



# Changes in Hematological and Biochemical Parameters of Periparturient Murrah Buffaloes during Summer and Winter Season: A Study to Assess Seasonal and Transitional Stress

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## ABSTRACT

**Background:** Environmental stress and transition period in buffaloes imposes significant changes in hemato-biochemical parameters. On this background the present study was attempted to evaluate biochemical parameters in transition buffaloes during summer and winter seasons.

**Methods:** Thirty six advanced pregnant and non-pregnant Murrah buffaloes of around 5 years of age were randomly selected. Whole blood was collected from twelve buffaloes each in their transition period during winter and summer season for a period of five weeks and six non pregnant buffaloes for each season served as control. Haematological, biochemical parameters were estimated and THI was calculated in both the seasons.

**Result:** The mean THI, TEC, Hb, MCH, MCHC, SOD activity and MDA levels were significantly higher ( $p < 0.05$ ) in summer compared to winter, while MCV and GSH activity was significantly higher ( $p < 0.05$ ) in winter compared to summer. No significant ( $p > 0.05$ ) difference between seasons was observed with respect to TLC, monocyte and lymphocyte per cent. Moreover, Hb, SOD, GSH and MDA concentration differed significantly ( $p < 0.05$ ) throughout the transition period during summer and winter seasons, while TEC, MCV, MCH and MCV concentration did not change ( $p > 0.05$ ) with transition period in both the seasons. The present study indicated that summer season had profound effect on hemato-biochemical parameters. While SOD, GSH and MDA could be used as markers of transitional stress in Murrah buffaloes.

**Key words:** Biochemical, Haematology, Murrah buffaloes, Season, Stress, Transition.

## INTRODUCTION

Livestock species usually experience environmental stress of varying degree, while being thermoregulators they initially try to cope up with stress via numerous behavioral and circulatory adjustments which are aimed at maintenance of homeostasis. When the thermoregulatory mechanisms of heat gain and heat loss are impaired the animal enters in to a state of heat/cold stress which adversely affects animal production. In tropical countries like India cold stress is less pronounced, while high ambient temperature is the major constraint on animal productivity (Shelton, 2000), with humidity aggravating the effects of heat stress (Marai *et al.* 2008). Heat stress effects are more pronounced in buffaloes than cattle because of differences in their heat regulating mechanisms (Salah *et al.* 1995). Under heat stress, the animals exhibit various physiological, haematological, biochemical and endocrine adjustments and thus heat stress could be assessed by changes in these variables since these are of values in determining the adaptation of animal to existing environment (Rasooli *et al.* 2004; Kumar *et al.* 2010; Nikhil *et al.* 2018).

Transition period is the most stressful in the life of a dairy animal as it induces rapid physiological changes for the onset of lactation and is associated with oxidative stress (Konvicna *et al.* 2015; Ambily *et al.* 2019). This makes the animals highly susceptible to negative energy balance (Mohamed *et al.* 2015). Under physiological conditions,

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sufficient antioxidant reserves are there to meet the free radicals produced (Castillo *et al.* 2001). However, when free radicals generated exceed the body's antioxidant capacity, oxidative stress develops. Initiation of lactation and tissue repair during postpartum period might cause more powerful oxidative stress to the animals than advanced pregnancy (Sharma *et al.* 2011). This when combined with the extreme

climatic conditions of summer further impairs the homeostasis and makes the animals more susceptible to diseases. Hence it is essential to study the levels of malondialdehyde (MDA), a marker of oxidative stress and first line defense antioxidants *i.e.* superoxide dismutase (SOD) and reduced glutathione (GSH) to assess the levels of oxidative stress and the cumulative effect of heat stress and transitional stress during different stages of periparturient period. The metabolic pattern of buffaloes is quiet different in comparison to others (Fiore *et al.* 2017) and the studies on physiology of the transition period in buffaloes are scanty (Abdulkareem, 2013; Fiore *et al.* 2017). Hence, current study aims to explore the physiology of transition period in Murrah buffaloes with the hypothesis that transition period with high temperature and humidity, might produce much more pronounced adverse effects and are reflected as altered haematological and biochemical parameters (Kumar *et al.* 2010).

## MATERIALS AND METHODS

### Animals and management

Thirty six advanced pregnant and non-pregnant Murrah buffaloes in II or III parity, aged around 5 yrs were selected from the buffalo herd of Livestock Farm Complex, NTRCVSc, Gannavaram and nearby farm. Twelve buffaloes each in transition period during the winter season (November to January) and the summer season (April to June) were used for the experiment. Six non pregnant healthy buffaloes each were used as a control during summer and winter seasons. The animals were maintained in loose housing system. The animals were routinely fed with concentrate mixture and green fodder as per the nutrient requirements of ICAR (2003). Fresh water was available for drinking to all the animals round the clock. All the buffaloes were identified with ear tags.

### Experimental design

The experiment was conducted in two different seasons *viz.*, winter season (November to January) and the summer season (April to June). Ambient temperatures and relative humidity was recorded using digital thermo hygrometer, daily throughout the experimental period. Temperature humidity index (THI) was calculated using the formula (Johnson 1963).

$$THI = 0.72 (T_{db} + T_{wb}) + 40.6$$

Where,

$T_{db}$  = dry bulb temperature ( $^{\circ}\text{C}$ );  $T_{wb}$  = wet bulb temperature ( $^{\circ}\text{C}$ )

### Blood collection and analysis

Blood samples were collected at 8 a.m. on weekly intervals during the experimental period of five weeks from all the animals from jugular vein in sterile heparinised vacutainer tubes (BD Vacutainer, Franklin Lakes) and serum vacutainers (BD Vacutainer, Franklin Lakes) from both the groups, with minimum disturbance to animal. Samples were transported to the laboratory in chilled ice box soon after collection. Heparinised blood was used for estimation of hematological parameters and antioxidant parameters by

hemolysate preparation, while another set of serum samples were centrifuged at 3000 rpm at room temperature for 25 minutes, serum was separated and used from total protein estimation which is necessary in calculation of SOD activity.

The heamatological parameters such as total erythrocytic count (TEC), heamoglobin (Hb), packed cell volume (PCV) were estimated using Mindray heamo analyzer BC 2800. On the basis of TEC, Hb and PCV, red cell indices *i.e.* Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated. Erthyocytic MDA (Balasubramanian *et al.* 1988), SOD (Madesh and Balasubramanian, 1998), GSH (Ellman, 1959) were estimated.

### Statistical analysis

The experiment was conducted for a period of five weeks; two weeks prepartum to three weeks postpartum. The data obtained on various parameters were statistically analysed using two way analysis of variance (ANOVA) (Snedecor and Cochran, 1994). The whole data was analysed using computerized software programme SPSS Ver.20.0.

## RESULTS AND DISCUSSION

### Meteorological data

Temperature humidity index (THI) was calculated using the following formula of Johnson (1963) in both summer and winter. The results of the THI in both summer and winter are given in Table 1. Significant difference ( $p < 0.05$ ) was evident in minimum, maximum and mean THI of both the seasons. High environmental temperature has adverse effects on the physiological functions of dairy animals and affects their productivity (Polsky and von Keyserlingk, 2017). This can further be aggravated when it is accompanied by high humidity (Collier *et al.* 2017). To measure the intensity of environmental stress, THI has been developed (Alhussien and Dang, 2018). It is one of the best methods to evaluate heat stress in animals (Marai and Haebe, 2010). The THI is a result of the combined effect of temperature and relative humidity (Dikmen and Hansen, 2009) which determine the microclimatic conditions of the animal. When the THI reaches 72, cows change their behaviour (Kamal *et al.* 2018; Herbut and Angrecka, 2018), yield and composition of milk gets altered (Liu *et al.* 2017). Based on the THI index, (Helal *et al.* 2010) a THI of 74 or less considered as normal, 75-78 alert, 79-83 danger and 84 and above an emergency. THI values more than 72 stressful, while THI above 78 indicated severe heat stress to buffaloes (Payne, 1990). THI values higher than 80 units have been classified as danger zone to the well-being and productivity of cattle (Segnalini *et al.* 2013). In the present study, animals calving in winter were found to be within the thermo comfort zone, while those calving in summer were under danger condition with a very high THI, this indicated that the animals were under severe heat stress. The buffaloes were more affected by heat stress compared to cows which indicate that buffaloes are more prone to stress due to high environmental variables (Hady

*et al.* 2018). The high susceptibility of buffaloes to thermal stress ; could be attributed to low potentiality to dissipate heat from the body surface due to fewer sweat glands and black colored skin.

### Haematological parameters

The haematological parameters were used to assess the physiological homeostasis of the animals (Abd Ellah *et al.* 2013). The results of the effect of season and transition on haematological parameters were presented in Table 2 and 3. In winter, no significant difference ( $p>0.05$ ) was observed in TEC, TLC, Hb, monocyte %, PCV, MCV, MCH, MCHC among all the weeks of transition period as well control ( $p>0.05$ ). In summer, no significant difference ( $p>0.05$ ) was observed in TEC, TLC, monocyte %, PCV, MCV, MCH, MCHC among all the weeks of transition period as well control ( $p>0.05$ ). The Mean  $\pm$  SE of the TEC in two weeks prepartum, day zero and control were significantly higher ( $p<0.05$ ) in summer compared to winter. In the present study, transition period did not affect the TEC significantly in the seasons studied. Similar findings were reported earlier in buffaloes (Abdulkareem, 2013 and Abdelrazek *et al.* 2018); cattle (Hadj *et al.* 2015); sheep (El-Ebissy, 2011); goat (Daramola *et al.* 2004). In winter, no significant difference in Hb was observed among all the weeks of transition period

as well in control, while in summer, the Hb concentration was significantly lower ( $p<0.05$ ) in 1 week prepartum compared to 2 weeks prepartum and control. The Hb concentration was significantly higher ( $p<0.05$ ) in 2 weeks prepartum compared to 1 week prepartum, 2<sup>nd</sup> and 3<sup>rd</sup> weeks postpartum. Also no significant difference in Hb concentration from one week prepartum to 3<sup>rd</sup> week postpartum was observed. No change in TEC and Hb during transition period in the present may be attributed to similar threshold of stress throughout the transition period (Vasantha *et al.* 2020).

The TEC values in 2 weeks of prepartum, day zero and control were significantly higher ( $p<0.05$ ) in summer compared to winter. This effect could be attributed to increased levels of cortisol during summer which enhance erythroid progenitor proliferation and positively influence erythropoiesis (Voorhees *et al.* 2013). During summer, to alleviate heat stress, cutaneous blood flow increases to dissipate heat. Increased cutaneous vascular flow up-regulates intracutaneous erythropoietin production that in turn contributes to the increased erythropoietin plasma levels (Ralf Paus *et al.* 2009). Increased erythrocyte count and haemoglobin concentration in the blood during summer compared to winter could be ascribed to increased

**Table 1:** Minimum, Maximum and Mean ( $\pm$ SE) values of THI recorded during summer and winter seasons during the study period (n=90).

Parameters	Season	Minimum	Maximum	Mean
THI	Summer	66.63 <sup>Ab</sup> $\pm$ 1.79	93.05 <sup>Aa</sup> $\pm$ 0.98	80.38 <sup>A</sup> $\pm$ 1.11
	Winter	59.21 <sup>Bb</sup> $\pm$ 1.95	83.06 <sup>Aa</sup> $\pm$ 2.08	71.03 <sup>B</sup> $\pm$ 1.17

The mean with same superscript (a,b) in a row and (A,B) in a column do not differ significantly,  $p<0.05$ .

**Table 2:** Mean ( $\pm$ SE) values of hematological parameters during summer and winter seasons in transition Murrah Buffaloes (n=12).

Parameters	Season	*Stage of transition period						Control
		-2 week	-1 week	Day 0	+1 Week	+2 Week	+3 week	
TEC millions/cmm	Summer	7.21 <sup>Aa</sup> $\pm$ 0.19	7.15 <sup>Aa</sup> $\pm$ 0.22	7.02 <sup>Aa</sup> $\pm$ 0.16	6.70 <sup>Aa</sup> $\pm$ 0.10	6.53 <sup>Aa</sup> $\pm$ 0.13	6.60 <sup>Aa</sup> $\pm$ 0.16	7.08 <sup>Aa</sup> $\pm$ 0.10
	Winter	5.88 <sup>Ba</sup> $\pm$ 0.24	6.06 <sup>Aa</sup> $\pm$ 0.50	5.94 <sup>Ba</sup> $\pm$ 0.28	5.99 <sup>Aa</sup> $\pm$ 0.31	6.10 <sup>Aa</sup> $\pm$ 0.22	6.20 <sup>Aa</sup> $\pm$ 0.26	5.70 <sup>Ba</sup> $\pm$ 0.26
Hemoglobin (g/dl)	Summer	10.75 <sup>Aa</sup> $\pm$ 0.15	9.82 <sup>Ac</sup> $\pm$ 0.19	10.57 <sup>Aabc</sup> $\pm$ 0.21	10.47 <sup>Aabc</sup> $\pm$ 0.16	9.87 <sup>Abc</sup> $\pm$ 0.21	9.88 <sup>Abc</sup> $\pm$ 0.24	10.68 <sup>Aab</sup> $\pm$ 0.16
	Winter	7.93 <sup>Ba</sup> $\pm$ 0.12	7.78 <sup>Ba</sup> $\pm$ 0.16	7.95 <sup>Ba</sup> $\pm$ 0.05	7.75 <sup>Ba</sup> $\pm$ 0.12	8.33 <sup>Ba</sup> $\pm$ 0.34	8.23 <sup>Ba</sup> $\pm$ 0.39	8.18 <sup>Ba</sup> $\pm$ 0.16
PCV (%)	Summer	28.78 <sup>Aa</sup> $\pm$ 0.69	29.22 <sup>Aa</sup> $\pm$ 0.49	30.71 <sup>Aa</sup> $\pm$ 0.80	29.39 <sup>Aa</sup> $\pm$ 0.83	30.62 <sup>Aa</sup> $\pm$ 0.72	30.55 <sup>Aa</sup> $\pm$ 1.11	31.74 <sup>Aa</sup> $\pm$ 0.62
	Winter	29.20 <sup>Aa</sup> $\pm$ 0.70	29.70 <sup>Aa</sup> $\pm$ 0.80	30.92 <sup>Aa</sup> $\pm$ 0.63	29.92 <sup>Aa</sup> $\pm$ 0.75	30.53 <sup>Aa</sup> $\pm$ 0.87	29.77 <sup>Aa</sup> $\pm$ 1.00	30.22 <sup>Aa</sup> $\pm$ 0.59

The mean with same superscript (a,b,c,d,e) in a row (stages of transition period) and (A,B) in a column (season) do not differ significantly,  $p<0.05$ .

**Table 3:** Mean ( $\pm$ SE) values of hematological indices during summer and winter seasons in transition Murrah Buffaloes (n=12).

Parameters	Season	*Stage of transition period						Control
		-2 week	-1 week	Day 0	+1 Week	+2 Week	+3 week	
MCV (fl)	Summer	40.66 <sup>Ba</sup> $\pm$ 1.53	41.82 <sup>Aa</sup> $\pm$ 1.99	44.14 <sup>Ba</sup> $\pm$ 1.41	44.78 <sup>Aa</sup> $\pm$ 1.64	46.84 <sup>Aa</sup> $\pm$ 1.33	45.23 <sup>Aa</sup> $\pm$ 1.72	42.71 <sup>Ba</sup> $\pm$ 1.09
	Winter	48.27 <sup>Aa</sup> $\pm$ 0.91	48.20 <sup>Aa</sup> $\pm$ 3.46	52.24 <sup>Aa</sup> $\pm$ 2.70	49.77 <sup>Aa</sup> $\pm$ 3.13	50.56 <sup>Aa</sup> $\pm$ 2.34	49.49 <sup>Aa</sup> $\pm$ 1.67	56.31 <sup>Aa</sup> $\pm$ 2.97
MCH (pg)	Summer	14.97 <sup>Aa</sup> $\pm$ 0.47	13.81 <sup>Aa</sup> $\pm$ 0.56	15.09 <sup>Aa</sup> $\pm$ 0.48	15.64 <sup>Aa</sup> $\pm$ 0.26	15.15 <sup>Aa</sup> $\pm$ 0.45	15.01 <sup>Aa</sup> $\pm$ 0.41	15.10 <sup>Aa</sup> $\pm$ 0.34
	Winter	13.60 <sup>Aa</sup> $\pm$ 0.48	13.16 <sup>Aa</sup> $\pm$ 0.82	13.53 <sup>Aa</sup> $\pm$ 0.61	13.07 <sup>Ba</sup> $\pm$ 0.56	13.72 <sup>Aa</sup> $\pm$ 0.59	13.31 <sup>Ba</sup> $\pm$ 0.45	14.45 <sup>Aa</sup> $\pm$ 0.52
MCHC(gm/dl)	Summer	36.89 <sup>Aa</sup> $\pm$ 0.78	33.17 <sup>Aab</sup> $\pm$ 1.05	34.25 <sup>Aab</sup> $\pm$ 0.94	35.12 <sup>Aab</sup> $\pm$ 1.17	32.38 <sup>Ab</sup> $\pm$ 0.68	33.42 <sup>Aab</sup> $\pm$ 1.49	35.40 <sup>Aab</sup> $\pm$ 0.63
	Winter	28.15 <sup>Ba</sup> $\pm$ 0.78	27.43 <sup>Ba</sup> $\pm$ 0.62	25.97 <sup>Ba</sup> $\pm$ 0.66	26.45 <sup>Ba</sup> $\pm$ 0.67	27.28 <sup>Ba</sup> $\pm$ 1.25	27.05 <sup>Ba</sup> $\pm$ 1.30	25.83 <sup>Ba</sup> $\pm$ 0.74

The mean with same superscript (a,b,c,d,e) in a row (stages of transition period) and (A,B) in a column (season) do not differ significantly,  $p<0.05$ .

erythropoietin that in addition to conferring protection to tissues also stimulated erythropoiesis.

In the present study, the TLC in all the weeks of transition period and control did not differ significantly in between summer and winter. In support to the present findings Aengwanich *et al.* (2011) in cattle and Hassan *et al.* (2013) in goats also reported the same. The lack of significant difference between the seasons under study might be attributed to the adaptation of the species to the increasing THI during summer season (Hassan *et al.* 2013).

The Mean  $\pm$  SE of the Hb in all the weeks of transition period and control were significantly higher ( $p < 0.05$ ) in summer compared to winter. Similar findings in cattle were reported in the studies of Shrikande *et al.* (2008) and AL-Saeed (2009). In contrary to our results, significantly decreased Hb was noticed in summer compared to winter in cattle (Kumar and Pachauri, 2000; Al-Saeed *et al.* 2009 and Mirzadeh *et al.* 2010).

The PCV in all the stages of transition period and control had no significant difference ( $p > 0.05$ ) in both the seasons. Similar to our findings Nikhil *et al.* (2018) reported that season had no effect on haematological parameters such as Hb, PCV, MCV, MCH, MCHC while TEC was significantly lower in monsoon compared to pre and post monsoon seasons in cross bred calves. Abdelrazek *et al.* (2018) revealed non-significant ( $p > 0.05$ ) changes in TEC, PCV, Hb, MCV, MCH, MCHC along transition period during postpartum period in buffaloes.

The MCV values on two weeks prepartum, day zero and control were significantly higher ( $p < 0.05$ ) in winter compared to summer. The MCH levels in one week and third week postpartum samples were significantly higher ( $p < 0.05$ ) in summer compared to winter. The Mean  $\pm$  SE of the MCHC in all the weeks of transition period and control were significantly higher ( $p < 0.05$ ) in summer compared to winter. The variation in MCV might be due to physiological adaptation of the animals or that indices like MCV might not have been affected by heat stress. The increased MCHC in our study during summer could be attributed to increased erythropoiesis. The sampling interval, methodology used for the experiment, numbers of animals sampled and/or degree of metabolic disturbances might be contributing factors that brought about variation in results of certain haematological parameters observed in our study (Hadj *et al.* 2015). Also, the non-significant difference observed in few of the stages

of transition period and in between the seasons might also be attributed to variation in individual level of physiological adaptation of the animals.

### Biochemical parameters

The results of the effect of season and transition on biochemical parameters were presented in Table 4. In winter, the SOD levels on two weeks postpartum, were significantly higher ( $p < 0.05$ ) than two weeks prepartum, day zero and one week postpartum. The SOD levels on one week postpartum was significantly lower ( $p < 0.05$ ) compared to one week prepartum, two week, three week postpartum and control. In summer, the SOD levels in two weeks prepartum, one week prepartum were significantly higher ( $p < 0.05$ ) compared to other weeks and control. The SOD levels in transitional and control buffaloes were significantly higher ( $p < 0.05$ ) in summer than winter. Similar to our results of SOD in winter, Agarwal *et al.* (2012) evidenced decreased erythrocytic SOD activity in transition period, towards calving and the value further declined after parturition. The findings of Singh *et al.* (2017) were similar to our results, with a significant decrease in levels of SOD throughout the transition period. The reason behind the decreased SOD activity, around calving may be due to altered homeostatic control (Agarwal *et al.* 2012). Usually prooxidants and antioxidants are in equal concentrations maintaining the oxidative balance, under stressful conditions the system tries to combat oxidative stress by elevating the concentrations of antioxidant enzymes such as SOD, GPx and catalase, Whereas under extreme stressful ambience the antioxidant system fails to combat pro-oxidants resulting in oxidative stress which is usually termed as altered hemostasis. Kumar *et al.* (2011) also similarly observed significantly ( $p < 0.05$ ) increased erythrocyte SOD activity in buffaloes exposed to hot dry and hot humid conditions in a climate chamber. As the calving approached, the reserves of the antioxidant defence gets exhausted due to excessive lipolysis induced by persistently high cortisol levels and thereby excess generation of free radicals (Hady *et al.* 2018). Hence parturition combined with seasonal stress significantly decreased the SOD levels to the day of calving which indicated that the combined effect was more pronounced on the day of calving.

The GSH levels on day zero of parturition was significantly lower ( $p < 0.05$ ) compared to other weeks of

**Table 4:** Mean ( $\pm$ SE) values of biochemical parameters during summer and winter seasons in transition Murrah Buffaloes (n=12).

Parameters	Season	*Stage of transition period						
		-2 week	-1 week	Day 0	+1 Week	+2 Week	+3 week	Control
SOD (U/mg of protein)	Summer	6.71 <sup>Ab</sup> $\pm$ 0.06	6.44 <sup>Ab</sup> $\pm$ 0.09	5.72 <sup>Ab</sup> $\pm$ 0.09	5.66 <sup>Ab</sup> $\pm$ 0.05	5.58 <sup>Ab</sup> $\pm$ 0.06	5.46 <sup>Ab</sup> $\pm$ 0.12	5.45 <sup>Ab</sup> $\pm$ 0.07
	Winter	3.29 <sup>Bbcd</sup> $\pm$ 0.03	3.33 <sup>Babc</sup> $\pm$ 0.02	3.24 <sup>Bcd</sup> $\pm$ 0.07	3.08 <sup>Bd</sup> $\pm$ 0.04	3.55 <sup>Ba</sup> $\pm$ 0.07	3.38 <sup>Babc</sup> $\pm$ 0.06	3.48 <sup>Bab</sup> $\pm$ 0.06
GSH (mM/L of blood)	Summer	4.37 <sup>Ab</sup> $\pm$ 0.35	3.68 <sup>Ab</sup> $\pm$ 0.16	1.39 <sup>Bc</sup> $\pm$ 0.07	3.24 <sup>Bb</sup> $\pm$ 0.16	3.96 <sup>Ab</sup> $\pm$ 0.24	4.19 <sup>Ab</sup> $\pm$ 0.30	5.16 <sup>Ba</sup> $\pm$ 0.24
	Winter	4.04 <sup>Ab</sup> $\pm$ 0.42	4.10 <sup>Ab</sup> $\pm$ 0.26	2.66 <sup>Ac</sup> $\pm$ 0.07	4.09 <sup>Ab</sup> $\pm$ 0.19	4.60 <sup>Ab</sup> $\pm$ 0.42	4.36 <sup>Ab</sup> $\pm$ 0.39	5.94 <sup>Aa</sup> $\pm$ 0.15
MDA ( $\mu$ moles)	Summer	0.03 <sup>Ad</sup> $\pm$ 0.003	0.06 <sup>Ac</sup> $\pm$ 0.003	0.10 <sup>Aa</sup> $\pm$ 0.004	0.09 <sup>Ab</sup> $\pm$ 0.003	0.05 <sup>Ac</sup> $\pm$ 0.003	0.01 <sup>Ae</sup> $\pm$ 0.002	0.03 <sup>Ad</sup> $\pm$ 0.003
	Winter	0.02 <sup>Bd</sup> $\pm$ 0.001	0.04 <sup>Bc</sup> $\pm$ 0.003	0.09 <sup>Ba</sup> $\pm$ 0.002	0.06 <sup>Bb</sup> $\pm$ 0.002	0.02 <sup>Bd</sup> $\pm$ 0.004	0.01 <sup>Ad</sup> $\pm$ 0.001	0.02 <sup>Bd</sup> $\pm$ 0.001

The mean with same superscript (a,b,c,d,e) in a row (stages of transition period) and (A,B) in a column (season) do not differ significantly,  $p < 0.05$ .



transition period and control in both the seasons. Sharma *et al.* (2011) and Singh *et al.* (2017) also reported decreased GSH concentration in early lactation compared to advanced pregnancy. The GSH levels on day zero, one week postpartum and control were found to be significantly higher ( $p < 0.05$ ) in winter than summer. The present results were contrary to the findings of Kumar *et al.* (2007) and Kumar *et al.* (2011) in buffaloes. It implied that production of GSH from GSSH (oxidized glutathione) was more prior to parturition and less after parturition. The higher levels of GSH in summer compared to winter could be due to involvement of GSH dependent enzymes glutathione peroxidase and glutathione reductase to control oxidative stress leading to intense regeneration of GSH from GSSH obtained after reduction of peroxides into alcohols (Kizil *et al.* 2007). The variations in GSH reported in our study was attributed to age, breed, timing and frequency of sampling, nutrition, environment, analytical procedures adopted to measure and management practices (Castillo *et al.* 2006).

The MDA concentration on day zero of parturition was significantly higher ( $p < 0.05$ ) when compared to other weeks of transition period and control in both the seasons. The MDA concentration in transition and control buffaloes except in the three weeks postpartum were significantly higher ( $p < 0.05$ ) in summer compared to winter. The findings of Abdelrazek *et al.* (2018) and Hady *et al.* (2018) were similar to ours with significant increase in MDA postpartum compared to late gestation. Significantly higher levels of MDA on day zero of parturition reflected the increased oxidative stress in the animals. The decreasing trend in MDA during the post transition period might be due to the gradual adaptation of the animal's body to the metabolic alterations leading to the animal regaining homeorhesis (Abuelo *et al.* 2015). The MDA levels in all the different weeks of transition period except in the three weeks postpartum were significantly higher ( $p < 0.05$ ) in summer compared to winter. In line with our results, Kumar *et al.* (2011) reported increased TBARS in adult buffaloes in both hot dry and hot humid conditions induced in a climate chamber. The animals in our study initially during the prepartum stage efficiently overcame the stress with increased levels of SOD, GSH and with less MDA formed. However, at parturition, the free radicals might have increased, anti oxidant reserves decreased (reduced SOD, GSH levels) and increased lipid peroxides (increased MDA concentration). During the postpartum period the animals were relieved from stress as indicated by the decreased MDA concentration.

## CONCLUSION

The present study indicated SOD, GSH and MDA could be used as markers of transitional stress in Murrah buffaloes. Moreover, summer season had profound effect on hemato-biochemical parameters. When thermal stress is accompanied with transitional stress the changes are much more pronounced. Hence various precautionary measures including managemental and nutritional strategies may be adopted during the transition period for mitigating the effects

of increasing THI during summer for optimum production and reproduction in buffaloes.

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## Compliance with ethical standards

The animal studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

## Conflict of interest

The authors declare that there is no any conflict of interest for this manuscript.

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