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Effect of herbal bio choline supplementation on oxidative stress and biochemical parameters in transition dairy cows

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Abstract
The study was undertaken to evaluate the effect of herbal biocholine supplementation on oxidative stress and hemato-biochemical parameters in dairy cows. Twenty four cows were divided into four groups and were supplemented for 42 days during transition period i.e from 3 weeks before parturition 'Far off dry' (FOD) to 3 weeks post parturition '>21 days in milk' (>21 DIM). Group I was kept as untreated control group. The animals in the group II and III were supplemented with herbal biocholine powder @ 15g and 20g per 100kg body weight per day, respectively. Animals of group IV were supplemented with Herbal biocholine @ 20g per 100 kg body weight per day + Herbal liver tonic @ 10 g per 100 kg body weight per day. There was significant increase in the mean Non esterified fatty acid (NEFA) levels of control group from FOD to >21 DIM, whereas, in treatment group 3, there was significant decline in the mean NEFA levels from FOD to >21 DIM. A significant increase was observed in the mean β-hydroxy butyric acid (BHBA) levels of control group, but in treatment group 3, there was significant decline in the mean BHBA levels. A significant decline in the mean blood glucose levels was observed in all groups from FOD to > 21 DIM. There was significant decline in mean reduced glutathione (GSH) and suoeroxidase dismutase (SOD) levels of control group from FOD to > 21 DIM. A significant increase in the mean lipid per oxidation (LPO) level was observed during > 21 DIM in control group, whereas, in treatment group 3, there was significant decline in LPO during > 21 DIM.

Keywords: Oxidative stress, transition, lipid peroxidation, superoxidase dismutase, reduced glutathione, herbal biocholine

Introduction
Oxidative stress is a product of increased reactive oxygen species (ROS) and free radicals or a decrease in the antioxidant defense mechanisms of body, which, in turn, lead to change in the activity of the immune system against host of stressors and in the basic biopolymers structure, ultimately leading to various health disorders (Abd Ellah, 2010) [1]. Although during metabolic processes free radicals are produced continuously in animal's body and are actively involved in important physiological events like immune response, fatty acid metabolism and various inflammatory reactions but their levels increase abruptly during transition period in dairy cows.

During transition period from late gestation to early lactation, a degree of Negative energy balance (NEB) is a normal physiological phenomenon and during this time homeorhetic regulation of various metabolic processes is essential in order to meet ever increasing metabolic demands of parturition and lacto genesis (Petit, 2009) [2]. Dairy cows are potentially subjected to a battery of stressors like increased free radical production, the inevitable metabolic adaptive stressors and heat/cold stress, overcrowding, changes in social structure, housing, uncomfortable stalls or footing and infectious challenges during transition period (Celi, 2011 and Placer *et al.*, 1966) [3, 4]. Oxidative stress sets in if the amount of free radicals produced exceeds the antioxidant capacity of the body, and can negatively impact the health status and immune response thus increasing the susceptibility of the transition animals to various metabolic diseases. Bio choline is a unique herbal animal feed supplement that contains selected herbs rich in non-toxic and highly bioavailable choline in conjugated / esterified form (Phosphatidylcholine) and phospholipids. Glycerols, phosphatidyl inositol and phosphatidylserine in herbal Choline play significant role in metabolism, enzymic modulation and biosynthesis of phosphatidylcholine and to produce significant growth response as well as to augment the bio-activity of herbal bio choline.

Recently, many scientists are focussing on the role played by oxidative stress in the development of various metabolic diseases (Singh *et al.*, 2015) [5]. Thus, keeping in view the role played by oxidative stress during transition period, the current study was carried out to assess efficacy of herbal bio choline supplementation at different doses and combinations in reducing the oxidative stress in cross bred dairy cows by measuring the activity of lipid peroxidation (LPO), superoxide dismutase (SOD) and reduced glutathione (GSH) in hemolysate, along with concurrent assessment of various hemato-biochemical and plasma mineral parameters during different transition stages.

Materials and Methods

Animals

Twenty four multiparous crossbred cows in advanced pregnancy were taken up for the study. From each animal, blood was collected thrice during different stages of periparturient period viz.

1. 3 weeks before parturition i.e., Far off Dry (FOD)
2. Soon after parturition i.e., Fresh
3. 3 weeks after parturition i.e., >21 Days in milk (DIM)

Blood collection

For estimation of various parameters, blood was collected from each dairy cow selected for sampling. Approximately 10ml of blood was collected in a 15 ml stopper sterile graduated plastic heparinised vials by puncturing the jugular vein aseptically after swabbing the blood collection site with spirit swab. To prevent haemolysis, the collected samples were immediately transported to laboratory packed in ice packs in a container box. For haematology (Hb, PCV, TEC and TLC), 3 ml of blood was collected in (plastic) di-sodium EDTA vials. For glucose estimation, 2 ml of blood sample was collected in separate vials containing sodium fluoride as anticoagulant. For estimation of various biochemical parameters, 2 ml of blood sample was collected in separate clot activator vials.

Oxidative stress

Lipid peroxidation (LPO) was estimated by methods of placer *et al.* (1966) [4]. The activity of SOD in haemolysate was measured by method of Nishikimi *et al.* (1972) [6]. The assay is based on the principle that SOD inhibits the reduction of nitro blue tetrazolium (NBT) by reduced NBT with reduced nicotineamide adenine dinucleotide (NADH) mediated by phenazine metho sulphate (PMS) under aerobic conditions. Reduced glutathione was estimated by the method of Hafeman *et al.* (1974) [7].

Hematology

Haematological parameters were estimated using automated haematologic analyzer (ADVIA 2120, SIEMENS Haematology Analyzer, USA) in the Diagnostic Laboratory of Department of Teaching Veterinary Clinical Complex, GADVASU, and Ludhiana.

Biochemistry

Estimation of biochemical parameters viz. Total plasma proteins (TPP), albumin, blood urea nitrogen (BUN), creatinine, glucose, cholesterol, Total Bilirubin along with enzymes viz. Aspartate amino transferase (AST), Triglyceride (Tg), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALKP) were analyzed with the help of Orthodiagnostic's Vitros 350 biochemistry analyzer using commercial kits in Department of Teaching Veterinary Clinical complex, GADVASU. NEFA and BHBA was estimated using RANDOX ELISA kit

Prophylactic trail

Twenty-four cattle were divided into four groups randomly and were supplemented for 42 days i.e. from 3 weeks before parturition to 3 weeks post parturition as mentioned below:

Group I was not supplemented with any supplement and was taken as untreated positive control group. The selected animals in the test group II and III were supplemented with herbal choline powder ([*1] Biocholine) @ 15g and 20g per 100kg body weight per day, respectively. Group IV animals were supplemented with Herbal choline ([*1]Biocholine) @ 20g per 100 kg body weight per day + Herbal liver tonic ([*2] LivoLiv-250) @ 10 gm per 100 kg body weight per day. All the supplements were manufactured and supplied by Indian Herbs Specialities Pvt Ltd, Saharanpur. Sampling was carried out thrice at different stages of peri-parturient period i.e. just before being dry, immediately after parturition and 3 weeks post parturition. Biocholine powder- Biocholine powder consists of selected herbs containing natural, stable and highly bio-available choline in conjugated/esterified form (phosphatidyl choline, lecithins and equivalents) along with other phospholipids and PUFA's.

A substantial proportion of choline is lost from synthetic choline salts due to conversion into trimethyl amine (TMA) which is toxic and imparts fishy odor, while negligible quantity of choline is lost from esterified choline in Biocholine. Presence of highly hygroscopic synthetic choline chloride in the premix or diet enhances oxidative destruction of other vitamins and feed additives.

Phosphatidyl choline present in Biocholine also helps in conversion of homo-cysteine into methionine for recycling of dietary and supplementary methionine.

[*2] LivoLiv-250 Powder-Major herbal ingredients in LivoLiv-250 are *Andrographispaniculata, Phyllanthusniruri* and *Azadirachta Indica.*

Statistical Analysis

The statistical analysis was carried out using SPSS (16.0). ANOVA followed by Duncan's multiple range test (DMRT) was used to estimate significant difference at $P<0.05$.

Results and discussion

Oxidative stress

Reduced Glutathione

There were significant decline in the mean GSH levels from FOD to > 21 DIM, in control group throughout the transition period, whereas a non-significant decline was observed in mean GSH values in treatment group I and II from FOD to > 21 DIM. In treatment group III, there was non-significant decline the mean GSH values from FOD to Fresh, but the mean GSH values increased significantly during > 21 DIM (Table 1). Similar to the present study, Singh *et al.* (2015) [5] observed significantly low mean GSH levels from far off dry to fresh stage in buffaloes alongwith significant increase in ROS and decrease in GPx and SOD activity during postpartum period. Similarly, 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 was also reported by Kincaid (2000) [8].

Table 1: Oxidative stress parameters values of control and different treatment groups at different sampling periods in cows supplemented with Biocholine (Mean±S.E)

Parameters	Period	Control	Treatment 1	Treatment 2	Treatment 3
GSH (mM)	FOD	2.8±0.08 ^{Ax}	2.63±0.09 ^{Ax}	2.62±0.09 ^{Ax}	2.7±0.06 ^{Ax}
	FRESH	2.4±0.07 ^{Ay}	2.43±0.08 ^{Ax}	2.47±0.07 ^{Ax}	2.3±0.07 ^{Ay}
	>21 DIM	2.3±0.06 ^{By}	2.48±0.11^{ABx}	2.37±0.13^{ABx}	2.65±0.08 ^{Ax}
LPO (n mol/g Hb)	FOD	156.92±2.54^{Az}	150.93±4.87^{Az}	149.25±3.85^{Az}	152.13±5.17 ^{Ay}
	FRESH	192.65±4.33 ^{Ay}	198.87±4.03 ^{Ay}	200.07±5.36 ^{Ay}	195.1±3.63 ^{Ax}
	>21 DIM	268.08±10.61 ^{Ax}	258.03±6.06 ^{Ax}	255.23±7.72 ^{Ax}	135.62±5.53^{Bz}
SOD (U/ mg Hb)	FOD	58.23±4.09 ^{Ax}	59.8±3.94 ^{Ax}	63.8±3.37 ^{Ax}	61.05±5.29 ^{Ax}
	FRESH	55.52±3.42^{Axy}	60.3±3.49 ^{Ax}	55.2±2.62 ^{Ay}	57.12±3.85 ^{Ax}
	>21 DIM	48.17±0.94 ^{By}	47.83±0.83 ^{By}	47.42±2.46 ^{By}	61.78±3.28 ^{Ax}

Values bearing different superscript in capital letters (A, B, C) across the row vary significantly (p<0.05)
Values bearing different superscript in small letters (x, y, z) down the column vary significantly (p<0.05)

Lipid peroxidation

There was significant increase in the mean LPO levels in control group, treatment group I and II from FOD to > 21 DIM (Table 1), which indicate rise in oxidative stress during early lactation. In treatment group III, though there was an significant increase in the mean LPO values from FOD to Fresh, the mean LPO values significantly decreased to 135.62 ± 5.53 n mol/g Hb during > 21 DIM, the results indicate the effectiveness of treatment III (80g Biocholine + 40g Livo-Liv/day). Similar to the present study, previous studies (Singh *et al.*, 2015 and Adella *et al.*, 2006) [5, 9] reported significantly high mean LPO levels from far off dry to fresh stage in buffaloes and stated maximum lipid peroxidation during first week of lactation. In treatment group III, the FOD value was similar to those of other groups but there was significant decrease in the mean LPO values from Fresh period to>21 DIM, indicating marked reduction in oxidative stress after supplementation of Biocholine @ 80g/day along with Livo-Liv @ 40g/day.

Likewise, some researchers like Adella *et al.* (2006) [9], Castillo *et al.* (2003) [10] and Saleh *et al.* (2007) [11] also used lipid peroxidation as a marker of oxidative stress in cattle and found an increase in the LPO levels after calving, as lipids were most susceptible to per-oxidative damage due to the presence of unsaturated bonds. The significant increase in the lipid peroxidation at fresh period after calving could be due to the enhanced metabolic demands imposed on the cow by Production of colostrum and the onset of lactation that over exceeded the demands of the fetus.

Superoxidase Dismutase

There was significant decline in the mean SOD values from FOD to > 21 DIM in control, treatment group I and II. In treatment group III, though there was non-significant decline in the mean SOD values from FOD to Fresh, but the mean SOD values increased to 61.78 (Table 1). The SOD being a major intracellular enzymatic antioxidant is the first defense against pro-oxidants that converts the superoxide (O_2^-) to hydrogen peroxide (H_2O_2), which is further converted into less lethal forms by other antioxidants (Halliwell and Chirico, 1993) [12]. Similar to the present study, significantly low mean SOD levels from far off dry to fresh stage in buffaloes were previously reported by Singh *et al.* (2015) [5].

Hematological indices

In treatment group III, there was significant increase in the mean Hb and PCV values from FOD to > 21 DIM (Table 2). There was a significant increase in the mean platelet count in third sampling i.e. > 21 DIM in both control and treatment groups I and II, while treatment group III had a significant increase of mean platelet count from FOD to >21 DIM. Similar to the present findings, a gradual decrease in the mean Hb during approaching parturition and it gradually increased with advancing days in milk was previously recorded by Mir *et al.* (2008) [13].

Table 2: The haematological values of control and different treatment groups at different sampling periods of cows supplemented with Biocholine (Mean ± S.E).

Parameters	Period	Control	Treatment 1	Treatment 2	Treatment 3
TLC (x 10^3cells/µl)	FOD	8.53±0.65 ^{Bx}	10.37±0.42^{Bx}	16.66±3.51^{Ax}	11.42±1.32^{ABx}
	FRESH	11.50±1.63^{Ax}	10.77±0.74^{Ax}	16.71±3.93^{Ax}	11.50±1.16^{Ax}
	>21 DIM	9.65±1.12 ^{Bx}	10.03±0.54^{ABx}	15.26±3.06^{Ax}	10.18±1.07^{ABx}
TEC (x 10^6cells/µl)	FOD	5.60±0.36 ^{Ax}	5.73±0.17^{Ax}	6.19±0.41^{Ax}	6.00±0.15^{Ax}
	FRESH	5.73±0.22 ^{Ax}	5.76±0.47^{Ax}	5.95±0.24^{Ax}	5.79±0.21^{Ax}
	>21 DIM	5.61±0.30 ^{Ax}	5.47±0.10^{Ax}	5.99±0.41^{Ax}	6.67±0.32^{Ax}
Haemoglobin (g/dl)	FOD	9.38±0.25 ^{Ax}	8.78±0.33^{Ax}	8.65±0.51^{Ax}	8.48±0.22 ^{Ay}
	FRESH	9.31±0.26^{Ax}	9.00±0.31^{Ax}	8.91±0.35^{Ax}	8.86±0.27^{Axy}
	>21 DIM	9.15±0.40^{Ax}	8.45±0.26^{Ax}	8.93±0.46^{Ax}	9.43±0.24^{Ax}
PCV (%)	FOD	26.01±0.85^{Ax}	24.98±0.91^{Ax}	24.36±1.21^{Ax}	23.53±0.51 ^{Ay}
	FRESH	26.78±0.85^{Ax}	25.90±0.88^{Ax}	25.00±0.83^{Ax}	24.86±0.68 ^{Ay}
	>21 DIM	26.18±1.03^{Ax}	23.70±0.780^{Bx}	25.60±1.20^{ABx}	26.91±0.45^{Ax}
MCV (fl)	FOD	45.20±2.06^{Ax}	43.03±1.76^{Ax}	40.58±1.49^{Ax}	40.35±1.25^{Ax}
	FRESH	47.15±1.26^{Ax}	45.18±1.28^{Ax}	42.15±1.65^{Ax}	43.20±1.97^{Ax}
	>21 DIM	46.80±0.78^{Ax}	45.95±1.22^{Ax}	42.83±1.77^{Ax}	43.35±1.64^{Ax}
MCH (pg)	FOD	16.06±0.70^{Ax}	15.03±0.38^{Ax}	14.65±0.60^{Ax}	14.68±0.49^{Ax}
	FRESH	16.38±0.43^{Ax}	15.68±0.45^{Ax}	15.03±0.60^{Ax}	16.80±1.75^{Ax}
	>21 DIM	16.31±0.24^{Ax}	15.80±0.50^{Ax}	15.25±0.63^{Ax}	16.80±1.78^{Ax}
MCHC (g/dl)	FOD	35.86±0.47^{Ax}	35.56±0.44^{Ax}	35.76±0.44^{Ax}	46.23±10.30^{Ax}

	FRESH	34.70±0.19 ^{By}	34.75±0.30^{Bxy}	35.66±0.26^{Ax}	35.68±0.24^{Ax}
	>21 DIM	34.88±0.18^{ABy}	34.43±0.24 ^{By}	35.93±0.56^{Ax}	35.15±0.41^{ABx}
Platelets(x 10^3cells/µl)	FOD	181.51±18.33^{Ay}	198.16±17.45^{Ay}	219.50±22.27 ^{Ay}	210.33±29.06 ^{Ay}
	FRESH	198.83±18.81^{Ay}	171.16±17.41 ^{Ay}	227.83±30.71 ^{Ay}	222.33±33.64 ^{Ay}
	>21 DIM	282.16±26.30^{Bx}	327.83±56.52^{Bx}	335.66±52.35^{ABx}	473.33±45.35^{Ax}

Values bearing different superscript in capital letters (A, B, C) across the row vary significantly (p<0.05)
Values bearing different superscript in small letters (x, y, z) down the column vary significantly (p<0.05)

Biochemical profile

Significant increase in mean values of ALKP, AST, NEFA, BHBA, Cholesterol, GGT and significant decline in mean value of Glucose was observed in control group from FOD to >21 DIM (Table 3). Significant decline in mean values of AST (treatment group III), NEFA (treatment group (II and III), BHBA (treatment group III), SUN (treatment group I and III), Cholesterol and GGT (treatment group III) along with significant increase in mean values of NEFA and Glucose (treatment group I and treatment group III, respectively) was observed from FOD to >21 DIM. Similar to the present study, significantly high levels AST during first 3 weeks of postpartum were recorded by Mir *et al.* (2008) [13], while another study by Schulz *et al.* (2014) [14] recorded that AST values were not significantly higher during the early lactation period when fed with choline orally during prepartum period. The diffusion of NEFA into the blood provides energy to these tissues throughout the body. In excess, they may become toxic (Herdt, 1988) [15] as the bovine liver has a very limited capacity to metabolize NEFA into TAG. They can be either oxidized or exported as very low density lipoproteins (Spain and Scheer, 2001) [16]. However, when the threshold is crossed, the TAG accumulates in the liver and acetyl COA that is not utilized in the tri carboxylic acid cycle (TCA) is converted into ketone bodies, such as acetone, acetoacetate and beta-hydroxy butyrate which may appear in the blood, milk, and urine (Goff and Horst, 1997) [17]. Excessive accumulation of TAG in the liver parenchyma of cows impairs its normal functioning and predisposes them to development of fatty liver syndrome (Jorritsma *et al.*, 2001, Grummer, 1993, Vanden, 1995 and Bryers, 1999) [18-21]. Similar to present findings, previous study by Taghipour *et al.* (2010) [22] reported high NEFA and BHBA concentration at parturition, high AST levels during 1st – 2nd week post lambing whereas, another study by Ospina *et al.* (2010) [23] reported >15 percent of sampled

Table 3. Biochemical values of control and different treatment groups at different sampling periods in cows supplemented with Biocholine (Mean±S.E).

Parameters	Period	Control	Treatment 1	Treatment 2	Treatment 3
Albumin(g/dl)	FOD	2.58±0.07^{Bxy}	2.47±0.08 ^{By}	2.9±0.04 ^{Ax}	2.52±0.13^{Bx}
	FRESH	2.22±0.08 ^{Ay}	2.27±0.08 ^{Ay}	2.38±0.13 ^{Ay}	2.32±0.11 ^{Ax}
	>21 DIM	2.9±0.18 ^{Ax}	3.03±0.3 ^{Ax}	2.87±0.03 ^{Ax}	2.6±0.07 ^{Ax}
ALKP(U/L)	FOD	47±3.52 ^{Ay}	38.67±3.04 ^{Ay}	45±2.02 ^{Ay}	43.33±4.88 ^{Ax}
	FRESH	63.17±1.72 ^{Ax}	64±1.91 ^{Ax}	60.17±4 ^{Ax}	47±3.42^{Bx}
	>21 DIM	67±6.67 ^{Ax}	37.5±1.8 ^{By}	31.33±2.14^{Bz}	36.67±4.18^{Bx}
AST(U/L)	FOD	57.33±4.52 ^{By}	57.67±5.16 ^{By}	79.67±7.99 ^{Ax}	91.83±7.3 ^{Ax}
	FRESH	86.5±4.01 ^{Ax}	79.33±4.9^{ABx}	73±4.34^{ABx}	71.17±5.41 ^{By}
	>21 DIM	79.17±7.17 ^{Ax}	54.17±2.97 ^{By}	69.33±6.8^{ABx}	56.83±2.56 ^{By}
BHBA(mmol/l)	FOD	0.55±0.11 ^{Ax}	0.68±0.08 ^{Ay}	0.67±0.06^{Az}	0.92±0.06 ^{Ax}
	FRESH	0.9±0.14 ^{Ax}	0.72±0.12 ^{Ax}	0.65±0.06 ^{Ax}	0.72±0.05 ^{Ay}
	>21 DIM	0.8±0.09 ^{Ax}	0.65±0.08^{Axy}	0.56±0.05 ^{Ay}	0.55±0.08 ^{Ay}
BUN (mg/dl)	FOD	7.33±1.36^{Bx}	8.67±0.92^{ABx}	4.17±0.75^{Cxy}	10.5±0.56 ^{Ax}
	FRESH	7.67±1.15 ^{Ax}	8.17±1.11 ^{Ax}	5.67±1.52 ^{Ax}	7.83±0.4 ^{Ay}
	>21 DIM	5.17±1.11 ^{Ax}	2.17±0.83 ^{By}	2±0.37 ^{By}	5±1.26^{Az}
Cholesterol (mg/dl)	FOD	77.33±5.68 ^{By}	74.5±1.78 ^{By}	115.5±11.29 ^{Ax}	125.67±11.07 ^{Ax}
	FRESH	90.17±7.96 ^{Ay}	94.83±4.71 ^{Ax}	86.5±4.3 ^{Ay}	95.33±6.46 ^{Ay}
	>21 DIM	126.17±10.11^{Ax}	70.5±7.48 ^{By}	92.67±8.77^{Bxy}	88±7.68 ^{By}
Creatinine (mg/dl)	FOD	1.72±0.15 ^{Ax}	1.8±0.2 ^{Ax}	1.42±0.07^{ABxy}	1.28±0.11^{Bx}
	FRESH	1.88±0.39 ^{Ax}	2.12±0.41 ^{Ax}	2.1±0.41 ^{Ax}	1.03±0.08^{Axy}
	>21 DIM	1.75±0.07 ^{Ax}	1.6±0.15 ^{Ax}	1.27±0.08 ^{By}	1±0.07 ^{By}
GGT (U/L)	FOD	29.67±5.22^{Bx}	30.83±2.89 ^{By}	28.83±3.99 ^{By}	50.17±4.28 ^{Ax}
	FRESH	43.67±3.13 ^{Ax}	50.33±2.11 ^{Ax}	43±4.25 ^{Ax}	43.5±2.31^{Axy}
	>21 DIM	43.17±5.94 ^{Ax}	27.83±2.63 ^{By}	24.5±1.86 ^{By}	35.5±3.68^{ABy}
Glucose (mg/dl)	FOD	45.83±7.35^{Bx}	64.5±6.54 ^{Ax}	69±4.28 ^{Ax}	56.67±1.43^{ABx}
	FRESH	28.33±1.33 ^{By}	27.83±1.51 ^{By}	28.5±1.59 ^{By}	33.33±1.09 ^{Ay}
	>21 DIM	29.67±1.74 ^{By}	23.5±2.33 ^{Cy}	31.33±1.12 ^{By}	43.67±0.8^{Az}
NEFA (mmol/l)	FOD	0.33±0.05 ^{By}	0.38±0.08 ^{By}	0.7±0.12^{Axy}	0.72±0.11^{Axy}
	FRESH	0.62±0.04^{Bx}	0.63±0.04^{Bx}	0.83±0.08 ^{Ax}	0.88±0.06 ^{Ax}
	>21 DIM	0.75±0.06 ^{Ax}	0.78±0.04 ^{Ax}	0.53±0.07 ^{By}	0.53±0.06 ^{By}
Total bilirubin (mg/dl)	FOD	0.72±0.1 ^{Ax}	0.85±0.03 ^{Ax}	0.38±0.08^{Bx}	0.7±0.06 ^{Ax}
	FRESH	0.75±0.12 ^{Ax}	0.78±0.13 ^{Ax}	0.5±0.09 ^{Ax}	0.65±0.07 ^{Ax}
	>21 DIM	0.73±0.11 ^{Ax}	0.9±0.04 ^{Ax}	0.37±0.05^{Bx}	0.75±0.06 ^{Ax}
Total Serum Proteins(g/dl)	FOD	6.9±0.26 ^{Ax}	7.05±0.2 ^{Ay}	5.97±0.21 ^{By}	6.57±0.31^{ABx}
	FRESH	6.78±0.23 ^{Ax}	6.65±0.22 ^{Ay}	6.12±0.15^{Axy}	6.75±0.29 ^{Ax}
	>21 DIM	7.2±0.56^{ABx}	8.08±0.3 ^{Ax}	6.6±0.23^{Bx}	7.23±0.41^{ABx}

across the row vary significantly (p<0.05) Values bearing different superscript in small letters (x, y, z) down the column vary significantly (p<0.05)

animals in herd with > 0.27 mEq/L prepartum and >0.70 mEq/L post partum NEFA levels and > 12 mg/dl BHBA in ketotic animals. Greater urea concentration in lactating animals could be a result of muscle protein catabolism when large amounts of body reserves get mobilized for meeting the lactation demands (Sreedhar *et al.*, 2013) [24]. Stressed cows had increased BUN levels as compared to control, which could be due to the higher utilization of amino acids as energy source in response to persistent negative energy balance (Shwartz *et al.*, 2009 and Gerardo *et al.*, 2009) [25, 26].

In the present study, the plasma glucose concentration showed a decrease starting from the FOD period up to the early lactation which could be associated with fetal development leading to mobilization of maternal glucose to fetal blood circulation (Jacob and Vadodaria, 2001) [27] during advanced pregnancy and a high demand for lactose synthesis and/or insufficient gluconeogenesis during early lactation (Pambu-Gollah *et al.*, 2000) [28].

Conclusion

In conclusion, the study highlights the importance of oxidative stress and other hemato-biochemical parameters in transition crossbred cows. It was concluded that due to negative energy balance and other metabolic derangements during the transition period LPO increases, whereas, SOD and GSH decreases with increase in oxidative stress in transition crossbred cows. Based on this study it is recommended that, supplementation with herbal choline (*[1]Biocholine) @ 20g per 100 kg body weight per day + Herbal liver tonic (*[2]LivoLiv-250) @ 10 gm per 100 kg body weight per day during transition period can help reduce oxidative stress in Crossbred Cows.

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