

To Study the Effect of Meteorological Variables on Oxidative Stress Parameters in Balck Bengal Goat

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ABSTRACT

Harsh environments create stressful conditions to animals raised in extreme climatic areas, and as a result, animals develop various adaptive mechanisms that enable them to survive under conditions of extreme heat or extreme cold. The present study entitled, "The effect of Meteorological Variables on Oxidative Stress in Black Bengal Goat" was conducted on twelve adult apparently healthy Black Bengal Goats. The does were investigated for their antioxidant's profiles during autumn (Oct to Nov) and winter (Dec to Jan) seasons. Meteorological parameters like dry bulb temperature and relative humidity were recorded daily for two times at 6 am and 3 pm for estimation of THI throughout the study period. In the present study, the mean THI found during autumn and winter season was 70.45 ± 0.60 and 57.58 ± 0.91 respectively while the mean THI for last one years was 72.09 ± 0.38 during autumn and 62.14 ± 0.28 during winter season. All the antioxidants that studied (SOD, LPO and GPx) showed significant ($p < 0.05$) variations during autumn and winter season. The SOD (IU/ml), LPO (nmol of MDA/ml of packed cells) level was observed to be higher during winter season than the autumn season whereas GPx (IU/ml) level was found lower during winter. Thus, the SOD and LPO was inversely proportional to THI and GPx was directly proportional to the THI. The study revealed that some of the parameters studied were visibly drifted from normal values as a result of climate stress during winter season. The results also highlighted drastic variations in the values of some of the antioxidants in animals. The information obtained is useful in understanding the adaptive physiology of the Black Bengal goats during environmental stress. The data generated may also help to distinguish and discriminate healthy animals from stressed ones under special physiological status of goats.

Keywords: Temperature humidity index, Dry bulb temperature, Wet bulb temperature, Antioxidants, Superoxide dismutase, Glutathione peroxidase.

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INTRODUCTION

The term “Climate change” can be described as long-term imbalance of customary weather conditions such as temperature, radiation, wind and rainfall characteristics of a particular region. The changes definitely have an enormous impact on health and biology of both human and animal kingdom. Out of various breeds of goat in India Black Bengal goat is observed to be highly tolerant to changing weather condition. The breed is found to be confined in north eastern India mostly in west bengal, jharkhand, bihar and orissa regions. The importance of goat in the rural economy is evidenced by its unparalleled economic traits, ability to get acclimatized under diversified agro-climatic condition, unfastidious type in choosing the available forage, high fertility and short generation interval. The breed occupies a unique position since they are used as a multipurpose animal for production of milk, meat and fiber besides hides and skin. The Black Bengal Goats adapt poorly to cold climates but relatively more adapted to areas of high temperatures (Shelton & spiller, 1977). Goats appear to have superior adaptation to arid tropics because of its ability to conserve water, travel well, graze selectively and take willingly a wide Variety of vegetation (Meferlane & Howard, 1972; Maloity & Taylor 1971 & Merrill et al., 1965). Adaptation of animals for ecological stress makes them better survivors for which the metabolic strength of blood is highly important. The seasonal rythms reflect the endogenous adaptive mechanism to react in advance to the regular environmental changes associated with the seasons (Piccione et al., 2009). Goats adapted to a harsh environment, perform better than other domesticated ruminants (Shkolnik & Silanikove, 1981; King, 1983; & Devendra, 1990). The range of thermo neutrality for goats is an environment having an air temperature of 13–27°C, relative humidity of 60-70 per cent (Mishra & Puneet, 2009). The temperature below and above this range makes animal stressful and reduces productivity. The breed has developed various adaptive mechanisms that allow their survival

at very high as well as very low temperatures (Al-Tamimi, 2007). In the case of short-day breeders, the reports are inadequate to depict the role of free radicals in the regulation of immunity. Various kinds of stress viz., heat and cold stress leads to the production of reactive oxygen species (ROS) such as superoxide, peroxide, hydroxyl radical and singlet oxygen species. Reactive oxygen species damage, biomolecules such as DNA/RNA, proteins and lipid peroxidation of membranes and disruption of normal cell metabolism (Rehman, 1984). Superoxide dismutase (SOD), Catalase (CAT), Lipid peroxidase (LPO) and glutathione peroxidase (GPX) are important antioxidant enzymes. Antioxidants are the substances that significantly delay or inhibit oxidation of a substrate. The production of free radicals and their neutralization by antioxidants is a normal body process. Ambient stress can reduce antioxidant activity of blood (Harmon et al., 1997) resulting in oxidative stress. Antioxidants enzymes protect the cell against cellular oxidants and prevent their accumulation. Excessive production of reactive oxygen species depletes such antioxidants and induces oxidative stress which suppresses the immune system and reduces productivity of animal (Kumar et al., 2011a).

MATERIALS AND METHODS

The present study was conducted on Black Bengal Goat in the Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, Birsa Agricultural University, Kanke, Ranchi-6, Jharkhand from October 2018 to January 2019. The average daily temperature at this period varied from 16.7°C to 23.6°C with average relative humidity percentage 65%.

2.1 Experimental Animals:

Apparently healthy, Black Bengal does n=12, having approximately average body weight of 8 to 14 kg and age 8-24 months, reared under uniform managemental husbandry practices were selected from, Instructional Farm of Small Ruminants (I.F.S.R.) College of

Veterinary Science and A.H. Birsa Agricultural University, Kanke, Ranchi, Jharkhand for the present experiment. Selected does were isolated from the herd at least fifteen days prior to start of the experiment. Design of experiment had been approved by the Institutional Animal Ethics Committee vide letter no- 528/CPC8EA.

2.2 Composition of the ration and management:

The does were provided green fodder, routine grazing (daily for four to six hours) with balanced ration. Animals were maintained on a standard ration at the rate of 250 gm/animal/day with green fodder and the water was provided *ad-libitum*. The animals were dewormed with anthelmintic f mebendazole @ 10mg/kg of body weight 15 days prior to the start of experiment.

2.3 Experimental Design:

The selected twelve animals were maintained at normal animal husbandry practices. The animals were kept in Instructional Farm of Small Ruminants; RVC. The experiment was conducted during two seasons i.e., autumn (Oct-Nov) and winter (Dec- Jan).

2.4 Blood Sampling:

The blood samples with anticoagulant (EDTA) in vials were collected from jugular vein of

each animal on 0th day of experiment and thereafter on every 15th day up to 120 days for haematological examination. The collected blood samples were taken immediately to the laboratory in ice box.

2.5 Reagents:

Phosphate buffer saline (pH 7.4); 30 % Trichloroacetic acid solution; 0.1M EDTA; 0.67 % Thiobarbituric acid (TBA); MTT [3-(4-5 dimethyl thiazole 2-yl) 2.5 diphenyltetrazolium bromide]; Dimethyl sulfoxide (DMSO); Pyrogallol (100 Mm); 0.3 M Sodium tungstate; 0.1 M EDTA; 0.3 M Sodium tungstate and 0.1 M EDTA.

2.6 Meteorological Parameters:

Climatic variables viz temperature, relative humidity was recorded. Temperature humidity index was calculated as per Johnson formula (1972). $THI = 0.72 (DBT + WBT) + 40.6$ Were, THI = Temperature humidity index, DBT = Dry bulb temperature, WBT = Wet bulb temperature.

2.6.1 Ambient temperature (°C)

The following table 1 shows the ambient temperature of autumn & winter as recorded at 15th day interval at different period for the shades of experimental animals.

Table1: Ambient temperature (°C) of autumn and winter season at different periods (Mean±SE)

Days	AUTUMN	WINTER	t value (P critical)
15 th day	17.27±0.40 ^b	6.91±0.62 ^b	7.00 ^{**}
30 th day	15.29±0.53 ^b	3.55±0.60 ^a	7.74 ^{**}
45 th day	16.43±1.07 ^b	3.89±0.40 ^a	3.00 ^{**}
60 th day	10.74±1.03 ^a	5.16±0.88 ^{ab}	0.009 ^{**}
F value	12.79 ^{**}	5.53 ^{**}	

NS - $P \geq 0.05$; * $P < 0.05$ - Significant; ** $P < 0.01$ - Highly Significant

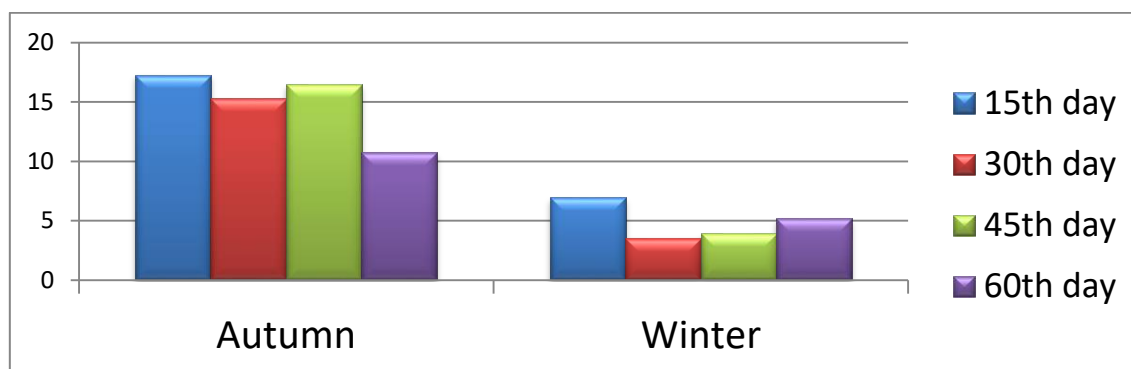


Fig. 1: Bar diagram depicting the ambient temperature (°C) of autumn and winter season at different periods (Mean ±SE)

As observed from table no-I, mean ambient temperature in autumn recorded 15th day interval was 17.27 ± 0.40 , 15.29 ± 0.53 , 16.43 ± 1.07 and 10.74 ± 1.03 at 15th, 30th, 45th and 60th day respectively (Table 1, Fig 1). The mean ambient temperature was found to be significantly lower ($p < 0.01$) on day 60th during autumn. Similarly, during winter season, the mean ambient temperature recorded on 15th day was found to be significantly ($p < 0.01$) higher as compared to that was on day 30th and 45th. The mean ambient temperature was found to be 6.91 ± 0.62 , 3.55 ± 0.60 , 3.89 ± 0.40 and 5.16 ± 0.88 on 15th day, 30th, 45th and 60th respectively. Significantly lower level of ambient temperature ($p < 0.01$) was observed on day 60 as compared to day 15, 30 and 45 during autumn. The difference in ambient temperature between autumn and winter was found to be highly significant on each

fortnight, it was higher during autumn whereas it was lower than the thermal comfort zone (12-24) during winter, and it was ≤ 5 which creates cold stress in animals.

2.6.2 Relative humidity (%)

Table-2 suggests that the difference between Relative humidity was non-significant between Autumn and Winter at all the fortnights except for that at day 15th the t-value (p critical) in that case was 0.04 ($p < 0.05$) and thus the relative humidity was significantly higher in Autumn (69 ± 0.33) than in winter (66.4 ± 1.22). Relative humidity on 15th, 30th, 45th and 60th day in Autumn was observed to be 69 ± 0.33 , 70.06 ± 1.29 , 66.4 ± 2.43 , and 65.26 ± 1.76 respectively (Table 2, Fig 2), while during winter, the relative humidity was found to be 66.4 ± 1.22 , 66.73 ± 1.65 , 67.8 ± 0.50 and 67.53 ± 0.74 on 15th, 30th, 45th and 60th day respectively.

Table 2: Relative humidity (%) of autumn and winter season at different periods (Mean \pm SE)

Days	AUTUMN	WINTER	t value (P critical)
15 th day	69 ± 0.33	66.4 ± 1.22	0.04*
30 th day	70.06 ± 1.29	66.73 ± 1.65	0.12 ^{NS}
45 th day	66.4 ± 2.43	67.8 ± 0.50	0.56 ^{NS}
60 th day	65.26 ± 1.76	67.53 ± 0.74	0.28 ^{NS}
F value	1.83 ^{NS}	0.34 ^{NS}	

NS - $P \geq 0.05$; * $P < 0.05$ - Significant; ** $P < 0.01$ - Highly Significant

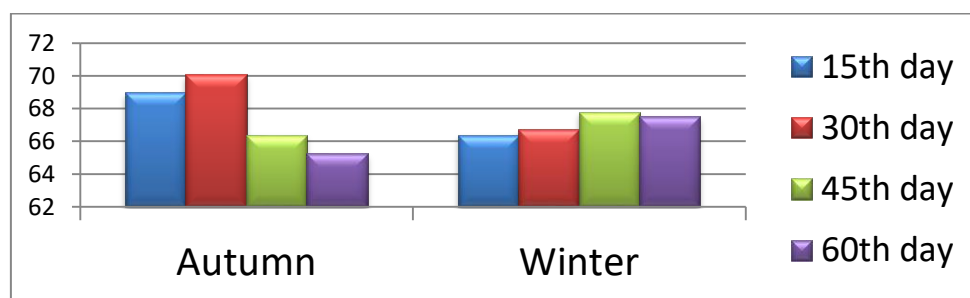


Fig. 2: Bar diagram depicting Relative humidity (%) of autumn and winter season at different periods (Mean \pm SE)

Statistically, non-significant differences were observed in average relative humidity (%) on different fortnights during autumn as well as during winter. There was a gradual fall in relative humidity during winter whereas a rise was observed on day 30 during autumn followed by a gradual fall on day 45 and 60. In our finding the average relative humidity during winter was higher than the findings of

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Rathwa et al. (2017), because of heavy rainfall in this region (tropical humid).

2.6.3 Temperature humidity index

The mean (Temperature humidity index) THI value have been found to be 72.48 ± 0.45 , 70.45 ± 0.60 , 72.73 ± 1.23 and 66.64 ± 0.97 for 15th, 30th, 45th and 60th day respectively. The F value of 10.54 ($P < 0.01$) was highly significant indicating significant variation in THI value at

periodical interval. The highest mean THI value was 63.87 ± 0.56 as recorded at 15th day of winter followed by mean value of 57.58 ± 0.91 , 58.29 ± 0.68 and 60.89 ± 1.13 at 30th, 45th and 60th day respectively. The difference between THI value of autumn and

winter was found to be highly significant ($P < 0.01$) at 15th, 30th, 45th day interval and significant ($P < 0.05$) at 60th day interval. Although the (Table 3, Fig 3) THI value of autumn was higher than winter at all the four fortnightly intervals.

Table 3: Temperature humidity index of autumn and winter season at different periods (Mean \pm SE)

Days	AUTUMN	WINTER	t value (P critical)
15 th day	72.48 ± 0.45^b	63.87 ± 0.56^c	0.00**
30 th day	70.45 ± 0.60^b	57.58 ± 0.91^a	0.00**
45 th day	72.73 ± 1.23^b	58.29 ± 0.68^a	0.00**
60 th day	66.64 ± 0.97^a	60.89 ± 1.13^b	0.01*
F value	10.54**	11.29**	

NS - $P \geq 0.05$; * $P < 0.05$ - Significant; ** $P < 0.01$ - Highly Significant

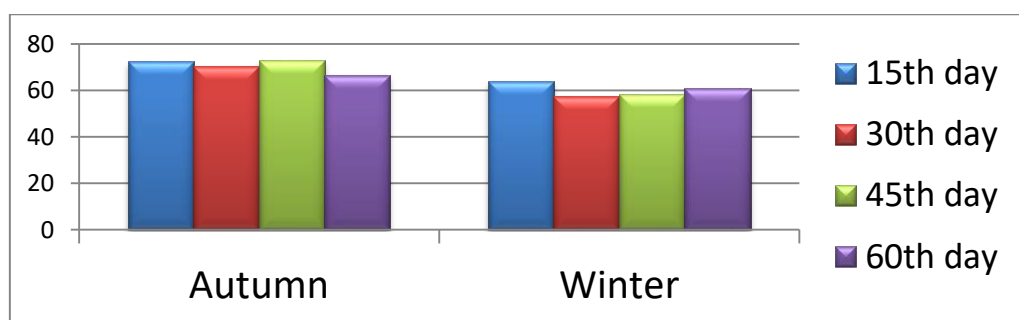


Fig. 3: Bar diagram depicting Temperature humidity index of autumn and winter season at different periods (Mean \pm SE)

In the present study, significant differences were observed within season as well as between seasons. Significantly lower level of THI ($p < 0.01$) was observed on day 60 during autumn whereas significantly lower level of THI ($p < 0.01$) was observed on day 30 and 45 as compared to day 60 and 45. The THI was significantly higher during autumn as compared to winter is in consistent with the findings of Rathwa et al. (2017) who also reported a higher THI during summer.

2.7 Oxidative Stress Parameter:

2.7.1 Lipid peroxidation (LPO)

Lipid peroxidation in tissue homogenate was estimated in terms of malondialdehyde (MDA) production by the modified method of Stock

and Dormandy (1971) as described by Jain (1988).

0.5 ml of tissue homogenate was suspended in 0.5 ml of PBS. To this 0.5 ml of 30 % TCA was added. Tubes were vortexed and allowed to stand on ice for at least 2 h. Tubes were centrifuged at 2000 rpm for 15 minutes. 1 ml of the supernatant was transferred into another tube and to this 0.075 ml of 0.1 M EDTA and 0.25 ml of 0.67 % thiobarbituric acid in 0.05 M NaOH was added. It was mixed and the tubes were kept in a boiling water bath for 15 min and then allowed to cool at room temperature. Absorbance was read at 532 nm. The amount of lipid peroxidation is expressed in nanomoles of MDA formed per mg of protein.

$$\text{nM/mg protein} = \frac{\text{OD}}{\epsilon} \times \frac{\text{Total vol. in cuvette}}{\text{Vol. of sample}} \times \frac{\text{Path length (cm)}}{\text{mg/protein/ml sample}}$$

2.7.2 Superoxide Dismutase Activity (SOD)

Superoxide dismutase (SOD) was estimated as per the method described by Mahesh and Balasubramanian (1998). It involves

generation of superoxide by pyrogallol autoxidation and the inhibition of superoxide dependent reduction of the tetrazolium dye MTT [3-(4-5 dimethyl thiazole 2-yl) 2,5

diphenyltetrazolium bromide] to its formazan, measured at 570 nm. The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helps to solubilize the formazan formed. Briefly, the reaction mixture contained (0.65 ml PBS), pH 7.4, 30 μ l 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT; 1.25 Nm), 75 μ l pyrogallol (100 Mm), and 10 μ l hemolysate or tissue homogenate. The mixture

was incubated at room temperature for 5 min and the reaction was stopped by adding 0.75 ml of dimethyl sulfoxide. The absorbance was read at 570 nm in a spectrophotometer. The enzyme activity has been expressed as uM MTT formazan formed/min/mg protein, using a molar extinction coefficient of $17000 \text{ M}^{-1} \text{ cm}^{-1}$. The colour evolved is stable for many hours, and expressed as uM of MTT formazan formed/mg protein.

$$\mu\text{M MTT formazan} = \frac{\text{ODt-ODc}}{\epsilon} \times \frac{\text{Total vol. in cuvette}}{\text{Vol. of sample}} \times \frac{\text{Path length (cm)}}{\text{mg/protein/ml sample}}$$

2.7.3 Reduced Glutathione (GSH)

GSH was estimated by the method of Prins and Loos (1969). Briefly, 0.2 ml of whole blood or tissue homogenate was mixed with 4 ml of 0.08 N H_2SO_4 . After 10 min, 0.5 ml of tungstate solution (0.3 M Sodium tungstate and 0.1 M EDTA) was added and mixed vigorously for 5 min to clear the brown hemolysate. After allowing it to stand for 5 min to avoid crust formation on top of the supernatant, it was centrifuged for 20 min at 2000 rpm at room temperature. Two ml of supernatant was mixed with 2.5 ml of Tris buffer (1M; pH 8.0 and 0.2 ml of 5, 5-dithiobis-2-nitrobenzoic acid reagent. The absorbance was read immediately at 412 nm in spectrophotometer. The GSH content has been expressed as mM/ml blood or mM/g tissue by using a molar extinction coefficient of $13.6 \times 103 \text{ M}^{-1} \text{ cm}^{-1}$.

RESULT AND DISCUSSION

Climatic changes have range of impact on physical, mental & community health of animals. The present experiment was conducted to study the effect of meteorological variables on oxidative stress in Black Bengal Goat. Harsh environments constitute stressful conditions to animals raised in extreme climatic areas, and as a result, animals develop various adaptive mechanisms that enable them to survive under conditions of extreme heat or extreme cold.

3.1 Lipid Peroxidation (mol of MDA produced /ml)

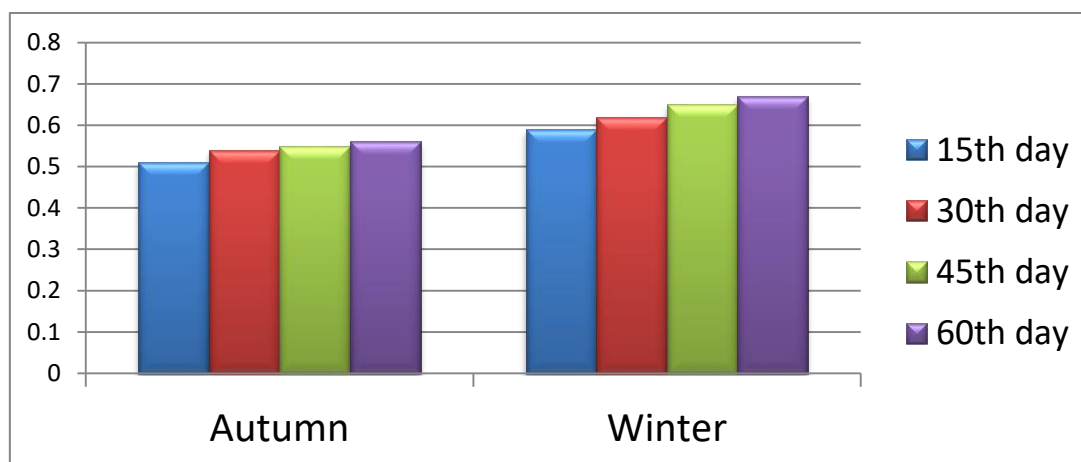
The process of lipid peroxidation is one of oxidative conversion of polyunsaturated fatty acids known as malondialdehyde (MDA), which is the most commonly studied, biologically relevant free radical reaction (Esterbauer et al., 1991 & Celi, 2011) and considered to be the best indicators of oxidative stress (Nielsen et al., 1997 & Georgieva, 2005).

Lipid peroxidation level (mol of MDA) in Black Bengal goats was compared during autumn and winter. Using independent t- test, the periodical variation in the lipid peroxidation level at fortnightly interval was analyzed with one way ANOVA. The effect of 15-day interval was highly significant during both autumn as well as during winter ($P < 0.01$). In the present study, the mean value of lipid peroxidation level (nmol of MDA/ml of packed cells) of Black Bengal Goat was 0.51 ± 0.01 , 0.54 ± 0.008 , 0.55 ± 0.006 , 0.56 ± 0.005 in Autumn and 0.59 ± 0.009 , 0.62 ± 0.012 , 0.65 ± 0.012 , 0.67 ± 0.011 in Winter (Table 4, Fig 4) on day 15th, 30th, 45th & 60th. The independent t test (t value P critical) shows that the effect of season on the above-mentioned parameter was highly significant ($P < 0.01$) at all the periodical intervals. While the mean value of lipid peroxidation (mol of MDA) in winter was greater than autumn at all the periodical interval mentioned.

Table 4: Lipid peroxidation (nmol of MDA/ml) of Black Bengal Goats in autumn and winter season at different periods (Mean±SE)

Days	AUTUMN	WINTER	t value (P critical)
15 th day	0.51±0.01 ^a	0.59±0.009 ^a	0.000 ^{**}
30 th day	0.54±0.008 ^b	0.62±0.012 ^{ab}	0.000 ^{**}
45 th day	0.55±0.006 ^b	0.65±0.012 ^{bc}	0.000 ^{**}
60 th day	0.56±0.005 ^b	0.67±0.011 ^c	0.000 ^{**}
F value	7.55 ^{**}	10.12 ^{**}	

NS - P≥0.05; *P<0.05 - Significant; **P<0.01- Highly Significant

**Fig. 4: Bar diagram depicting Lipid peroxidation (nmol of MDA) of Black Bengal Goats in autumn and winter season at different periods (Mean±SE)**

Increasing trend was observed in the level of LPO during winter. Significantly higher levels of LPO were observed on each fortnight during winter as compared to autumn. Which indicates stress level was increased during this period. However, the level was lower in comparison to the findings of Rathwa et al. (2017).

3.2 Superoxide dismutase (IU/ml)

Super dismutase (SOD) is the important antioxidant enzyme present in the body and provides defensive mechanism against free radical mediated oxidative damage. Antioxidant activity of SOD mediated by dismutation reaction, where SOD scavenges highly reactive superoxide radical and converts it to oxygen molecule and less reactive H₂O₂ molecule (Fridovich, 1972). Superoxide dismutase (U) of Black Bengal Goats in Autumn and Winter season was found out at different periods (Mean±S.E) namely 15th, 30th, 45th and 60th day i.e at an interval of 15 days between the two periods. The effect was found to be highly significant between autumn and winter season (P<0.01) at

all the periodical intervals on the basis of t - values. The mean values were greater during autumn than winter in all the cases. The one-way ANOVA predicted non- significant effect during autumn and highly significant effect during winter for the variation due to 15-day interval.

The mean value of SOD in the present study was 616.42±15.30, 563.23±14.32, 505.59±14.06 and 485.76±18.39 during autumn while the mean value of SOD during winter was 696.32±19.59, 689.39±19.49, 676.96±20.72 and 651.08±17.10 respectively (Table 5, Fig 5) on day 15th, 30th, 45th and 60th. Decreasing trend was observed in the level of SOD, which was significantly higher on day 15 and 30 as compared to day 60 during autumn. Our findings were in agreement with the findings of Rathwa et al. (2017) in sheep. The higher SOD activity in black Bengal goats during winter season was probably a response to higher superoxide generated in these animals. Superoxide dismutase catalyzes the dismutation of superoxide to oxygen and hydrogen peroxide. Thus, a higher SOD

activity leads to increase in hydrogen peroxide level in the cells which is metabolized by intra cellular components by GPx which protecting

the organism against deleterious effects of free radicals.

Table 5: Superoxide dismutase (IU) of Black Bengal goats in autumn and winter season at different periods (Mean±SE)

Days	AUTUMN	WINTER	t value (P critical)
15 th day	616.42±15.30 ^c	696.32±19.59	0.000 ^{**}
30 th day	563.23±14.32 ^b	689.39±19.49	0.000 ^{**}
45 th day	505.59±14.06 ^a	676.96±20.72	0.000 ^{**}
60 th day	485.76±18.39 ^a	651.08±17.10	0.000 ^{**}
F value	14.32 ^{**}	1.06 ^{NS}	

NS - P≥0.05; *P<0.05 - Significant; **P<0.01- Highly Significant

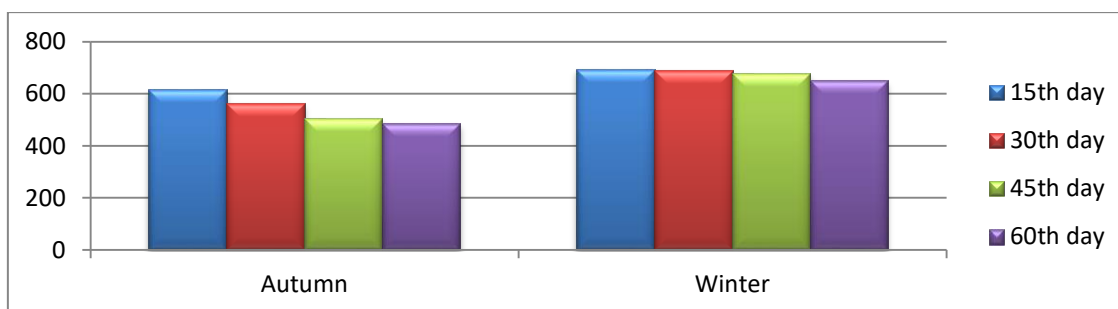


Fig. 5: Bar diagram depicting Superoxide dismutase (IU/ml) of Black Bengal goats in autumn and winter season at different periods (Mean±SE)

3.3 Reduced Glutathione (IU/ml)

A review of the result as presented above in table 6 shows that the effect of season on reduced glutathione concentration was highly significant. A pair wise comparison also revealed that autumn had higher mean reduced glutathione level in the Black Bengal Goats at all the 15-day intervals under study. In both the cases the mean value recorded was highest at 15th day and lowest at 60th day. To see the within season variation, one way ANOVA was used and the difference w.r.t. 15- day interval was significant during autumn (F= 2.84, P<0.05) and highly significant during winter season (F= 8.35, P<0.01).

In the present study, the mean value of GPx (U/ml) of Black Bengal Goat was

192.81±0.52, 191.20±1.37, 189.38±1.95, 184.86±3.26 and 176.64±2.87, 163.35±2.63, 159.72±2.94, 158.12±3.17 (Table 6, Fig 6) respectively during autumn and winter on day 15th, 30th, 45th & 60th. The level of GPx gradually decreased till day 60th during autumn as well as during winter. Significantly lower level of GPx was observed on each fortnight during winter than autumn season. In present study decreased level of GPx was observed during winter that may be due to glutathione-associated metabolism which is a major mechanism for cellular protection against agents which generate oxidative stress. In the present finding, the level was higher than the findings reported by Rathwa et al. (2017) in indigenous sheep.

Table 6: Reduced glutathione (U/ml) of Black Bengal goats in autumn and winter season at different periods (Mean±SE)

Days	AUTUMN	WINTER	t value (P critical)
15 th day	192.81±0.52 ^a	176.64±2.87 ^b	0.000 ^{**}
30 th day	191.20±1.37 ^{ab}	163.35±2.63 ^a	0.000 ^{**}
45 th day	189.38±1.95 ^b	159.72±2.94 ^a	0.000 ^{**}
60 th day	184.86±3.26 ^b	158.12±3.17 ^a	0.000 ^{**}
F value	2.84 [*]	8.35 ^{**}	

NS - P≥0.05; *P<0.05 - Significant; **P<0.01- Highly Significant

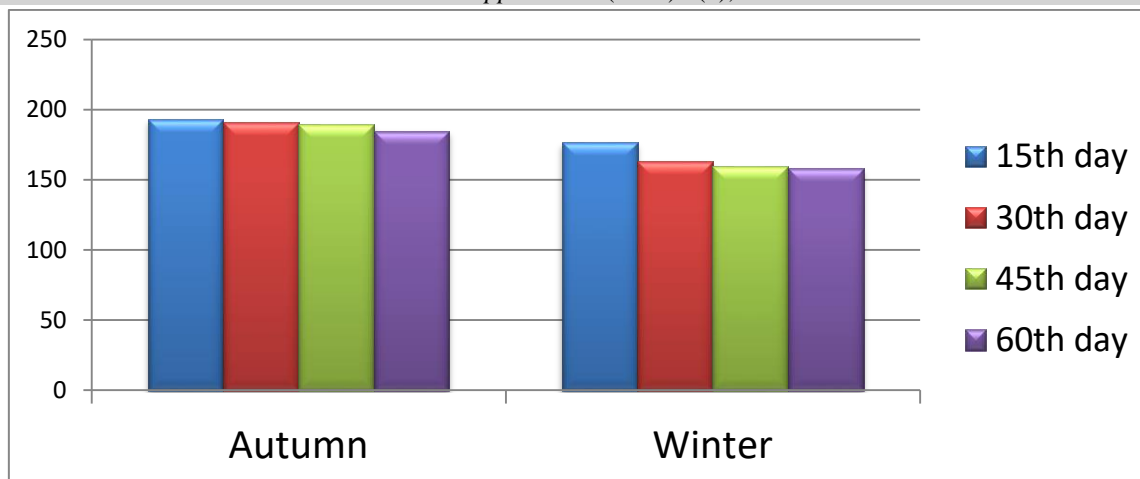


Fig. 6: Bar diagram depicting glutathione (IU/ml) of Black Bengal Goats in autumn and winter season at different periods (Mean \pm SE)

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