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Effect of season and age on biochemical, antioxidant and oxidative profiles in mithun bull

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ABSTRACT

Mithun (*Bos frontalis*) is a domesticated free-range bovine species primarily used as a meat animal and is a pride of North Eastern Hilly regions of India. The present study was conducted to measure the effect of season on biochemical, and antioxidant and oxidative stress profiles for different age groups at different seasons in mithun bulls. A total of 30 mithun males were selected from the mithun breeding farm, ICAR-NRC on Mithun, Medziphema, Nagaland and were equally divided into five classes based on their age. Each group consisted of six animals and the groups were Gr A, Gr B, Gr C, Gr D and Gr E. Seasons were categorised into winter, spring, summer and autumn based on the meteorological parameters such as temperature humidity index (THI) and sunshine hours. Biochemical indices such as total protein, albumin, globulin, glucose and total cholesterol; antioxidant profiles such as total antioxidant capacity (TAC), catalase (CAT), glutathione (GSH), glutathione reductase (GSHR) and superoxide dismutase (SOD); and oxidative profile such as malondialdehyde (MDA) were estimated. Statistical results revealed that these experimental profiles differed significantly between the different age groups for the different seasons and between the seasons for different age groups. Blood biochemical indices increased significantly as age advanced and higher concentration in spring and winter than in summer season. TAC, CAT, GSH, GSHR and SOD were significantly greater and MDA was significantly lower in spring and winter than in summer season. The antioxidants increased significantly from Gr A to Gr B and then reduced gradually to Gr E, whereas concentration of MDA significantly increased as the age advanced. It can be concluded that spring and winter season has significantly greater beneficial effects than summer season on production and reproduction programme in semi-intensive management of mithun in tropical humid hilly ecosystem of Nagaland.

Keywords: Age, Antioxidant, Biochemical profiles, Blood, Mithun, Season

Mithun is a unique domestic free-range ruminant bovine species primarily used as a meat animal and is the pride of North Eastern Hilly (NEH) states of India (Arunachal Pradesh, Mizoram, Manipur and Nagaland). It also has an important place in the social, cultural, religious and economic life of the tribal population in NEH region. It is a potential source of delicious meat and can also be used as a draught and pack animal due to its sure footedness on steep slopes of its home tract. The massive unique beautiful animal is well adopted anatomically and physiologically at an altitude ranged from 300–3,000 m msl. Mithun is a new introduction to the field of scientific animal husbandry, hence, a holistic approach from all corners of animal husbandry and veterinary programme are to be made to exploit production and reproduction potential of mithun. Although mithun is not yet endangered, it suffers from severe non-cyclical population fluctuations on a local/regional basis as per the livestock census of Government of India. Statistics show that the mithun population is decreasing gradually due to lack of suitable breeding bulls, increase in intensive inbreeding practices and lack of suitable breeding management in this region. Therefore, greater efforts are required from all the quarters to preserve the mithun population to enhance the socio-economic status and livelihood of this region. A good understanding on the effects of age and season on biochemical and antioxidant and oxidative profiles is important to consider while taking efforts to preserve mithun germplasm.

Biochemical and antioxidant profiles are influenced by various factors like breed, age, physiological status, season, housing system, starvation, stress and transportation (Casella *et al*. 2016, Arfuso *et al*. 2016). Biochemical composition of serum can provide valuable information related to nutrition, sex, age and physiological status of the animal and analysis of these are very useful to get insight in the metabolic and health status of animals (Osman and Al-Busadah 2003). Serum biochemical profiles were consistent with the seasonal trend in rainfall and feed availability, indicating that these blood metabolites are

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sensitive to seasonal variations in feed availability and protein intake. Supplementation of anti-heat stress preparations to heat stressed buffaloes has significantly increased the serum biochemical profiles (Abou-Zeina *et al.* 2009). Seasonal variation in the antioxidant and oxidative status was reported due to variation in the environmental variables such as ambient temperature, relative humidity and photo period (Bello-Klein *et al.* 2000). Heat stress induces enhanced production of free radicals and ROS resulting in oxidative stress and a decrease in antioxidant defense mechanisms in farm animals. Increased environmental temperature during summer season causes reduced gastrointestinal activity and depressed appetite which inturn forced the animals to lipolysis its body reserves to meet their energy demand, which in turn elevated ROS production and reduced antioxidant status (Trevisan *et al.* 2001). Similar type of studies were conducted in livestock species, buffaloes (Nili-Ravi: Das *et al*. 2013, African buffalo: Couch *et al.* 2017, Egyptian buffalo: Hady *et al.* 2018, Murrah: Lakhani *et al*. 2018), cattle (Hariana and Sahiwal: Kumar *et al*. 2017, Holstein Friesian: Cerutti *et al*. 2018, crossbred cattle: Hady *et al*. 2018), goat (Beetal and Toggenberg: Kour *et al*. 2017), sheep (West *et al.* 1999, Rathwa *et al*. 2017) and pig (Iveta *et al*. 2011). Perusal of available literature on similar line of investigation revealed no information in mithun males of different age groups in different seasons under humid tropical hilly ecosystem of Nagaland. Therefore, the present study was designed to assess the effect of season and age classes on biochemical, and antioxidant and oxidative stress profiles in mithun under tropical humid hilly ecosystem of Nagaland.

MATERIALS AND METHODS

Study area: Present study was conducted at Mithun breeding farm, ICAR-National Research Centre on Mithun, Medziphema, Nagaland, India which is located between 25°54″30″ North latitude and 93°44″15″ East longitude and at an altitude range of 250–300 m msl. For calculation of temperature humidity index (THI), meteorological factors (ambient temperature and relative humidity) were obtained from meteorology station of ICAR-ICAR Research Complex for NEH Region, Nagaland, India, located at close proximity of the experimental station. Season-wise THI was calculated for five whole calendar years and one year was divided into four seasons namely spring (February to April; THI: 63.51±0.48), summer (May to July; THI: 76.06±0.45), autumn (August to October; THI: 74.67±0.38) and winter (November to January; THI: 54.41±0.28) with an average THI of 66.89±0.39. THI differed significantly between the experimental seasons. Temperature humidity index was calculated by using the following formula: THI= 0.72 (W + D + 40.6), where W stands for wet bulb temperature (°C) and D stands for dry bulb temperature (°C) (Kadzere *et al*. 2002). Similarly, sunshine hours also differed significantly 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: The present research was carried out on 30 mithun males of different age groups randomly selected from the mithun breeding farm, ICAR-National Research Centre on Mithun, Medziphema, Nagaland, India and were maintained under the same feeding, housing, lighting and management conditions. The feeding schedule was followed as per the farm management for each experimental animal. The mithun males were distributed equally into five different groups Gr A (0.1–1.0 year), Gr B (1.1–2.0 years), Gr C (2.1-3.0 years), Gr D (3.1–5.0 years) and Gr E (5.1–6.0 years) based on their age and consisted of six animals per group.

Estimation of biochemical profiles: The blood samples were collected by venipuncture of jugular vein in heparin tubes (20 IU of heparin/mL of blood) from the experimental mithun bulls at 4 h interval throughout the day during the different seasons. The blood samples were centrifuged at 1200 × g for 15 min at 4°C. The plasma samples were separated rapidly, labelled properly and preserved at –80°C in deep freezer for further analysis.

The biochemical indices such as total protein, albumin, glucose and total cholesterol were estimated with commercially available diagnostic kits (ERBA Mannheim, Germany) using biochemistry analyser (Automated Clinical Chemistry Analyser EM200, ERBA Diagnostics Mannheim GmbH, Germany).

Estimation of antioxidant profiles

Total antioxidant capacity (TAC): Antioxidants in blood plasma were estimated by TAC colorimetric assay kit (BioVision, Mountain View, CA, USA) as per the manufacturer's guidelines. In this assay, Trolox was used as an antioxidant standard. The Cu^{2+} ion was converted to Cu^{+} by antioxidants. The reduced Cu^{+} ion was chelated with a colorimetric probe giving a broad absorbance peak at 570 nm (Thermo Scientific Multiskan GO Microplate Spectrophotometer, USA), proportional to the TAC (mmol/mL).

Superoxide dismutase (SOD): Superoxide dismutase is an enzyme that catalyzes the dismutation (or partitioning) of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide, produced as a by-product of oxygen metabolism, causes many types of cell damage. SOD in blood plasma was estimated by SOD colorimetric assay kit (Cayman Chemical Company, USA) as per the manufacturer's instructions. SOD assay kit utilizes a tetrazolium salt for detection of SOD radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The absorbance was measured at 440–460 nm (Thermo Scientific Multiskan GO Microplate Spectrophotometer, USA). SOD activity was expressed in U/mL.

Glutathione (GSH): Glutathione in the blood plasma was estimated by Cayman's GSH assay kit (Cayman Chemical Company, USA) as per the manufacturer's guidelines. This

assay utilizes an optimized enzymatic recycling method using GSH reductase for the quantification of GSH. The sulfhydryl of GSH reacts DTNB [(5, 5′-dithio-bis-2- (nitro benzoic acid); Ellman's reagent] and produces a yellow coloured 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB (between GSH and TNB) that is concomitantly being produced, is reduced by GSH reductase to recycle the GSH and produces more TNB. The rate of TNB production is directly proportional to this recycling reaction which in turn is directly proportional to the concentration of GSH in the sample. The absorbance of TNB was measured at 405–424 nm (Thermo Scientific Multiskan GO Microplate Spectrophotometer, USA) to estimate the GSH in the sample. GSH activity was expressed in µmol/mL.

Glutathione reductase (GSHR): Glutathione reductase in the blood plasma was estimated by Cayman's GSHRx assay kit (Cayman Chemical Company, USA) as per the manufacturer's guidelines. GSH serves as an essential electron donor to GSH peroxidases in the reduction of hydroperoxides. Glutathione reductase (GSHR) is a flavoprotein that catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to GSH. This enzyme is essential for the GSH redox cycle which maintains adequate levels of reduced cellular GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress. The oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm and is directly proportional to the GSHR activity in the sample. The absorbance was measured at 340 nm (Thermo Scientific Multiskan GO Microplate Spectrophotometer, USA) to estimate the GSHRx in the sample. GSHRx activity was expressed in nmol/min/mL.

Catalase (CAT): Catalase in the blood plasma was estimated by using Cayman's Catalase assay kit (Cayman Chemical Company, USA) as per the manufacturer's guidelines. This assay kit utilizes the peroxidase function of CAT for determination of enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured colorimetrically with 4-aminos-3-hydrazino-5-mercapto-1, 2, 4-triazole (Purpald) as the chromogen. Purpald specifically forms a bicycle heterocycle with aldehydes, which upon oxidation changes from colourless to a purple colour. The absorbance was measured at 540 nm (Thermo Scientific Multiskan GO Microplate Spectrophotometer, USA) to estimate the activity of CAT in the sample. CAT activity was expressed in nmol/min/mL.

Estimation of oxidative profile

Malondialdehyde: Malondialdehyde in blood plasma was separated and determined as conjugate with TBA. Plasma proteins were precipitated by TCA and then removed by centrifugation. The MDA–TBA complex was measured at 534 nm (Thermo Scientific Multiskan GO Microplate Spectrophotometer, USA) (Shah and Walker 1989).

Statistical analysis: The statistical analysis of the data was performed as per standard procedures. Analysis of variance (ANOVA) was performed using a generalized liner model (Statistical Analysis System for Windows, SAS Version 9.3; SAS Institute, Inc., Cary, NC, 2001) and treatment means were separated using Student-Newman-Keuls (SNK) multiple range test. The data used in the study were tested for normality before analysis using Shapiro-Wilk statistics. Means were analyzed by two-way analysis of variance (ANOVA), followed by the Tukey's post hoc test to determine the significant differences between the different seasons and between the age groups on different seasons on the selected blood parameters using the SAS software/PC computer program. The per cent data were subjected to arcsine (angular) transformation before proceeding to general linear model. Differences with values of $p<0.05$ were considered to be statistically significant after arcsine transformation of percentage data. Correlation between age and biochemical, antioxidant and oxidative profiles and between the THI and biochemical, antioxidant and oxidative profiles were established with correlation coefficient being done as per Pearson's method.

RESULTS AND DISCUSSION

Statistical results revealed that these experimental profiles differed significantly ($p<0.05$) between the different age groups for the different seasons and between the seasons for different age groups. Blood biochemical indices significantly ($p<0.05$) increased as the age advanced and were higher in spring and winter than in summer. The total protein was significantly higher in Gr E and lower in Gr A in winter (21.76 and 18.08%), spring (20.47 and 18.41%), summer (23.11 and 16.39%) and autumn (21.58 and 17.79%). Similarly, significantly higher total protein was observed in spring and lowest in summer in Gr A (30.31 and 18.82%), Gr B (30.07 and 19.78%), Gr C (29.63 and 20.18%), Gr D (28.82 and 21.02%) and Gr E (27.70 and 21.80%). Albumin was significantly higher in Gr E and lower in Gr A in winter (22.46 and 17.05%), spring (23.33 and 16.96%), summer (25.17 and 13.03%) and autumn (22.10 and 17.83%) seasons. Similarly, significantly higher total protein was observed in spring and lowest in summer in Gr A (29.92 and 16.17%), Gr B (29.38 and 18.53%), Gr C (28.16 and 21.27%), Gr D (28.30 and 22.13%) and Gr E (29.12 and 22.06%). Globulin was significantly higher in Gr E and lower in Gr A in winter (21.18 and 18.94%) and summer (21.38 and 19.21%), and higher in Gr C and lower in Gr A in spring (21.45 and 19.62%) and autumn (21.24 and 17.79%) seasons. Similarly, significantly higher globulin was observed in spring and lowest in summer in Gr A (30.60 and 20.77%), Gr B (31.23 and 21.65%), Gr C (30.83 and 19.29%) and Gr D (29.32 and 19.95%). In Gr E, winter has higher and summer has lower value (27.34 and 21.55%). Glucose was significantly higher in Gr E and lower in Gr A in winter (21.87 and 17.82%), spring (22.92 and 17.31%), summer (22.82 and 17.02%) and autumn (22.36 and 17.75%) seasons. Similarly, significantly higher

glucose was observed in spring and lowest in summer in Gr C (26.53 and 23.36%), Gr D (26.20 and 24.09%) and Gr E (26.88 and 23.86%) and winter had higher and summer had lower value in Gr A (26.36 and 22.89%) and Gr B (26.61 and 23.15%). Total cholesterol was significantly higher in Gr E and lower in Gr A in spring (21.46 and 17.87%), summer (21.34 and 17.66%) and autumn (20.64 and 19.45%) seasons and in winter, Gr C had higher and Gr A had lower value (21.15 and 18.42%). Similarly, significantly higher total cholesterol was observed in winter and lowest in summer in Gr A (26.63 and 23.20%), Gr B (26.33 and 23.52%), Gr C (27.29 and 24.89%) and Gr D (26.32 and 24.26%) and spring had higher and summer had lower value in Gr E (26.15 and 24.47%) (Fig. 1).

TAC, CAT, GSH, GSHR and SOD were significantly ($p<0.05$) higher in spring and winter seasons than in summer season whereas MDA was significantly ($p<0.05$) higher in summer than in spring and winter seasons. These antioxidants increased significantly from Gr A to Gr B and then reduced gradually to Gr E, whereas concentration of MDA increased as the age advanced. TAC was significantly higher in Gr B and lower in Gr D in winter (23.69 and 16.90%), spring (24.33 and 15.73%) and autumn (24.55 and 16.58%) and in summer, Gr B had higher and Gr E had lower value (24.78 and 16.84%). Similarly, significantly higher TAC was observed in spring and lowest was in summer in Gr A (30.98 and 20.27%), Gr B (29.09 and 21.74%), Gr C (27.62 and 21.66%), Gr D (27.60 and 22.25%) and Gr E (30.23 and 20.58%). CAT was significantly higher in Gr B and lower in Gr A in winter (20.81 and 18.98%), higher in Gr C and lower in Gr A in spring (21.38 and 20.67%), higher in Gr B and lower in Gr E in summer (21.31 and 18.97%) and autumn (21.50 and 18.68%) seasons. Similarly, significantly higher CAT was observed in spring and lowest was in summer in Gr A (28.13 and 21.05%), Gr B (27.81 and 21.42%), Gr C (29.31 and 20.68%), Gr D (27.97 and 21.93%) and Gr E (28.29 and 20.67%). GSH was significantly higher in Gr C and lower in Gr E in winter (24.69 and 15.64%) and summer (27.68 and 14.28%), higher in Gr B and lower in Gr E in spring (24.61 and 16.77%) and higher in Gr C and lower in Gr A in autumn (23.95 and 14.36%). Similarly, significantly higher GSH was observed in spring and lowest in autumn in Gr A (33.97 and 16.02%), Gr B (35.86 and 13.54%), Gr D (32.33 and 13.38%) and Gr E (34.09 and 12.85%) and higher in winter and lower summer in Gr C (32.39 and 16.90%). GSHR was significantly higher in Gr B and lower in Gr A in winter (26.05 and 16.32%) and spring (26.97 and 16.15%), higher in Gr B and lower in Gr E in summer (29.48 and 15.23%) and autumn (30.64 and 14.47%). Similarly, significantly higher GSHR was observed in spring and lowest was in summer in Gr A (28.43 and 19.97%), Gr B (28.72 and 20.45%), Gr C (28.95 and 19.84%) and Gr E (33.48 and 17.74%) and higher in winter and lower summer in Gr D (31.46 and 17.51%). SOD was significantly higher in Gr A and lower in Gr E in winter (24.18 and 15.03%), spring (23.67 and 14.78%) seasons,

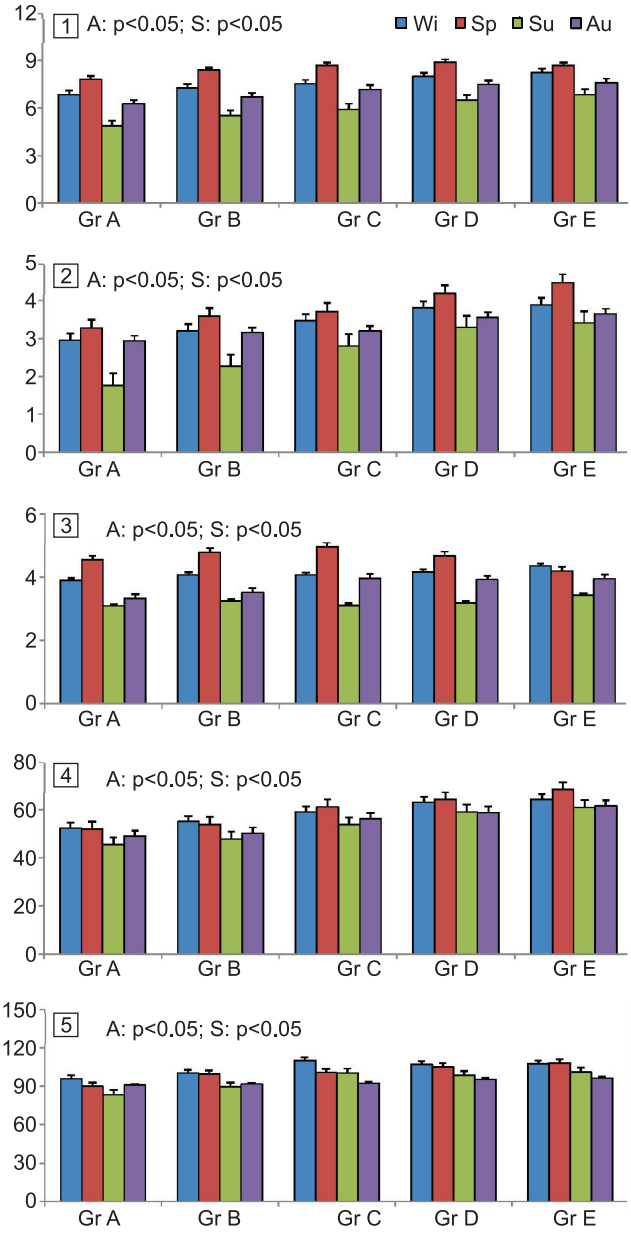

Fig. 1. Effect of seasons and age on biochemical profiles in mithun males (mean±SE). Vertical bar on each point represents standard error. 1, Total protein (g/dl); 2, Albumin (g/dl); 3, Globulin (g/dl); 4, Glucose (mg/dl) and 5, Total cholesterol (mg/dl). Gr A (10.70 months), Gr B (21.08 months), Gr C (32.82 months), Gr D (53.03 months) and Gr E (69.48 months). A, age effect; S, season effect; n= 6 for each group. Seasons: Wi, Winter; Sp, Spring; Su, Summer and Au, Autumn.

higher in Gr A and lower in Gr D in summer (31.85 and 13.93%) and higher in Gr B and lower in Gr E (22.64 and 15.53%). Similarly, significantly higher SOD was observed in spring and lowest was in autumn in Gr A (34.36 and 13.79%), Gr B (37.10 and 16.01%), higher in spring and lower in summer in Gr C (36.56 and 14.84%), Gr D (37.89 and 13.80%) and Gr E (35.47 and 16.84%). MDA was significantly higher in Gr E and lower in Gr A in winter (25.42 and 16.09%), spring (23.30 and 17.01%) and summer (24.34 and 16.07%) seasons and higher in Gr D

and lower in Gr A in autumn (23.44 and 16.79%). Similarly, significantly higher MDA was observed in summer and lowest was in winter in Gr A (28.83 and 21.24%) and Gr C (30.21 and 20.45%), higher in summer and lower in spring in Gr B (28.96 and 21.44%), Gr D (29.43 and 20.65%) and Gr E (30.04 and 20.73%) (Fig. 2).

The correlation analysis revealed that significant positive correlation ($p<0.05$) between age and total protein, albumin, globulin, glucose, total cholesterol and MDA and negative correlation between age and antioxidant profiles in winter, spring, summer and autumn seasons in mithun. Again THI had a significant ($p<0.05$) positive correlation with MDA and negative correlation with the antioxidant profiles and biochemical profiles.

Although several studies were conducted to assess the effect of season and age on biochemical, and antioxidant and oxidative stress profiles in different livestock species, similar line of studies were lacking in mithun under humid tropical hilly ecosystem of Nagaland. Serum biochemistry profiles are often used to determine the physiological abnormalities in animals that may indicate disease and these parameters vary between the populations, age groups and across the seasons (Couch *et al*. 2017). Biochemical profiles such as total protein, albumin, globulin, glucose and total cholesterol concentration were decreased significantly in dry hot summer stressed animals. In general, serum biochemical profiles were consistent with this seasonal trend in rainfall and feed availability, indicating that these blood metabolites are sensitive to seasonal variations in feed availability and protein intake. The decreased serum proteins concentration during dry summer season can be partially due to decline in feed consumption and marked increase in respiratory activity with rising temperature (Fox *et al*. 1988). Plasma total protein level tended to be lower during maximum heat load (Gudev *et al.* 2007). El-Nouty *et al.* (1980) reported that plasma total protein content was decreased by 11.9% in buffaloes when exposed to direct solar radiations. In lactating buffaloes, El-Khashab (2010) reported that total protein, albumin and globulin were improved significantly by application of cooling methods. Higher eosinophil count in dry season indicates that these animals suffered parasite infestation which inturn chronic stimulation of immuno system, which increased utilization of globulin into gamma globulin (Otto *et al*. 2000) leads to deficiency of globulin in dry summer season. Moreover, during summer season, the protein intake was decreased, that may be reason, total protein and albumin levels have been reduced. In one study, supplementation of anti-heat stress preparations like zinc, sodium selenite and vitamin E to heat stressed buffaloes significantly increased the total serum proteins and total globulins (Abou-Zeina *et al.* 2009). Similar decrease of total protein concentration under heat stress condition was also recorded by Haeeb *et al.* (2007). Blood cholesterol was lowered during summer than rainy season in the present study and similar observation was reported in lactating buffaloes (Verma *et al.* 2000). Haeeb *et al*. (1996) showed that cholesterol concentration

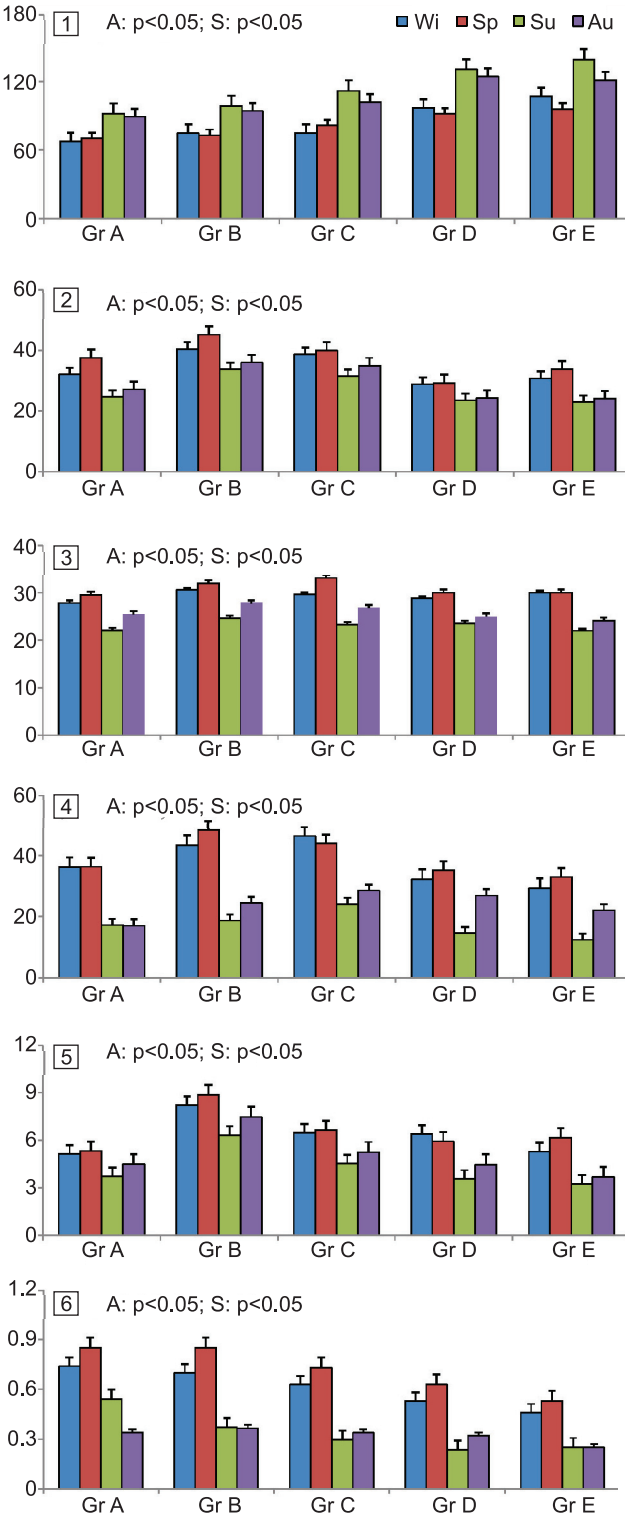

Fig. 2. Effect of seasons and age on antioxidant and oxidative stress profiles in mithun males (mean±SE). Vertical bar on each point represents standard error. 1, Malondialdehyde (nmol/L); 2, Total antioxidant Capacity (nmol/µL); 3, Catalase (nmol/min/L); 4, Glutathione (nmol/L); 5, Glutathione Reductase (nmol/min/L) and 6, Superoxide dismutase (nmol/min/L). Gr A (10.70 months), Gr B (21.08 months), Gr C (32.82 months), Gr D (53.03 months) and Gr E (69.48 months). (A, age effect; S, season effect; n= 6 for each group). Seasons: Wi, Winter; Sp, Spring; Su, Summer and Au, Autumn.

decreased markedly with increase in environmental temperature. Marai and Haeeb (2010) explained that the decrease of cholesterol might be due to dilution resulting from increase of total body water or to the decrease in acetate concentration, a primary precursor for the synthesis of cholesterol. Similarly, Hooda and Singh (2010) reported that when animals were exposed at 40°C for 4 h for 16 days in a psychrometric chamber, blood total cholesterol decreased significantly at day 8 and 16 after exposure. Similar observation was also reported in mithun in psychrometric chamber that higher THI (78.29) had reduced the level of blood cholesterol (Khate 2015). In another study, Gudev *et al.* (2007) reported that plasma cholesterol tended to be lower during the exposure to direct solar radiation as compared to the morning levels. Such reduction of cholesterol may be due to increase in utilization of fatty acids for energy production as a consequence of the decrease in glucose level. During heat stress, feed consumption decreases, which comparatively lower the blood glucose level (Kataria *et al*. 2002) or it might be due to increased glucose oxidation (Collier *et al*. 2008) during summer stress. Decreased gluconeogenesis and glycogenolysis were observed in cows during heat stress (Itoh *et al*. 1998). The present study finding was similar to the observations of Kataria *et al.* (1993) in Marwari goats and Rasooli *et al*. (2004) in heifers. Similar total protein was reported in bovine species by Otto *et al*. (2000) and Surya Prakash *et al*. (2018) and Xuan *et al*. (2018), Mamun *et al*. (2013) and Suharti *et al*. (2017) reported higher value and Mahima *et al*. (2013) reported lower value than in the present study. Albumin value in our study is similar to that reported by Otto *et al*. (2000), Mahima *et al*. (2013) and Mamun *et al*. (2013) whereas lower values were reported by Surya Prakash *et al*. (2018) and Suharti *et al*. (2017) and higher value reported by Xuan *et al*. (2018) than in the present study. Similarly, Otto *et al*. (2000) and Xuan *et al*. (2018) reported similar values of globulin as in the present study for mithun whereas Mahima *et al*. (2013) reported lower value than in the present study. With regards to glucose, Otto *et al*. (2000) and Mamun *et al*. (2013) reported similar values, Suharti *et al*. (2017) and Surya Prakash *et al*. (2018) reported lower value than in the present study.

In physiological conditions, there is a balance between the factors that promote the formation of free radicals and the levels of antioxidants. The body contains an elaborate antioxidant defense system that depends on dietary intake of antioxidant vitamins and minerals and the endogenous production of antioxidant compounds such as GSH. Reactive oxygen species are scavenged by enzymatic antioxidants like SOD, GSHR, CAT (Halliwell and Gutteridge 2006) and by small molecular antioxidants such as GSH. GSH appears to be essential for the activation and maintenance of cellular defences against oxidative stress, since it provides the substrate for glutathione peroxidase to detoxify peroxides. Animals in dry summer season have significantly lower amount of antioxidants and higher concentration of MDA which indicated that these animals were under with severe oxidative stress in tropical humid hilly ecosystem of Nagaland (Ghosh *et al*. 2013). In the present study, the free radical production was significantly higher in animals in dry summer season as similar report was observed that heat stress/stress stimulates excessive production of free radicals (Bernabucchi *et al*. 2002, Sivakumar *et al*. 2010). Moreover, exercise in summer season is postulated to generate free radicals by (a) increases in epinephrine and other catecholamines that can produce oxygen radicals when they are metabolically inactivated, (b) production of lactic acid that can convert a weakly damaging free radical (superoxide) into a strongly damaging one (hydroxyl), and (c) inflammatory responses to secondary muscle damage incurred with overexertion (Sen 1995). Seasonal variation in the antioxidant and oxidative status is due to weather or environmental variables such as ambient temperature, relative humidity and photo period (Bello-Klein *et al.* 2000). Deficiency of antioxidants may occur due to different kinds of stress (McDowell *et al*. 2007). These free radical oxidations are activated in animals under various types of stresses and lipid peroxidation products accumulate in various organs. Among the stressors, heat stress has been of major one in reducing animal productivity in tropical, sub-tropical and arid areas (Silanikove *et al.* 1997). Heat stress induces oxidative stress reaction causes excessive production of free radicals which in turn damage the cell membrane and molecular structures (Ghosh *et al*. 2013). Zuo *et al.* (2000) and Mujahid *et al.* (2005) proved that heat induces increased ROS production. Antioxidants prevent or inhibit oxidation of the substrate (lipids, protein or DNA) by donating electrons (Halliwell and Gutteridge 1995). TAC, SOD, CAT, GSH and MDA are well known markers of oxidative stress (Pathan *et al*. 2009). Increased environmental temperature during dry summer season causes reduced gut motility, rumination, ruminal contractions and depressed appetite which have a direct negative effect on appetite centre of the hypothalamus (Zha *et al*. 2009) and the animal is forced to lipolysis its body reserves to meet their energy demand, which in turn elevated the levels of reactive oxygen species production and reduced antioxidant status (Trevisan *et al*. 2001). Similar findings were observed that higher MDA and lower antioxidant level was in buffaloes affected with summer hot stress (Chaudhary *et al*. 2015). Similar results were observed in the present study in the mithun species under humid tropical hilly ecosystem. The stress can be counteracted by supplementation of antioxidants are very helpful in animal species (Sejian *et al*. 2012) as because the antioxidants are compounds or systems that delay autoxidation by inhibiting the formation of free radicals or by interrupting propagation of the free radical by several mechanisms (Brewer 2011). This in turn helps to protect from cellular damage during any stressful condition.

The correlation studies revealed that significant positive correlation ($p<0.05$) between age and total protein, albumin, globulin, glucose, total cholesterol and MDA and negative correlation between age and antioxidant profiles in winter,

spring, summer and autumn seasons in mithun were observed. Again THI had a significant ($p<0.05$) positive correlation with MDA and negative correlation with the antioxidant profiles and biochemical profiles. These correlation results in the present study were in agreement with other workers, they got similar negative correlation between higher THI and the health and physiological status of bovine species (Ghavi Hossein-Zadeh *et al*. 2013, Alhussien and Dang 2017, Polsky and von Keyserlingk 2017).

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