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Oxidative stress, hemato-biochemical and mineral profile in crossbred cows during transition period from Punjab

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Abstract
Twenty-two crossbred dairy cows undergoing transition from two different farms of district Jalandhar, Punjab were taken-up to evaluate the oxidative stress and establish normal values for various metabolic parameters during different stages of the transition ie., far-off dry (FOD), close-up dry (COD) and fresh (F). Significant increase was observed in lipid peroxidation from FOD to CUD and fresh stage in both farms whereas, significantly low levels of superoxidase dismutase were observed during FOD stage in both farms with increasing trend in subsequent stage and again followed by declining trend after parturition. Significantly low levels of reduced glutathione were observed from FOD and CUD to fresh stage in farm 1 whereas, significantly low levels were observed between FOD and fresh stage in farm 2. Baseline values of various metabolic profile parameters (Hb, PCV, TEC, TLC, total protein, albumin, plasma urea nitrogen, creatinine, glucose, BHBA, NEFA, Ca, Mg, Na, K, Cu, Fe, Zn and inorganic phosphorus) were established during three different stages of transition period for both the farms for future reference.

Keywords: Oxidative stress, transition period, metabolic profile, crossbred cows

Introduction
Oxidative stress is caused by either a decrease in the antioxidant defense mechanism or a relatively increased production of free radicals and reactive oxygen species (ROS), which impair DNA, enzymes, and membranes. These changes in biopolymer structure and immune system activity ultimately result in a variety of health disorders. Free radicals are produced continuously by metabolic processes in animals' bodies and are crucial for physiological processes like immunological response, the metabolism of unsaturated fatty acids, and inflammatory reactions. However, in dairy animals, free radical levels rise sharply during transition period (Singh *et al.*, 2015) [33].
During the shift from late gestation to early lactation, dairy cattle naturally experience negative energy balance, or NEB. Homeostatic control of metabolic processes is required during this phase, which is commonly thought to last from three weeks prior to parturition to three weeks following parturition, in order to meet the demands of lactogenesis and parturition (Payne *et al.*, 1970; Singh *et al.*, 2018) [25, 35]. A variety of stressors may affect cows during the transition period, such as increased production of free radicals, the unavoidable metabolic adaptive stressors, heat and cold stress, overcrowding, changes in social structure and grouping, housing modifications, uncomfortable stalls or footing, infectious challenges, and mycotoxin exposure, among others. Oxidative stress can affect an animal's health, weaken its immune system, and make transition animals more vulnerable to a range of production disorders if the quantity of free radicals it produces outweighs its antioxidant capacity (Singh *et al.*, 2017) [34]. Although there has not been much research done in India on oxidative stress and metabolic profile in transition dairy cows, there has been some recent attention paid to the role oxidative stress plays in the emergence of several production disorders.
Keeping in view the significance of oxidative stress and metabolic profiling during the transition period, the current study was carried out to evaluate the oxidative stress in dairy cows by measuring the activity of reduced glutathione (GSH), lipid peroxidation (LPO), and superoxide dismutase (SOD) in hemolysate. Concurrently, various hemato-biochemical and

plasma mineral profiles were assessed in order to establish normal values for various metabolic parameters during different stages of the transition.

Materials and Methods

A total of 22 crossbred dairy cows from two dairy farms in their last trimester of pregnancy were sampled during the study period. Management practices in both the farms were same and included loose housing system with head to head feeding. Water was available *ad-lib* and milking was done in milking parlors twice a day with machines. Both dry and pregnant cows were kept in a separate pen from the lactating cows. Dry pregnant cows were fed 30 kg of silage, 4 kg of feed and 2 kg wheat straw and lactating cows were fed 8 kg of feed, 35 kg of corn silage and 3 kg of wheat straw. Further, all the included animals did not show any sign of disease/ metabolic derangement throughout the study period.
From each animal blood samples were collected thrice during different stages of periparturient period, viz:

Far off dry (FOD): > 10 days following dry off and not < 30 days prior to calving.

Close up dry (CUD): Between 3 and 21 days prior to calving.

Fresh (F): 3 to 30 days in milk.

Sample Collection

For the estimation of various parameters of metabolic profile testing, blood was collected from jugular vein from each cow. Samples for hematology were collected in EDTA vials having purple caps whereas, samples for mineral estimation were taken in heparin coated glass vials.

Hematology

Hematological parameters were estimated on the hematological analyzer (ADVIA 2120, SIEMENS Hematology Analyzer, USA.) in the Diagnostic Laboratory of Department of Teaching Veterinary Clinical complex, GADVASU, Ludhiana.

Mineral analysis

Plasma

Concentrations of copper, iron and zinc

Concentrations of various minerals viz. Cu, Zn and Fe were estimated by Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 700, USA).

Concentrations of Calcium and Magnesium

Concentrations of minerals viz. Ca and Mg were estimated by Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 700, USA).

Concentration of Sodium and Potassium

Both sodium and potassium were analyzed using Orthodiagnostic's Vitros 350 biochemistry analyzer using commercial kits.

Plasma Inorganic Phosphorus (Pi)

Plasma Pi was determined using method given by Tausky and Shorr (1953) [34].

Beta Hydroxy Butyric Acid (BHBA) and Non-esterified Fatty Acids (NEFA)

Both BHBA and NEFA were estimated on the ELISA plates from the plasma samples with the help of kits provided by Diasys Diagnostics systems, Germany.

Biochemistry

Estimation of Biochemical parameters viz. Total Plasma Proteins (TPP), albumin, plasma urea nitrogen (PUN), creatinine, glucose was analyzed using Orthodiagnostic's Vitros 350 biochemistry analyzer using commercial kits.

Oxidative Stress Parameters

Estimation of Lipid Peroxidation (LPO)

Lipid peroxidation (LPO) was assayed by the methods of Placer *et al.* (1966) [26].

Superoxide Dismutase (SOD) Activity

The activity of SOD in hemolysate was measured by the method of Nishikimi *et al.* (1972) [21].

Estimation of Reduced Glutathione (GSH)

Reduced glutathione was estimated by the method of Hafeman *et al.* (1974) [12].

Statistical Analysis

Data was analyzed statistically by using SPSS software (version 16.0; Microsoft).

Results and Discussion

Base line study

Hematological Parameters

Hemoglobin (Hb) and packed cell volume (PCV)

The mean Hb and PCV values from two different farms are presented in Table 1. A non-significant increasing pattern was seen from FOD to Fresh stage in Hb and PCV in both farms with no significant difference in Hb and PCV within both farms.

Total Erythrocytic Count (TEC)

The mean TEC values from two different farms are presented in Table 1. Level in farm 1 was significantly lower than farm 2 during FOD stage.

Total Leucocyte Count (TLC)

The mean TLC values from two different farms are presented in Table 1 representing no significant changes within farms and between different transition stages.
The elevated total leucocyte count (TLC) during the advanced stage of pregnancy may be explained by the body's natural release of adrenaline, which increased the mobilization of neutrophils in the bloodstream (Roy *et al.*, 2010) [29]. Similar to present study, Meglia *et al.* (2005) [20] also reported higher WBC counts in parturient cows than earlier in the dry period.

Plasma biochemical parameters

Total plasma protein

The mean TPP values from two different farms show significant decrease in levels of TPP at CUD and Fresh period in farm no 1 and significant decrease at fresh period from FOD and CUD period (Table 2). Since determining protein status is more challenging than determining energy balance, so a variety of metabolite markers, such as BUN, creatinine, total proteins, and albumin, must be used for evaluating the protein status of the body. (Van Saun, 2004) [35].

In their individual studies, different authors have provided a variety of explanations for the decline in plasma protein levels, including the need for immunoglobulins to be transported from serum to the mammary gland (Weaver *et al.,* 2000) [39], for optimal gonadotropin-releasing factor secretion, Antunovic *et al.,* 2002) [2], and it is also required for increased fetal growth, fetal muscle development, and for gluconeogenesis. (Lone *et al*., 2003) [19].

Similar results were observed by Roubies *et al.* (2006) [28] in their respective studies as they also observed a decrease in plasma proteins from late pregnancy to early lactation as compared to the mid lactation reflecting the maternal requirement of proteins for the onset of milking and providing immunoglobulins.

Albumin

The overall mean albumin level of cows from both farms is presented in Table 2. A significant decrease was observed in the overall mean albumin level ($p<0.05$) at fresh period as compared to FOD and CUD period. The inability of the cows to consume enough dietary proteins to meet their needs for both mammary and extra-mammary amino acids, including a high demand for hepatic gluconeogenesis, may be the cause of the drop in plasma proteins and albumin values during the periparturient period. This would result in a significant, progressive mobilization of tissue proteins during this time.

In addition to the periparturient cow's current endocrine status, significant drops in insulin and insulin-like growth factor-I in plasma levels as well as insulin resistance in peripheral tissues further causes net mobilization of amino acids, which further lowers plasma protein and albumin levels. (Bell *et al.*, 2000) [4]. Significant decreasing trend in serum albumin with advancing pregnancy had been reported in indigenous cows by Kumar *et al.* (2001) [18] and in Jersey crossbred cattle by Lone *et al.* (2003) [19]

Plasma Urea Nitrogen (PUN)

The mean PUN values from two different farms are presented in Table 2 and show no significant change in levels between two farms and during different transition stages. During the late pregnancy, there in increased catabolism of amino acids at the expense of protein synthesis, due to which there is increased urea production Shwartz *et al.* (2009) [32], and also for meeting the early lactation demands, there is increased muscle protein catabolism due to which there is increased urea concentration in lactating animals (Sreedhar *et al.,* 2013) [36]. Similar results were reported by Kulkarni *et al.* (2010) [17] who reported an increase in BUN levels during the early lactation period.

Creatinine

There was no significant change in mean creatinine levels between two farms and during different transition stages (Table 2).

Glucose

The mean glucose values in both farms were significantly low at fresh stage compared to FOD stage (Table 2). The current study found that the plasma glucose concentration decreased from the fetal development period until early lactation. This could be attributed to the mobilization of maternal glucose for the fetal blood circulation during advanced pregnancy (Jacob and Vadodaria, 2001) [15] and a high demand for lactose synthesis and/or insufficient gluconeogenesis during early lactation (Pambu-Gollah *et al.*, 2000) [24].

Beta Hydroxy Butyric Acid (BHBA) and Non-Esterified Fatty Acid (NEFA)

The mean BHBA values showed significant difference between FOD and fresh stage in farm 2 (Table 2). The mean NEFA values show significantly high levels during FOD stage in farm 2 as compared to farm 1 (Table 2). Within farms NEFA tend to increase significantly between FOD and fresh period.

Energy status of the dairy animals varies during different phases of the transition period, as during this period there is high demand of energy in the animal body which is required during parturition and also during early lactation. So in order to meet the demands of the body, through various undergoing metabolic pathways, there is increase production of NEFA and BHBA in the body. This increased levels of NEFA in the body indicated development of negative energy balance in the body. Similarly various other indicators of NEB are increased plasma concentration of BHB (Bell, 1995) [3], decreased plasma glucose concentration (Grum *et al.,* 1996) [11], decreased amount of insulin and insulin-like growth factor-1 (IGF-1) (Butler *et al.,* 2003) [6] and a decreased body condition score (BCS) (Oldick, 1999) [23].

Concentrations of Plasma Minerals

Calcium (Ca)

The mean plasma calcium levels show significant decrease from FOD and CUD to fresh stage in farm 2 (Table 3). Level of calcium in farm 2 at fresh stage was significantly higher than farm 1.

During the early lactation period, there is increased demand for calcium in the body, which is required for milk synthesis and also during this period there is slow rate of absorption of calcium from the intestinal tract due to which there is decrease in the levels of calcium in the body. Similarly Goff (2004) [8] also observed a drop in serum Ca concentration during the early milking period which was greater in older cows than in primiparous cows.

Magnesium (Mg)

The mean plasma magnesium levels are significantly high during CUD and fresh stage in farm 2 compared to farm 1 (Table 3). The level of magnesium in farm 2 at fresh stage was significantly lower than FOD/CUD stage.

Sodium (Na)

The mean plasma sodium levels from two different farms showed no significant change (Table 3).

Potassium (K)

The mean plasma potassium levels from two different farms showed significant high levels during fresh period in farm 2 as compared to farm 1 (Table 3). Certain workers (Dodamani *et al.,* 2009) [7] found non-significant differences in serum K levels during different stages of pregnancy.

Copper (Cu)

The mean plasma copper levels from two different farms showed significantly high levels throughout transition in farm 2 as compared to farm 1 (Table 3). Within farms, farm 2 was having significantly low levels of copper at fresh stage as compared to FOD and CUD stages.

During the late gestation period from seven month onwards, copper is transported through the placenta to the fetus and is stored in the fetus, even when the dam is fed a copper deficient diet, due to which there is decrease I plasma copper levels in dam Gooneratne *et al.,* (1986a and 1986b) [9, 10]. Similarly, Noaman (2013) [22] reported decreased Cu levels due to the higher Fe levels which caused inhibition of the intestinal Cu absorption. However, Cakir (2006) reported higher Cu levels in his study and stated that the reason for this could be due to increased Cu in the form of ceruloplasmin enzyme in response to increased blood estrogen or progesterone.

Iron (Fe)

The mean plasma iron levels from two farms showed significant high levels throughout transition in farm 2 as compared to farm 1 (Table 3). However, no significant differences were found within farms throughout transition. Many metabolic processes, including the electron transport system, oxidation-reduction reactions, energy and protein metabolism, antioxidant defense system, and hemo-respiratory carrier, depend on iron. (Andreiu 2008) [1]. Due to which, it is possible that it will affect the animals inadvertently during the periparturient phase.

Zinc (Zn)

The mean plasma zinc levels from two different farms showed no significant changes (Table 3). While the current study did not find a significant decrease in zinc levels, the lower mean plasma zinc concentration around the peripartum may be due to late-term fetuses accumulating zinc at a rate of approximately 12 mg/day (House and Bell, 1993) [14] and also zinc is necessary for the synthesis of colostrum during early lactation (Kincaid and Cronrath, 1992) [16]. Moreover, glucocorticoids decreased the absorption of zinc and in combination with various other stressors stimulate metallothionein synthesis, which in turn pulled zinc into cells.

Inorganic Phosphorus (Pi)

The mean plasma inorganic phosphorus levels from two different farms show significantly high levels in farm 2 throughout transition as compared to farm 1 (Table 3).

Erythrocytic Lipid Peroxidation (LPO)

Significant increase was observed from FOD to CUD and fresh stage in both farms (Table 4). The significant increase in the lipid peroxidation especially after calving could be due to the increased metabolic demands imposed on the cow by colostrum production and the onset of lactation that far exceeded the demands of the fetus. The metabolic adaptations to lactation were initiated in late pregnancy, especially during the CUD period. It might also reduce synthesis of metallothionein, a metal binding protein that scavenge hydroxide radicals (Prasad *et al.*, 2004) [27].

Superoxide Dismutase (SOD)

Significantly low levels were observed during FOD stage in both farms with increasing trend in subsequent stage and again followed by declining trend after parturition (Table 4). The SOD is a major intracellular enzymatic antioxidant considered as the first defense against pro-oxidants that converts the superoxide (O_2^-) to hydrogen peroxide (H_2O_2), which is further converted into less dangerous forms by other antioxidants (Halliwell and Chirico, 1993) [13]. The present findings were similar to previous studies (Bernabucci *et al.*, 2005) [5] where researchers also recorded a decrease in SOD with the advancement of lactation with lowest levels during the early lactation period. As is well known, SOD is a Cu/Zn-dependent enzyme and erythrocyte GSH-Px is a Se-dependent enzyme.

Reduced Glutathione (GSH)

Significantly low levels were observed from FOD and CUD to fresh stage in farm 1 whereas, significantly low levels were observed between FOD and fresh stage in farm 2 (Table 4). Reduced Glutathione is a major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms (Scholz *et al.*, 1989) [30]. Reduced levels of GSH were observed in the present study, which might be due to the decreased production or increased depletion. Similarly, Sharma *et al.* (2011) [31], also recorded a significant depletion in the blood GSH levels due to the increased production of ROM during the early lactation as compared to the advanced pregnancy, along with a significant positive correlation between the GSH and LPO during the early lactation period. Bernabucci *et al.* (2005) [5] reported highest levels of SH groups during the late pregnancy in comparison to the mid pregnancy and early lactation.

Table 1: Hematological indices in crossbred cows (Mean ± S.E.)

Parameters	Period	Farm 1 (n=10)	Farm 2 (n=12)	Overall (n=22)
Hb (g/dl)	FOD	9.72 ± 0.40^{Ax}	9.86 ± 0.27^{Ax}	9.80±0.23
	CUD	9.89 ± 0.30^{Ax}	9.71 ± 0.21^{Ax}	9.79±0.17
	Fresh	10.57 ± 0.40^{Ax}	9.92 ± 0.32^{Ax}	10.21±0.25
PCV (%)	FOD	28.91 ± 0.89^{Ax}	27.73 ± 1.37^{Ax}	28.26±0.84
	CUD	29.25 ± 0.75^{Ax}	27.71 ± 1.31^{Ax}	28.41±0.79
	Fresh	31.12 ± 1.33^{Ax}	28.18 ± 1.57^{Ax}	29.51±1.07
TEC ($x10^6/\mu l$)	FOD	5.09 ± 0.21^{Ax}	7.02 ± 0.69^{Ay}	6.14±0.43
	CUD	5.50 ± 0.22^{Ax}	6.28 ± 0.54^{Ax}	5.93±0.31
	Fresh	5.67 ± 0.18^{Ax}	5.57 ± 0.21^{Ax}	5.61±0.14
TLC ($x10^3/\mu l$)	FOD	8.55 ± 0.38^{Ax}	8.60 ± 0.50^{Ax}	8.58±0.32
	CUD	8.90 ± 0.37^{Ax}	8.43 ± 0.29^{Ax}	8.65±0.23
	Fresh	9.05 ± 0.49^{Ax}	9.10 ± 0.47^{Ax}	9.07±0.33
Values bearing different superscript in capital alphabets (A, B, C) down the column differ significantly ($p<0.05$) Values bearing different superscript in small alphabets (x, y, z) across the rows differ significantly ($p<0.05$)				

Table 2: Biochemical profile in crossbred cows from Jalandhar district (Mean± S.E.)

Parameters	Period	Farm No.1 (n=10)	Farm No.2 (n=12)	Overall (n=22)
TPP (g/dl)	FOD	7.48±0.18^{Ax}	7.55±0.11^{Ax}	7.51±0.10^{A}
	CUD	7.36±0.14A^{Bx}	7.42±0.12^{Ax}	7.39±1.09^{A}
	Fresh	6.92±0.17^{Bx}	7.04±0.14^{Bx}	6.98±0.10^{B}
Albumin (g/dl)	FOD	2.95±0.10^{Ax}	2.95±0.07^{Ax}	2.95±0.06^{A}
	CUD	2.92±0.09^{Ax}	2.90±0.06^{Ax}	2.90±0.05^{A}
	Fresh	2.62±0.14^{Ax}	2.59±0.09^{Bx}	2.60±0.07^{B}
PUN (mg/dl)	FOD	12.70±1.29^{Ax}	11.00±1.51^{Ax}	11.77±1.00^{A}
	CUD	13.00±1.49^{Ax}	10.91±1.53^{Ax}	11.86±1.07^{A}
	Fresh	15.30±1.77^{Ax}	12.00±1.83^{Ax}	13.50±1.30^{A}
Creatinine (mg/dl)	FOD	1.56±0.08^{Ax}	1.35±0.08^{Ax}	1.44±0.06^{A}
	CUD	1.56±0.20^{Ax}	1.28±0.06^{Ax}	1.40±0.10^{A}
	Fresh	1.33±0.17^{Ax}	1.13±0.11^{Ax}	1.22±0.09^{A}
Glucose (mg/dl)	FOD	76.60±2.35^{Ax}	74.83±2.45^{Ax}	75.63±1.68^{A}
	CUD	69.80±1.95^{Bx}	71.08±1.64^{ABx}	70.50±1.24^{B}
	Fresh	65.60±2.39^{Bx}	68.50±1.27^{Bx}	67.18±1.29^{B}
BHBA (mmol/L)	FOD	0.29±0.05^{Ax}	0.33±0.02^{Ax}	0.31±0.02^{A}
	CUD	0.33±0.04^{Ax}	0.40±0.03^{ABx}	0.37±0.02^{AB}
	Fresh	0.40±0.09^{Ax}	0.44±0.02^{Bx}	0.42±0.04^{B}
NEFA (mmol/L)	FOD	0.09±0.02^{Ax}	0.25±0.03^{Ay}	0.18±0.02^{A}
	CUD	0.20±0.04^{ABx}	0.29±0.03^{Ax}	0.25±0.02^{AB}
	Fresh	0.28±0.05^{Bx}	0.35±0.03^{Ax}	0.32±0.03^{B}
Values bearing different superscript in capital alphabets (A, B, C) down the column differ significantly (p<0.05) Values bearing different superscript in small alphabets (x, y, z) across the rows differ significantly (p<0.05)				

Table 3: Plasma mineral concentration in cross breed cows (Mean± S.E.)

Parameters	Period	Farm 1 (n=10)	Farm 2 (n=12)	Overall (n=22))
Ca (mmol/l)	FOD	2.29±0.12^{Ax}	2.50±0.04^{Ax}	2.40±0.06
	CUD	2.23±0.12^{Ax}	2.44±0.04^{Ax}	2.35±0.06
	Fresh	2.00±0.12^{Ax}	2.30±0.03^{By}	2.16±0.06
Mg (mmol/l)	FOD	1.01±0.10^{Ax}	1.21±0.03^{Ax}	1.12±0.05
	CUD	0.95±0.09^{Ax}	1.18±0.02^{Ay}	1.07±0.05
	Fresh	0.76±0.07^{Ax}	1.05±0.04^{By}	0.92±0.05
Na (mmol/l)	FOD	135.80±1.98^{Ax}	137.33±1.56^{Ax}	136.64±1.22
	CUD	135.30±2.07^{Ax}	135.83±1.48^{Ax}	135.59±1.21
	Fresh	13220±2.28^{Ax}	133.92±1.37^{Ax}	133.14±1.26
K (mmol/l)	FOD	4.35±0.16^{Ax}	4.75±0.20^{Ax}	4.56±0.13
	CUD	4.23±0.14^{Ax}	4.68±0.20^{Ax}	4.47±0.13
	Fresh	3.92±0.11^{Ax}	4.57±0.19^{Ay}	4.27±0.13
Cu (µmol/)	FOD	10.66±0.43^{Ax}	11.82±0.16^{Ay}	11.29±0.24
	CUD	10.35±0.42^{Ax}	11.56±0.13^{Ay}	11.01±0.24
	Fresh	9.64±0.39^{Ax}	10.70±0.22^{By}	10.22±0.23
Fe (µmol/)	FOD	122.28±4.74^{Ax}	141.11±2.20^{Ay}	132.55±3.15
	CUD	121.09±4.73^{Ax}	141.22±2.10^{Ay}	132.07±3.27
	Fresh	120.35±4.48^{Ax}	140.92±2.28^{Ay}	131.57±3.25
Zn (µmol/)	FOD	15.53±0.96^{Ax}	17.18±0.67^{Ax}	16.43±0.58
	CUD	15.34±0.93^{Ax}	16.96±0.66^{Ax}	16.22±0.57
	Fresh	14.71±0.99^{Ax}	16.66±0.67^{Ax}	15.77±0.60
Pi (mmol/l)	FOD	1.54±0.06^{Ax}	1.77±0.02^{Ay}	1.67±0.03
	CUD	1.51±0.06^{Ax}	1.77±0.03^{Ay}	1.65±0.04
	Fresh	1.46±0.06^{Ax}	1.70±0.03^{Ay}	1.59±0.04
Values bearing different superscript in capital alphabets (A, B, C) down the column differ significantly (p<0.05) Values bearing different superscript in small alphabets (x, y, z) across the rows differ significantly (p<0.05)				

Table 4: Parameters related to oxidative stress in crossbred cows (Mean± S.E.)

Parameters	Period	Farm No.1 (n=10)	Farm No.2 (n=12)	Overall (n=22)
LPO (n mol/g Hb)	FOD	167.44±19.90^{Ax}	187.59±15.44^{Ax}	178.43±12.25
	CUD	228.13±16.94^{Bx}	224.47±10.11^{Bx}	226.13±9.23
	Fresh	267.62±22.88^{Bx}	281.76±16.04^{Bx}	275.34±13.34
SOD (U/ mg Hb)	FOD	52.23±5.31^{Ax}	59.33±4.78^{Ax}	56.10±3.55
	CUD	90.33±9.22^{Bx}	91.46±4.49^{Bx}	90.95±4.72
	Fresh	75.80±5.07^{Bx}	74.84±3.42^{Cx}	75.28±2.89
GSH (mM)	FOD	2.75±0.20^{Ax}	2.99±0.19^{Ax}	2.88±0.13
	CUD	2.34±0.12^{Ax}	2.51±0.13^{ABx}	2.43±0.09
	Fresh	1.84±0.12^{Bx}	2.27±0.19^{Bx}	2.08±0.12
Values bearing different superscript in capital alphabets (A, B, C) down the column differ significantly (p<0.05) Values bearing different superscript in small alphabets (x, y, z) across the rows differ significantly (p<0.05)				

Conclusion

Establishing base line values for various metabolic parameters in crossbred cows during transition period will definitely help in predicting the future health and productivity in dairy animals which will ultimately help in increasing the economic benefits of the farmers.

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