in different seasons. Analysis was done in two different ways with respect to time (0800, 1200, 1600, 2000, 2400 and 0400 h) and day time (0800 to 1600 h) and night time (2000 to 0400 h) collections. The levels of OSBs differed significantly among the seasons, among the collection times and between day and night times. Winter and spring had recorded significantly higher antioxidants and lower MDA than summer. Levels of antioxidants were higher and MDA was lower during night as compared to day time collections. It was concluded that spring and winter seasons had greater beneficial effects than summer on health and wellbeing of the mithun.

Keywords: 24-h profiles, Antioxidant, Mithun, Oxidant, Seasonality

Mithun is free-range cattle in NEH region and suffers severe non-cyclical population fluctuations. Oxidative stress is due to imbalance between reactive oxygen species (ROS) production and neutralization capacity of antioxidants (Sies 1997), which leads to loss of their biological activities and death of organism (Inoue *et al.* 2009). Antioxidants catalyze the respective ROS (Lykkesfeldt and Svendsen 2006).

Day length and temperature humidity index (THI) are major components that determine the seasonality and functionality of different OSBs (Singh *et al.* 2014). THI value of ≤70 is considered as comfortable, 75–78 as stressful and >78 as extreme distressful where livestock lose their thermoregulatory mechanisms (Kadzere *et al.* 2002). Average THI of mithun breeding station during summer (76.06±0.45) and autumn (74.67±0.38) was >74; therefore, mithuns got exposed to higher heat stress during these seasons. Generally, mithun prefers cold or mild environment. Higher THI and longer photo period decrease antioxidant production and enhances oxidative stressors in summer (Bhatti *et al.* 2017). Therefore, mithun needs to be protected from changing climatic scenario.

Some sort of seasonality prevailed in mithun based on reproductive (Perumal *et al.* 2014, Perumal *et al.* 2017); however, mithun is a perennial breeder. Under clinical point of view, accurate estimation of biochemical profile depends on blood collection at an appropriate time of day relative to phase of the underlying biochemical rhythm. Oxidative load was higher during day whereas antioxidants were higher during night (Singh *et al.* 2014). Similarly, Benot *et al.* (1999) observed higher TAC at 01:00 than those at 13:00, 19:00 and 07:00 h in human. Di Trana *et al.* (2006) reported significant positive correlation between antioxidants and negative correlation between MDA and antioxidants. Though, several authors reported daily and seasonal pattern of OSBs in different species (goat: Singh *et al.* 2014; buffalo: Yatoo *et al.* 2014; cattle: Mirzad *et al.* 2018) and such information was lacking in mithun. Therefore, present study was designed to evaluate the OSBs in 24 h in adult mithun at different seasons and to examine their temporal relationships throughout the sampling period.

MATERIALS AND METHODS

Area of the study: Present investigation was conducted at ICAR-National Research Centre on Mithun, Medziphema, Nagaland,situated in between 25°54″30″ North latitude and 93°44″15″ East longitude with an altitude of 250–300 m msl. The present study was conducted in four different seasons such as winter (November to January; THI: 54.41±0.28), spring (February to April; THI: 63.51±0.48), summer (May to July; THI: 76.06±0.45) and autumn (August to October; THI: 74.67±0.38) with an average THI of 66.89±0.39. Similarly, sunshine hours

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significantly differed between winter (4.11±0.36), spring (4.81±0.28), summer (6.55±0.15) and autumn (6.32±0.28) seasons with an average of 5.45±0.34.

Experimental animals: Apparently healthy (n=6) intact adult mithun bulls aged 5–6 years with average body weight 510.83±4.72 kg (495–520 kg) and good body condition score (5–6) were randomly selected. These experimental mithuns were maintained under homogenous feeding, watering, lighting, grazing, housing and other managemental procedures as per the standard farm schedule.

Collection of blood and estimation of OSBs: Blood samples were collected from the experimental mithun bulls in heparin tubes (20 IU of heparin/mL of blood) at 0400 h interval (08:00, 12:00, 16:00, 20:00, 24:00 and 04:00 h) throughout the day in different seasons. These blood samples were centrifuged at 4°C with 1200 × g for 15 min. Plasma samples were separated, labelled and preserved in deep freezer at –80°C for further analysis. Samples were analysed in different times of collection (08:00, 12:00, 16:00, 20:00, 24:00 and 04:00 h) and between day (08:00 to 16:00 h) and night time (20:00 to 04:00 h) collections.

Antioxidant profiles: Total antioxidant capacity (K274; mmol/mL), superoxide dismutase (706002; U/mL), glutathione (703002; µmol/mL), glutathione reductase (703202; nmol/min/mL) and catalase (707002; nmol/min/mL) in blood plasma were estimated as per the manufacturer's guidelines (TAC: BioVision, Mountain View, CA, USA and SOD, GSH, GSHR and CAT: Cayman Chemical Company, USA) and measured by Thermo Scientific Multiskan GO Microplate Spectrophotometer (USA).

Oxidative profile: Malondialdehyde in blood plasma was determined as conjugate with TBA. Plasma proteins were precipitated by TCA and then removed by centrifugation. MDA–TBA complex was measured at 534 nm (Thermo Scientific Multiskan GO Microplate Spectrophotometer, USA).

Statistical analysis: Means were analysed by two way ANOVA followed by Tukey's post hoc test to determine the significant differences among the different seasons, among the collection times and between day and night collectionswith use of SAS software (SAS Version 9.3; SAS Institute, Inc., Cary, NC, 2001). Mean values with a significance of $p<0.05$ were considered as statistically significant.

RESULT AND DISCUSSION

OSBs of mithun differed significantly ($p<0.05$) among the collection times (Fig. 1) and between day and night collections (Fig. 2) in different seasons. Winter and spring had higher antioxidant concentration than summer whereas MDA was higher in summer than in winter and spring. Concentration of antioxidants was higher in night time than in day time. On contrary, MDA was higher in day than in night time collection in different seasons.

TAC concentration was decreased significantly from 08:00 to 12:00 h, increased from 12:00 to 16:00 h, decreased from 16:00 to 24:00 h and then increased from 24:00 to 04:00 h in winter, whereas in spring, summer and autumn seasons, its concentration decreased from 08:00 to 12:00 h, increased from 12:00 to 24:00 h and decreased from 24:00 to 08:00 h. CAT concentration decreased significantly ($p<0.05$) from 08:00 to 12:00 h and increased from 12:00

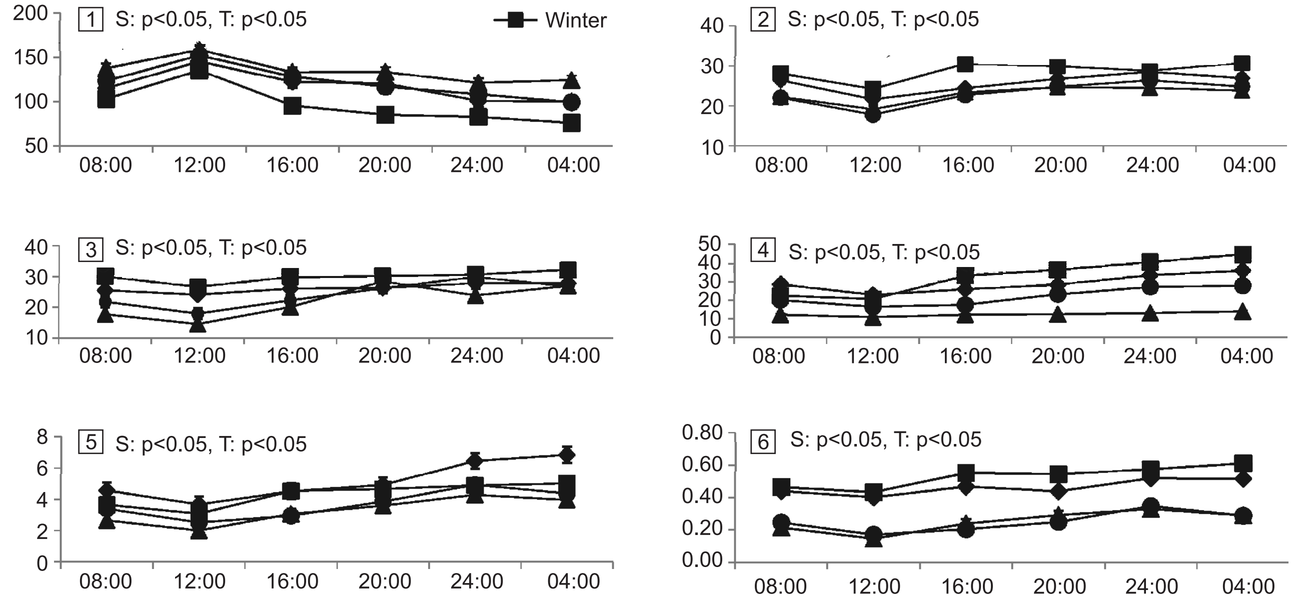

Fig. 1. Rhythmic changes of serum antioxidant and oxidative stress profiles in mithun bull. Vertical bar on each point represents standard error. Graph 1: Malondialdehyde, 2: Total antioxidant capacity, 3: Catalase, 4: Glutathione, 5: Glutathione reductase and 6: Superoxide dismutase. Winter: November–January, Spring: February–April, Summer: May–July and Autumn: August–October. Vertical bar on each point represents standard error of mean. Average Age: 53.03 months. T: time effect, S: season effect, n=6 for each group.

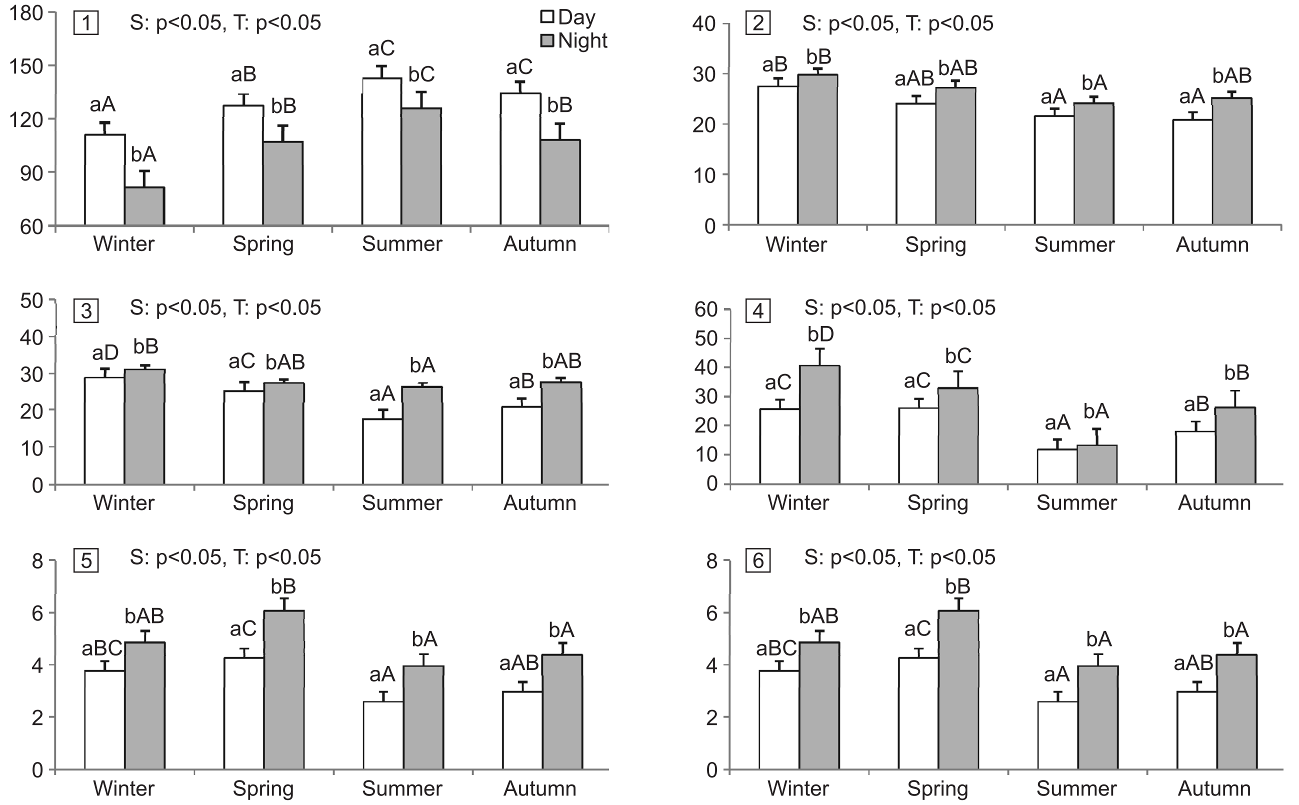

Fig. 2. Serum antioxidant and oxidative profiles at Day (D) and Night (N) time (T) in mithun bull. Vertical bar on each point represents standard error. Graph 1: Malondialdehyde, 2: Total antioxidant capacity, 3: Catalase, 4: Glutathione, 5: Glutathione reductase and 6: Superoxide dismutase. Winter: November–January, Spring: February–April, Summer: May–July and Autumn: August–October. Average Age: 53.03 months. S: season effect, n=6 for each group. Vertical bar with capital letters (A, B) indicates significant ($p < 0.05$) difference between seasons and small letters (a, b) indicates significant ($p<0.05$) difference between day and night for within the seasons for different seasons.

to 04:00 h in winter and spring.In summer, CAT concentration decreased from 08:00 to 12:00 h, increased from 12:00 to 20:00 h, decreased from 20:00 to 24:00 h and again increased from 24:00 to 04:00 h. Similarly, in autumn, concentration of CAT decreased significantly from 08:00 to 12:00 h, increased from 12:00 to 24:00 h and decreased from 24:00 to 04:00 h (Fig. 1).

GSH concentration decreased significantly from 08:00 to 12:00 h and increased from 12:00 to 04:00 h in winter, spring, summer and autumn seasons. GSHR concentration decreased significantly from 08:00 to 12:00 h and increased from 12:00 to 04:00 h in winter and spring seasons, whereas in summer and autumn seasons, GSHR concentration decreased from 08:00 to 12:00 h, increased from 12:00 to 24:00 h and then decreased from 24:00 to 08:00 h. SOD concentration decreased significantly from 08:00 to 12:00 h and increased from 12:00 to 04:00 h in winter and spring seasons whereas in summer and autumn seasons, its concentration decreased from 08:00 to 12:00 h, increased from 12:00 to 24:00 h and decreased from 24:00 to 08:00 h (Fig. 1).

In NEH region, mithun is an important meat animal. Mithun is a perennial breeder; however, some sort of seasonality prevailed as in other bovine species (Perumal *et al.* 2014, 2017). Seasonal mean THI in summer and autumn was 76.06±0.45 and 74.67±0.38, respectively in mithun experimental station, which is >74; therefore, these animals were exposed to thermal stress during these seasons. Generally, mithun lives in cold environment as during winter and spring, its moves towards plain areas whereas during summer, it retires in deep forest. Further, conception and pregnancy rate were decreased in bovine species when the THI was >72 (Dash *et al.* 2016), which indicates clearly that higher THI reduces antioxidants and increases ROS which inturn affects reproduction performances of animals. Though several authors reported daily and seasonal pattern of OSBs in different species (goat: Singh *et al.* 2014; buffalo: Yatoo *et al.* 2014; cattle: Mirzad *et al.* 2018), similar studies in mithun were lacking. OSBs had 24-h circulating patterns with phase specific or time specific relationships in mithun in our study. OSBs differed significantly between seasons, between collection times and between day and night within season. Variation in OSBs in our study with studies of others was most probably due to large differences in local experimental conditions. Day length and THI determine the concentration of OSBs. Higher THI and longer photo period decrease antioxidant concentration and enhance oxidative stressors in summer than winter (Bhatti *et al.* 2017), which could to be justified with the results of our study at least in part in mithun.

MDA is the end product of lipid peroxidation which measures the level of pro-oxidants indirectly in the physiological system. Variation in DNA damage, protein oxidation and lipid peroxidation were reported at different times of day, thus circadian oscillations of oxidative stressors were expressed (Tomás-Zapico *et al.* 2003). These circadian oscillations associate directly with daily rhythm of antioxidant expression. Inverse correlation was reported between lipid peroxidation and antioxidants. Lipid peroxide was low during night whereas antioxidants was higher during night (Singh *et al.* 2014). In our study, MDA was higher and antioxidant was lower in summer than in winter and spring. Higher MDA value could be due to reduced antioxidant in summer season. However, it is understood that heat stress can increase MDA production and induce oxidative stress which ultimately damage both the cellular and mitochondrial structures (Belhadj Slimen *et al.* 2016).

Thermal stress in summer increases lipid peroxide production which in turn increases antioxidants activities to neutralize that ROS (Kehrer and Smith 1994). Thus, significant positive association exists among antioxidants whereas negative association exists between ROS and antioxidants. Decreased antioxidants during summer might be due to deleterious thermal stress in our study. Significantly ($p<0.05$) higher MDA during summer suggested that higher oxidative damage under unfavourable higher temperature with longer photo period (Megahed *et al.* 2008). Thus, these antioxidant enzymes emerged as sensitive markers to assess the oxidative stress because their level may either increase or decrease according to the ROS. Further, higher antioxidants and lower MDA in winter and spring than in summer might be due to melatonin secretion. We could predict that in summer, mithun had lower melatonin concentration and TAC activity increased to counter act the effect of ROS, thus TAC level was reduced in summer. On the other hand, in winter, because of the higher melatonin concentration, TAC level in mithun was more preserved and TAC activity was reduced as compared to summer. Melatonin is not only protecting as an antioxidant, but also stimulates the production of antioxidants (Karbownik and Reiter 2000). Therefore, antioxidant level was higher in winter and spring as compared to summer (Adamczyk-Sowa *et al.* 2014). Similarly, in heifers, TAC level reduced in summer than winter (Turk *et al.* 2015) and ROS increased in summer in mid-lactating cows (Calamari *et al.* 1999). Higher oxidant and reduced antioxidant profiles have observed during summer in dairy and buffalo cows (Megahed *et al.* 2008). Higher MDA was observed in mithun during summer indicates mithun suffered higher oxidative stress than in winter season. Further, mithun has a magnificent body mass and their heat emission efficiency could be lower from their body surface, thus, this may be another reason for increased oxidative stress in mithun during summer (Sakatani *et al.* 2012). Higher oxidative stress index in summer than in winter and mid-day than at mid-night indicated that mithun experienced an oxidative challenge during summer and at noon time in present location supported by findings of Abuelo *et al.* (2013).

Antioxidants expressed a nyctohemeral rhythm with the higher value at mid-night with corresponding lower value at mid-day in all seasons. Results of our study pertaining to TAC is consistent with the earlier reports (goat: Singh *et al.* 2014; buffalo: Yatoo *et al.* 2014 and cattle: Mirzad *et al.* 2018) and indicated that serum TAC has a parallel rhythm with 24 h cycle of melatonin. Concentration of these antioxidants found higher during night (1800 to 0600 h) while MDA recorded higher during day time (Singh *et al.* 2014). Interestingly, antioxidants under study observed significantly ($p<0.05$) higher during winter and spring than in summer and autumn as reported in caprine species (Singh *et al.* 2014). Similarly, in human, TAC concentration was higher during nocturnal than diurnal time (Benot *et al.* 1999). Benot *et al.* (1999) observed significantly higher serum TAC at 01:00 than those at 13:00, 19:00 and 07:00 h in human. Further, these authors reported association of TAC rhythm with the rhythm of melatonin (Benot *et al.* 1999), therefore, TAC increased when melatonin increases and melatonin is higher in night time (nocturnal hormone); TAC is also higher in night time. Similar results were obtained in present study. Chandra and Aggarwal (2009) also reported higher CAT activity in crossbred cows during summer who reported its significantly reduced concentration during summer season.

One important group of antioxidant enzymes oscillate with circadian rhythmicity is SODs. These antioxidant enzymes protect against oxidative damage by catalyzing the dismutation of O_2^- into O_2 and H_2O_2. Daily rhythmicity of SOD activity was reported by Diaz-Munoz and co-workersin 1985. They again reported peak of SOD activity during dark phase in cerebral cortex of rat (Diaz-Munoz *et al.* 1985). CAT is another one antioxidant enzyme that protects further by catalyzing the decomposition of H_2O_2 to H_2O and O (Sani *et al.* 2006). CAT activity rhythmicity has been established more than 25 years ago and assessed in many different model organisms including humans (Sani *et al.* 2006). CAT activity at its peak reported in middle of the dark phase in mice (Sani *et al.* 2006).

GSH is a non-enzymatic low molecular weight antioxidant which neutralizes ROS (Mannervik 1987). Further, GSHP has more affinity for H_2O_2 than CAT and GSHP plays a much greater active role in H_2O_2 removal when its concentrationis lower (Pisoschi and Pop 2015). Circadian variation in GSH production observed first timeand reported in rat blood in 1967 (Calcutt 1967). Since then, circadian oscillation of GSH has been predicted and confirmed in several different species (Fanjul-Moles *et al.* 2009). Again GSSG also expressed daily rhythmic patterns (Atkinson and Babbitt 2009).

Study concluded that spring and winter had beneficial effects than summer on production and reproduction events in mithun. Loss of the antioxidant defense system can generate pathological lesions in cells which would be repaired with the changes in metabolic and molecular adaptation. Therefore, mithun should be supplemented with suitable antioxidants during summer season. Future study is needed to study the circadian rhythmic pattern of OSBs with sampling at 10–20 min intervals in mithun.

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