The Pharma Innovation Journal 2019; 8(8): 97-106

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(8): 97-106

www.thepharmajournal.com
Received: 22-06-2019
Accepted: 24-07-2019

Ambily TR
MVSc, Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

Beena V
Associate Professor, Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

Karthiayini K
Professor, Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

Ramnath V
Professor, Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

Uma R
Assistant Professor, Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

Siddaramesh AS
MVSc, Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

Sunanda C
Academic consultant, Department of Biostatistics, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

Correspondence
Ambily TR
MVSc, Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

Effect of supra-nutritional supplementation of vitamin E and/or selenium on oxidant-antioxidant profile in transition dairy cows

Ambily TR, Beena V, Karthiayini K, Ramnath V, Uma R, Siddaramesh A S and Sunanda C

Abstract
Transition period in dairy cow is associated with oxidative stress resulting from augmented metabolic demands. The study was conducted to assess and compare the changes in oxidative stress parameters during transition period in dairy cows, supplemented with/without antioxidants; vitamin E and/or selenium. Twenty four healthy pregnant dairy cows belonging to 2nd/3rd parity at 220th day of pregnancy were selected and divided randomly into four groups of six animals each, G_0 (control), G_1 (selenium @ 0.3 ppm/kg DM/day), G_2 (vitamin E @ 1000 IU/day) and G_3 (selenium @ 0.3 ppm/kg DM + vitamin E @ 1000 IU per day). The animals were maintained on uniform standard diet plan with specific supplementation as mentioned and uniform THI (average THI 79) during the entire study period (from 220th day of pregnancy to 30 days post-partum). Blood and serum samples were collected on 220th & 250th days of pregnancy, the day of calving and on the 30th day post-partum. Oxidative stress parameters (blood Glutathione peroxidase and Superoxide dismutase; serum catalase and malonaldehyde) haematological parameters (volume of packed red cells-VPRC, total erythrocyte count-TEC, haemoglobin concentration-Hb and total leucocyte count-TLC and serum biochemical parameters (total protein, albumin, globulin and albumin: globulin ratio) were estimated. We observed oxidative stress in G_0 and better antioxidant status in the supplemented groups on the day of calving and 30th day post-partum. The improvement in health status of supplemented group animals was evident from better oxidant-antioxidant and Hemato-biochemical profile. Thus based on this study supplementation of vitamin E/selenium may be recommended beneficial for dairy cows, as it alleviates oxidative stress and provide improved health status during transition period.

Keywords: Antioxidants, dairy cows, oxidative stress, selenium, transition period, vitamin E

1. Introduction
Management of dairy cows during transition period from late gestation to early lactation is of great importance for optimising the performance of animals. Animals in this period are typically in a negative energy balance as they fail to meet the increased nutrient requirements of fetal growth, calving and onset of lactation (Giulia *et al*., 2014). Changes in metabolic, endocrine and physiological functions are associated with several health disorders like ketosis, mastitis, metritis, retained fetal membranes, hypocalcemia. Reduction in immune responses during this period also predisposes animals to infectious diseases (Sordillo and Aitken, 2009) [37]. Increased metabolic rate causes augmented production of free radicals and whenever the rate of production of free radicals exceeds the antioxidant capacity of the animal, it suffersrom oxidative stress (Abuelo *et al*., 2015) [1]. So, assessing the oxidative status of dairy cows in the transition period will help to evaluate the extend of oxidative stress of the animals and to adopt ideal antioxidant supplementation strategies as present supplementation strategies are only meant to avoid deficiency disorders. So, the present study was designed to evaluate the stress alleviating effects of proven antioxidants vitamin E (α- tocopherol) and selenium (Se) when supplemented with the diet in transition dairy cattle. Vitamin E is a potent chain breaking, lipid soluble antioxidant and Se is a part of different selenoproteins including one of the most important antioxidant enzymes, glutathione peroxidase.

2. Materials and methods
2.1 Experimental animal and design
Twenty-four clinically healthy pregnant dairy cattle belonging to 2nd/3rd parity at 220 days of pregnancy were selected and were divided randomly into four groups and were randomly

allocated to one of the four dietary treatments G_0 (control), G_1 (selenium @ 0.3 ppm/kg DM/day), G_2 (vitamin E @ 1000 IU/day) and G_3 (selenium @ 0.3 ppm/kg DM + vitamin E @ 1000 IU per day). All the experimental animals were given pregnant cattle ration, fresh Hybrid Napier grass and *adlibitum* access to water. Supplementation started at 220 days of pregnancy and continued till 30 days post-partum. The study was conducted during the months of august to November during which the average temperature humidity index were around 79, to exclude variations caused due to thermal stress.

2.2 Sample collection

Approximately 5ml blood was collected on 220th & 250th days of pregnancy, day of calving and on 30th day post-partum in heparinised vacutainers and serum separation vials. Serum was separated by centrifuging and stored in -50° freezer for laboratory analysis.

2.3 Estimation of oxidative stress parameters

2.3.1 Sample preparation for GSH-Px

50 µl of the heparinised blood is mixed with 2 ml of diluting agent supplied along with GSH-Px assay kit supplied by RANDOX Laboratories Ltd, U.K. The samples were stored at -50ºC until analysis. Glutathione peroxidase activity was determined photometrically in semiautomatic bio chemical analyzer (Hospitex- Screen Master T) as per Paglia and Valentine (1967) [29] by using Ransel kit supplied by RANDOX Laboratories Ltd, U.K.

Sample preparation for SOD

500 µl of blood is taken in a glass tube and is centrifuged at 3000 rpm for 10 minutes to separate off the plasma. To get the RBC sediment washed, added 3 ml of normal saline and centrifuged at 3000 rpm for 10 minutes. Discarded the supernatant without disturbing the RBC sediment. This step was repeated for 3-4 times to make the RBCs devoid of any plasma. The final washed RBCs were made to 2 ml by adding cold double distilled water and were stored at -50ºC until analysis. Blood superoxide dismutase activity was determined photometrically in semiautomatic bio chemical analyzer (Hospitex- Screen Master T) by using Ransod kit supplied by RANDOX Laboratories Ltd, U.K.

2.3.2 Sample preparation for catalase

As soon as the blood in the serum separation vial got clotted it was centrifuged at 3000g for 15 minutes at 4 °C in a refrigerated centrifuge. Separated serum samples were collected in eppendorf tubes and were stored at -50ºC until analysis. Catalase activity was determined photometrically using the peroxidatic function of catalase for determination of enzyme activity in ELISA plate reader using Catalase assay kit supplied by Cayman Chemical Company, U.S.A.

Sample preparation for MDA

Level of lipid peroxides in serum was determined by estimating malonaldehyde (MDA) level by method of Yagi (1984) [42].

2.4 Estimation of Hematological Parameters

Hematological parameters like total erythrocyte count (TEC), total leucocyte count (TLC), Hemoglobin (Hb) gram per cent and volume of packed red cells (VPRC) were estimated as per the standard techniques described by Schalm (1986) [32] and expressed as millions/µL, leucocyte count/µL, per cent and per cent respectively.

2.5 Protein estimation

Total proteins and albumin of serum were estimated Photometrically (Hospitex- Screen Master T) based on biuret and bromocresol green method using kits supplied by Agappe diagnostics Ltd, Agappe hills, Ernakulam, Kerala and expressed as g/dL. Globulin concentration was calculated by subtracting the concentration of albumin from total protein concentration and using albumin and globulin values, ratio of albumin and globulin was calculated.

2.6 Statistical analysis

The data obtained on various parameters were statistically analysed using software SPSS version 24.0 by two factor ANOVA for repeated measures.

3. Results

3.1 Oxidative stress parameters

3.1.1 Blood Glutathione peroxidase

The mean GSH-Px values on 220th & 250th days of pregnancy, the day of calving and on the 30th day post-partum of all the four experimental groups are presented in Table 1. When analysed within period between groups, significant difference in blood GSH-Px activity was found on the day of calving and on 30 day post-partum. On the day of calving, cows in G_1 (0.3 ppm Se) had significantly ($P \leq 0.05$) higher GSH-Px activity compared to G_0 (control) and G_2 (1000 IU vitamin E). G_3 (selenium @ 0.3 ppm/kg DM + vitamin E @ 1000 IU per day) significantly differed from G_2 (1000 IU vitamin E) but not from G_1 (0.3 ppm Se).

Table 1: GSH-Px activity (U/L of haemolysate) (mean ± SE, n=6)

Group	220th day	250th day	Day of calving	30 day post-partum	P value
G_0	1045.43 ± 60.26^{A}	1235.25 ± 192.84^{A}	1087.58 ± 72.23^{abA}	789.15 ± 73.60^{aB}	0.015
G_1	1068.40 ± 53.51	1128.58 ± 77.23	1338.18 ± 120.79^{c}	1109.12 ± 103.41^{ab}	0.223
G_2	1121.48 ± 84.87	1015.85 ± 55.90	993.35 ± 28.01^{a}	920.33 ± 50.87^{a}	0.199
G_3	1033.60 ± 54.43	1021.35 ± 57.07	1240.72 ± 42.71^{bc}	1336.02 ± 191.74^{b}	0.185
P value	0.780	0.472	0.018	0.021	

G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group

*- significant at 5% level, means with same lower case as superscripts have no significant difference between the groups; means with same upper case as superscripts have no significant difference between the periods.

On 30 day post-partum, blood GSH-Px values were significantly ($p \leq 0.05$) high in G_3 animals compared to G_0 and G_2 animals and there was no significant difference between G_1 and G_3 animals.

When analysed within group between periods, significant difference in blood GSH-Px activity was found only on G_0 (control) animals with significantly low activity on 30 day post-partum compared to other periods.

3.1.2 Blood Superoxide dismutase

The average blood SOD activities on 220th & 250th days of pregnancy, the day of calving and on the 30th day post-partum of all the four experimental groups are presented in Table 2.

There was no significant difference in blood SOD activity between different periods in any of the experimental groups or between different groups in any of the experimental periods. The blood SOD activity showed an increasing trend from 220 days of pregnancy to 30 day post-partum in G_0 (control) and G_3 (Se @ 0.3 ppm + vitamin E @ 1000 IU/day) animals. In G_1 and G_3 the average blood SOD activities were high in all the experimental periods compared to G_0. In G_2 (vitamin E @ 1000 IU/day) animals, blood SOD values showed an increasing trend up to calving but decreased thereafter.

Table 2: Blood SOD activity (U/mL of blood) (mean ± SE, n=6)

Group	220th day	250th day	Day of calving	30 day post-partum	P value
G_0	314.33 ± 57.29	360.33 ± 20.99	409.43 ± 38.13	413.07 ± 72.81	0.447
G_1	431.85 ± 76.16	388.53 ± 46.73	415.68 ± 70.84	459.90 ± 60.82	0.878
G_2	291.2 ± 45.02	399.23 ± 40.92	429.78 ± 79.33	331.80 ± 35.56	0.090
G_3	466.83 ± 90.43	502.70 ± 68.96	528.67 ± 54.09	567.73 ± 92.34	0.554
P value	0.234	0.197	0.511	0.137	

G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group

3.1.3 Serum Catalase

The mean catalase values on 220th & 250th days of pregnancy, the day of calving and on the 30th day post-partum of all the four experimental groups are presented in Table 3. On within period between groups analysis, significant difference in serum catalase activity was found only on 30th day post-partum with G_0 having significantly high serum catalase activity compared to G_3 and G_2. There was no significant difference between G_0 and G_1 and G_1 was significantly high compared to G_3. On within group between period analysis, no significant difference could be observed between any periods in any of the experimental groups.

Table 3: Serum catalase activity (nmol/min/mL) (mean ± SE, n=6)

Group	220th day	250th day	Day of calving	30 day post-partum	P value
G_0	11.196 ± 2.599	9.129 ± 1.809	6.406 ± 0.760	9.149 ± 1.795^{a}	0.073
G_1	7.752 ± 2.843	5.605 ± 1.028	6.852 ± 0.948	7.98 ± 0.836^{ab}	0.636
G_2	10.815 ± 2.985	8.364 ± 1.482	5.927± 1.251	4.618 ± 0.781^{bc}	0.054
G_3	6.077± 1.546	5.803 ± 1.734	3.565 ± 0.487	4.013 ± 1.030^{c}	0.295
P value	0.445	0.290	0.079	0.014^{*}	

G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group
*- significant at 5% level, and means with same lower case as superscripts have no significant difference between the groups

3.1.4 Serum Malonaldehyde

The mean MDA values on 220th & 250th days of pregnancy, the day of calving and on the 30th day post-partum of all the four experimental groups are presented in Table 4 and demonstrated in Figure 1. When analysed within period between groups, on 30 days post-partum, the mean serum MDA values varied significantly ($p<0.01$) with significantly high levels for G_0 (control) compared to G_1 (0.3 ppm Se) and G_2 (1000 IU vitamin E). The mean serum MDA of G_1 (0.3 ppm Se) was significantly high compared to G_2 (1000 IU vitamin E). When analysed within group between periods, there was significant difference in serum MDA values in G_0 (control) and G_3 ($p<0.05$). In G_0, MDA values were significantly higher on the day of calving and 30th day post-partum compared to MDA values on the 220th day of pregnancy, but not with 250th day results. 30th day results were significantly high compared to all other experimental periods in G_0. In G_3 animals, similar pattern was observed except that the 30th day results were not significantly high from day of calving results. In G_1 and G_2 no significant difference was observed in serum MDA values between different periods of transition period.

Table 4: Serum malonaldehyde (nmol/mL of serum) (mean ± SE, n=6)

Group	220th day	250th day	Day of calving	30 day post-partum	P value
G_0	1.90 ± 0.31^{A}	5.41 ± 1.4^{AB}	6.45 ± 0.80^{B}	13.33 ± 1.82^{aC}	0.032^{*}
G_1	3.16 ± 0.99	7.8 ± 3.01	8.15 ± 2.69	9.06 ± 1.74^{b}	0.053
G_2	1.65 ± 0.34	4.26 ± 2.13	3.00 ± 0.48	4.00 ± 0.52^{c}	0.370
G_3	1.45 ± 0.32^{A}	3.55 ± 1.13^{AB}	3.63 ± 0.48^{B}	5.46 ± 0.84^{bcB}	0.016^{*}
P value	0.173	0.497	0.067	0.00^{**}	

G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group
**- significant at 1% level, *- significant at 5% level, means with same lower case as superscripts have no significant difference between the groups; means with same upper case as superscripts have no significant difference between the periods.

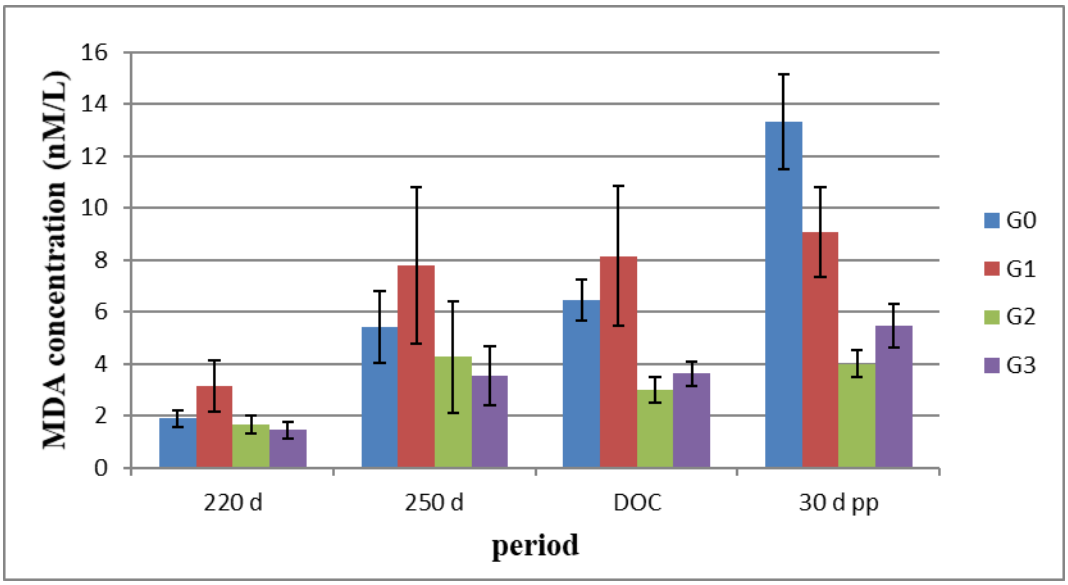

Fig 1: Serum malonaldehyde (nmol/mL of serum) (mean ± SE, n=6)

G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group

3.2 Hematological parameters

3.2.1 Volume of packed red cells

The mean VPRC values on 220th & 250th day of pregnancy, the day of calving and on the 30th day post-partum of all the four experimental groups are presented in Table 5. On period wise analysis, the mean VPRC values were significantly different on day of calving. G_3 animals were having significantly high mean VPRC values compared to G_0 and G_2. However, there was no significant difference between G_1 and G_3. No significant difference was observed between G_0, G_1 and G_2. No significant differences in mean VPRC values were noted on within group between period analysis.

Table 5: Volume of Packed Red Cells (%) (mean ± SE, n=6)

Group	220th day	250th day	Day of calving	30day post-partum	P value
G_0	32.50 ± 1.38	30.83 ± 1.54	29.33^{a} ±1.31	30.83 ± 1.08	0.229
G_1	30.67 ± 2.55	31.00 ± 1.13	31.50^{ab} ±1.20	32.17 ± 0.95	0.845
G_2	29.83 ± 2.07	30.33 ± 1.89	30.67^{a} ± 1.67	32.33 ± 1.71	0.462
G_3	34.83 ± 1.49	35.00 ± 1.21	35.17^{b} ± 0.79	35.83 ± 1.01	0.799
P value	0.297	0.126	0.026*	0.053	

G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group
*- significant at 5% level, and means with same lower case as superscripts have no significant difference between the groups

3.2.2 Total erythrocyte count

The mean TEC on 220th & 250th day of pregnancy, the day of calving and on the 30th day post-partum of all the four experimental groups are presented in Table 6. On within period between group analysis, mean TEC were significantly different on 250th day of pregnancy, day of calving and on 30th day post-partum. On 250th day of pregnancy, G_3 were having significantly (p<0.05) high TEC compared to G_0, G_1 and G_2. There was no significant difference between G_0, G_1 and G_2. On day of calving and on 30 days post-partum, G_3 was having significantly (p<0.01) high TEC compared to G_0, G_1 and G_2. No significant difference was noted between G_0, G_1 and G_2.

Table 6. Total erythrocyte count (millions/µl) (mean ± SE, n=6)

Group	220th day	250th day	Day of calving	30day post-partum	P value
G_0	4.92 ± 0.44	4.52^{a} ± 0.31	4.15^{a} ± 0.17	4.60^{a} ± 0.22	0.284
G_1	5.17^{AB} ± 0.41	4.48^{aA} ± 0.22	4.23^{aA} ± 0.34	5.25^{aB} ± 0.28	0.028*
G_2	4.2 ± 0.51	4.52^{a} ± 0.44	4.52^{a} ± 0.31	5.03^{a} ± 0.23	0.446
G_3	5.68^{AB} ± 0.46	5.78^{bA} ± 0.37	5.77^{bA} ± 0.39	6.23^{bB} ± 0.32	0.020*
P value	0.177	0.036*	0.005**	0.002**	

G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group
**- significant at 1% level, *- significant at 5% level, and means with same lower case as superscripts have no significant difference between the groups; means with same upper case as superscripts have no significant difference between the periods.

On within group between period analysis, there was no significant difference between different periods in G_0 and G_2. In G_1, mean TEC during 30th day post-partum were significantly high compared to 250th day of pregnancy and day of calving results. TEC on 220 day was not significantly different from any period. There was no significant difference between TEC values on 220th & 250th day of pregnancy and day of calving. In G_3, mean TEC on 30th day post-partum was significantly high compared to 250th day of pregnancy and day of calving values. Mean TEC on 220th day was not significantly different from any periods. No significant difference was found between 250th day and day of calving TEC values.

3.2.3 Hemoglobin Concentration

The mean Hb concentration on 220th & 250th day of pregnancy, the day of calving and on the 30th day post-partum of all the four experimental groups are presented in Table 7. On within group between period or within period between groups analysis, no significant difference was observed in mean Hb concentration.

3.2.4 Total leucocyte count

The mean TLC on 220th & 250th day of pregnancy, the day of calving and on the 30th day post-partum of all the four experimental groups are presented in Table 8. On within group between period or within period between groups analysis, no significant difference was observed in mean total leucocyte count.

Table 7: Haemoglobin concentration (g %) (mean ± SE, n=6)

Group	220th day	250th day	Day of calving	30day post-partum	P value
G_0	9.92 ± 0.39	9.52 ± 0.46	9.33 ± 0.36	9.48 ± 0.37	0.493
G_1	9.85 ± 0.69	9.67 ± 0.35	9.37 ± 0.28	9.78 ± 0.28	0.628
G_2	9.22 ± 0.52	9.15 ± 0.57	9.03 ± 0.82	9.17 ± 0.67	0.885
G_3	10.95 ± 0.49	10.75 ± 0.65	10.87 ± 0.65	10.89 ± 0.40	0.965
P value	0.179	0.188	0.134	0.072	

G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group

Table 8: Total leucocyte count (mean ± SE, n=6) G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group

Group	220th day	250th day	Day of calving	30day post-partum	P value
G_0	11033.3 ± 878.9	9716.67 ± 907.90	11300.0 ± 371.48	10966.7 ± 483.51	0.108
G_1	9550.0 ± 715.43	9166.67 ± 389.59	10950.0 ± 328.38	10350.0 ± 261.73	0.083
G_2	9150.0 ± 649.49	9233.33 ± 294.00	10533.3 ± 333.33	10100.0 ± 265.83	0.097
G_3	8216.67 ± 810.5	9250.0 ± 388.80	10033.3 ± 537.07	9400.0 ±393.70	0.098
P value	0.106	0.888	0.171	0.054	

3.3 Serum Biochemical Parameters

3.3.1 Total protein

The mean total protein concentration on 220th & 250th day of pregnancy, the day of calving and on the 30th day post-partum of all the four experimental groups are presented in Table 9.

On within period between group analysis, there was no significant difference in mean total protein concentration on 220th & 250th day of pregnancy and on 30th day post-partum. But on the day of calving total protein concentration varied significantly (p<0.01) between groups. G_0 and G_1 animals were having significantly high total protein concentration compared to G_3 animals. Total protein concentration of G_2 was not significantly different from any other groups. On within group between period analysis, there was no significant difference in mean total protein concentration between different experimental periods.

Table 9: Serum total protein concentration (g/dL) (mean ± SE, n=6)

Group	220th day	250th day	Day of calving	30day post-partum	P value
G_0	7.95 ± 0.57	8.57 ± 0.39	8.59^{a} ± 0.33	8.29 ± 0.44	0.510
G_1	8.25 ± 0.42	7.79 ± 0.35	8.51^{a} ± 0.19	8.12 ± 0.48	0.618
G_2	7.88 ± 0.17	8.78 ± 0.46	8.02^{ab} ± 0.26	7.84 ± 0.33	0.173
G_3	8.12 ± 0.27	8.01 ± 0.36	7.18^{b} ± 0.35	7.41 ± 0.33	0.215
P value	0.902	0.271	0.009**	0.457	

G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group

**- significant at 1% level, and means with same lower case as superscripts have no significant difference between the group

3.3.2 Serum albumin

The mean serum albumin values on 220th & 250th day of pregnancy, the day of calving and on the 30th day post-partum of all the four experimental groups are presented in Table 10. No significant difference in mean serum albumin values could be observed on group wise or period wise analysis.

Table 10: Serum albumin concentration (g/dL) (mean ± SE, n=6)

Group	220th day	250th day	Day of calving	30day post-partum	P value
G_0	3.76 ± 0.225	3.809 ± 0.125	3.767 ± 0.168	3.807 ± 0.216	0.959
G_1	3.913 ± 0.121	3.752 ± 0.110	4.258 ± 0.178	3.885 ± 0.235	0.300
G_2	3.768 ± 0.227	3.827 ± 0.084	3.92 ± 0.152	3.836 ± 0.123	0.924
G_3	3.88 ± 0.085	4.119 ± 0.105	4.219 ± 0.231	3.699 ± 0.121	0.134
P value	0.897	0.101	0.212	0.904	

G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group

3.3.3 Serum globulin

The mean serum globulin values on 220th & 250th day of pregnancy, the day of calving and on the 30th day post-partum of all the four experimental groups are presented in Table 11.

When analysed within period between groups, no significant difference could be observed during 220th & 250th day of pregnancy and on 30th day post-partum. But, on the day of calving there was significant difference in mean serum globulin values, G_3 animals were having significantly ($p \leq 0.01$) low concentration of serum globulin compared to G_0, G_1 and G_2. No significant difference could be found between G_0, G_1 and G_2. No significant difference in mean serum globulin values could be observed on within group between period analysis.

Table 11: Serum globulin concentration (g/dL) (mean ± SE, n=6)

Group	220th day	250th day	Day of calving	30 day post-partum	P value
G_0	4.189 ± 0.463	4.761 ± 0.419	4.85 [a] ± 0.417	4.48 ± 0.517	0.467
G_1	4.337 ± 0.386	4.039 ± 0.266	4.252 [a] ±0.174	4.23 ± 0.387	0.917
G_2	4.11 ± 0.291	4.949 ± 0.414	4.093 [a] ± 0.342	4.01 ± 0.377	0.232
G_3	4.24 ± 0.258	3.889 ± 0.402	2.961 [b] ± 0.409	3.714 ± 0.282	0.164
P value	0.975	0.163	0.009**	0.581	

G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group
**- significant at 1% level, and means with same lower case as superscripts have no significant difference between the groups

3.3.4 Albumin Globulin ratio

The mean A:G ratio on 220th & 250th day of pregnancy, the day of calving and on the 30th day post-partum of all the four experimental groups are presented in Table 12.

There was no significant difference in A: G ratio between different groups or between different periods.

Table 12: Serum albumin: globulin ratio (mean ± SE, n=6)

Group	220th day	250th day	Day of calving	30 day post-partum	P value
G_0	0.947 ± 0.115	0.837 ± 0.093	0.821 ± 0.108	0.92 ± 0.14	0.353
G_1	0.935 ± 0.080	0.946 ± 0.056	1.013 ± 0.067	0.96 ± 0.10	0.898
G_2	0.957 ± 0.118	0.792 ± 0.048	1.003 ± 0.112	1.01 ± 0.11	0.414
G_3	0.934 ± 0.064	1.121 ± 0.127	1.678 ± 0.413	1.03 ± 0.088	0.174
P value	0.998	0.065	0.064	0.910	

G_0- Control, G_1- Se supplemented group, G_2- vitamin E supplemented group, G_3- Se and vitamin E supplemented group

4. Discussion

4.1 Oxidative stress parameters

4.1.1 Blood Glutathione peroxidase

Glutathione peroxidase (GSH-Px), an antioxidant enzyme which was found to be essential in protecting erythrocytes from oxidative damage and thereby preventing hemolysis (Mills, 1957) [26]. Each mole of GSH-Px in red blood cells (RBC) contained 4g atom of Se and that Se was essential for the enzymatic activity of GSH-Px (Flohe *et al*., 1973) [14]. In rats Se supplementation at 1mg/l in drinking water resulted in significantly high erythrocyte GSH-Px values when compared to control ones (Doni *et al*., 1984) [10]. In ruminants, cytosolic GSH-Px was considered as one of the essential antioxidant systems, even though catalase also take part in the reduction of hydroperoxides formed inside mammalian cells. But catalase plays an important role in adaptive response to oxidative stress (Wichtel, 1998) [41].

Transition period in dairy cows is associated with oxidative stress (Umesh *et al*., 2010; Konvicna *et al*., 2015) [40, 20]. In the present study, significant increase in blood GSH-Px activity was noted among Se supplemented animals (Calamari *et al*., 2010; Kendall *et al*., 2012; Gong *et al*., 2014; Teixeira *et al*., 2014) [6, 19, 16, 39] during day of calving and 30th day post-partum. This indicated that initiation of lactation and tissue repair related to purpeureum might be inducing more powerful oxidative stress to the animals than advanced pregnancy (Sharma *et al*., 2011) [33]. So, among the transition period, the latter half (calving to 30 days post-partum) could be considered as the most crucial period as far as oxidative stress is concerned. When Se alone was given, though there was a trend in GSH-Px level increase, it was not statistically significant. But, when compared to other groups, Se supplementation could maintain a statistically significant (p<0.05) increase in GSH-Px activity on the day of calving. So it might be inferred that at the supplemented level, Se could maintain a better antioxidant status up to calving. But, at 30 days post-partum this antioxidant support reduced. This might be either due to the utilisation of Se for the process of milk secretion or due to excess utilisation of GSH-Px during increased oxidative stress. As milk production increases, animal would not be able to meet the increase in nutrient requirements and comes to a state of negative energy balance. This leads to increased mobilisation of fat reserves. Fat metabolism increases production of free radicals and lipid peroxides. Also there would be impairment in antioxidant defence system as Se would be utilised for tissue repair process and there would be reduction in vitamin E concentration due to reduced carrier protein synthesis by inflamed liver (Abuelo *et al*., 2015) [1]. The results of MDA estimation also supported this. But, when vitamin E alone was given, no statistically improved GSH-Px activity could be detected. This could be due to two probable reasons-(1) Vitamin E at the provided dose might not be enough to counteract the increased oxidative stress (2) Insufficient serum Se, as Se is preferentially utilised for tissue repair process and milk synthesis.

The increase in blood GSH-Px activity during calving in G_1 and G_3 might be due to extra nutritional back up provided by Se supplementation and also due to the increased level of oxidative stress during calving demanding an increased production (Bernabucci *et al*., 2005) [3]. On 30 days post-partum, only G_3 animals had significantly high blood GSH-Px activity. That means Se supplementation alone could not reduce oxidative stress during post-partum. Significant amounts of Se would be utilised for colostrum synthesis during post-partum, which also could be a reason for reduced GSH-Px activity during post-partum. As vitamin E is a potent antioxidant preventing propagation of free radicals and reducing formation of lipid peroxides (Abuelo *et al*., 2015) [1], it might be reducing the amount of oxidative radicals and thus sparing GSH-Px.

The significant decrease in GSH-Px activity in G_0 animals on 30th day post-partum compared to other periods of study indicates increased oxidative stress during this period (Sharma *et al*., 2011) [33].

4.1.2 Blood Superoxide dismutase

SOD is the antioxidant enzyme that catalyses the reaction of the highly reactive $O_2^{\bullet-}$ (superoxide anion) to O_2 and to the less reactive H_2O_2 and peroxide which can be further destroyed by catalase or GSH-Px reactions (Fridovich, 1995) [15]. In the current study, there was no significant difference in blood SOD values due to different physiological states of transition period or due to different treatments (Sharma *et al*.,

2011) [33]. But, Bernabucci *et al*. (2005) [3] reported that they could observe a progressively increasing blood SOD values from three weeks pre-partum to four days post-partum.
We couldn't observe any significant difference in blood SOD values during transition period with supplementation of either Se @ 0.3 ppm/day/head or with vitamin @ 1000 IU/day/head. Similar results were reported by Bicalho *et al*. (2014) [4]. Whereas, Machado *et al*. (2014) [23] reported that there was increase in SOD activities during entire transition period when supplemented with trace minerals like zinc, copper, manganese and selenium. The increase in blood SOD values during transition period in supplementation studies could be attributed to additional benefits provided by zinc, manganese and copper as all these are required co-factors for SOD.

4.1.3 Serum Catalase
Catalase convert hydrogen peroxide formed inside cells to water and oxygen. Catalase was found to be 14 times less potent than GSH-Px in protecting human fibroblasts against oxidative stress (Michiels *et al*. 1994) [25]. Wichtel (1998) [41] observed that even though cytosolic GSH-Px was considered as one of the essential antioxidant system, catalase played an important role in adaptive response to oxidative stress. It has got a complementary role with GSH-Px in counteracting the oxidative stress imposed on by organic hydroperoxides (Sunde and Hoekstra, 1980) [38].
In the current study, when analysed between groups, significant difference in serum catalase activity could be observed only on 30 days post-partum. G_0 animals were having significantly high catalase activity during 30 days post-partum. This could be due to the adaptive nature of catalase, which activity increased in unsupplemented animals during periods of extreme stress.
On within group between periods analysis there was no significant difference in serum catalase activity. This was in accordance with Sharma *et al*. (2011) [33]. In the earlier discussion on GSH-Px, the reduced level of GSH-Px on 30 days post-partum for control animals was thought to be due to two reasons: either due to the utilisation of Se for the process of milk secretion or due to excess utilisation of GSH-Px for counteracting increased oxidative stress. The result of catalase reinforces the above conclusion. This is in accordance with the findings of House and Bell (1994) [18]. So for giving the antioxidant support catalase has to be increased (Mates *et al*., 1999) [24]. Whereas in vitamin E supplemented groups (G2 and G3) due to the GSH-Px sparing activity of vitamin E, there was reduced catalase activity.

4.1.4 Serum Malonaldehyde
The serum malondialdehyde (MDA) levels on 30 days post-partum were significantly low for G_2 (vitamin E @ 1000 IU/day) animals compared to G_0 and G_1 animals. Doni *et al*. (1984) [10] in their study on rats fed with diets deficient in vitamin E and/or Se, they found a significantly increased heart, kidney and platelet MDA after three month deficient diet. They concluded that vitamin E was the best antioxidant that reduces lipid peroxidation from the finding that the rise in MDA levels was high for vitamin E deficient diet fed rats compared to Se deficient diet fed rats. Kumaraguruparan *et al*. (2002) [22] conducted a study on human patients with fibroadenoma of breast and they reported that estimation of lipid peroxides provides an estimate of extent of oxidative damage.
In the present study, it was found that though Se was an antioxidant capable of preventing lipid peroxidation, vitamin E was the best antioxidant with respect to protective effect against lipid peroxidation. No added advantage was there when these two antioxidants are given in combination before calving. The steady increase in the MDA level in control animals, indicated that the level of oxidative stress gradually increases from 220 days of pregnancy to day of calving and 30day post-partum. Similar results were reported by Castillo *et al*. (2005, 2006) [8, 9] that in dairy cows, plasma MDA increased around parturition with highest value one week after parturition. Sharma *et al*. (2011) [33] reported that MDA levels were significantly high in early lactation cows compared to those in advanced pregnancy. According to Saikumar *et al*. (2013) [31] and Konvicna *et al*. (2015) [20], the significantly high level of lipid peroxidation during immediate post-partum was suggestive of increased oxidative stress in that period.
The increasing trend of MDA during transition period was also observed within supplemented groups. But the increase in vitamin E supplemented group was much less compared to control or Se supplemented groups. Gong *et al*. (2014) [16] could observe a significant reduction in serum MDA levels in organic Se supplemented dairy cows indicating the better effect of organic Se in alleviating oxidative stress. It was also observed that, even after supplementation with most effective antioxidant vitamin E, the lipid peroxidation could not be reduced to the initial value of the experiment. Further studies are needed with increased vitamin E supplementation at different levels to assess whether the values can be brought back to initial levels. The study revealed that vitamin E is the better antioxidant in alleviating the ill effects of stress induced lipid peroxidation.

4.2 Hematological parameters
4.2.1 Volume of packed red cells
There was no significant difference in volume of packed red cells (VPRC) due to different physiological states associated with transition period in dairy cattle. According to Ate *et al*. (2009) [2] there was no significant difference in VPRC between advanced pregnant and early lactating dairy cows. But, Nazifi *et al*. (2008) [27] reported that VPRC were significantly higher in pregnant cows compared to post-partum results.
In the present study, there was significant (p<0.05) difference in VPRC on the day of calving between different treatment groups with highest value for G3 animals. The VPRC of G_3 animals was significantly (p<0.05) high compared to G_0 and G_2 animals. The high VPRC on the day of calving for G_3 animals might be due to the complementary hemopoetic activity of vitamin E and Se during this period.
4.2.2 Total Erythrocyte Count
On 250 days of pregnancy, day of calving and on 30 days post-partum, G_3 animals were having significantly high TEC compared to G_0, G_1 and G_2 animals, suggesting a positive effect of supplementation with both vitamin E (1000 IU/day) and Se (0.3 ppm/day) on TEC of dairy cattle. It could be due to membrane protective effect of vitamin E and better antioxidant protection provided by high GSH-Px activity through Se supplementation, which might have reduced the rate of hemolysis (Fibach and Rachmilewitz, 2008) [13]. The increased concentration of malonaldehyde seen during transition period indicates the intensity of oxidative stress and cell membrane damage during this period (Castillo *et al*., 2005; Sharma *et al*., 2011) [8, 33].

On group wise analysis, there was no significant difference in mean TEC values in G_0. Similar results were reported by Ate *et al*. (2009) [2] that there was no significant difference in TEC between animals in advanced pregnancy and early lactation. In G_1 and G_3 animals, there was significant increase in mean TEC during early lactation compared to advanced gestation, suggesting supporting role of Se and Se + vitamin E supplementation during increased oxidative stress (Necheles *et al*., 1968; Brownlee *et al*., 1977) [28, 5].

It can be inferred that Se is having a significantly high hemopoetic effect from 250 days of pregnancy to 30 days post-partum. Vitamin E is also playing a complementary role. At the same time vitamin E alone is not capable of producing significant impact. This can probably be due to the low level of vitamin E supplementation.

4.2.3 Hemoglobin Concentration

No significant difference in hemoglobin (Hb) concentration between group or between period was noticed. This was in accordance with the findings of Ate *et al*. (2009) [2], that there was no significant difference in Hb concentration between animals in advanced pregnancy and early lactation. But, Patel *et al*. (2017) [30] reported that there was significant increase in Hb concentration from 30 days pre-partum to day of calving and decreased during post-partum. The statistically non significant difference in the Hb concentration implicated that, supplementation of Se and vitamin E would not improve the Hb status of transition dairy cows.

4.2.4 Total Leucocyte Count

There was no significant difference in TLC between animals in advanced pregnancy and early lactation (Ate *et al*., 2009) [2] and between supplemented and non-supplemented animals. Calamari *et al*. (2011) [7] observed that the TLC were not affected by Se supplementation at 0.31 or 0.50 mg/kg dry matter. From the current study we could found that there was no significant effect for Se, vitamin E or Se + vitamin E supplementation on TLC of transition dairy cattle. Slightly higher value of TLC found on the day of calving might be due to the inflammatory changes associated with calving.

4.3 Serum Biochemical Parameters

4.3.1 Total Protein

Total protein concentration was significantly (p<0.01) low for G_3 on the day of calving. This could be due to significantly low serum globulin concentration (Table 11) found on the day of calving. Up to 80 per cent increase in secreted immunoglobulin levels was noted in colostrum in buffaloes supplemented with antioxidant vitamins one-month pre-partum (Sikka *et al*., 2002, 2006) [35, 36]. There was no significance difference in serum total protein concentration between Se supplemented and non-supplemented groups (El-Shahat and Abdel-Monem, 2011) [11]. No significant difference in total serum protein concentration was found between different periods of study among supplemented or control groups (Ate *et al*., 2009) [2].

4.3.2 Serum Albumin

In the current study, there was no significant difference in mean serum albumin values between different groups or periods (El-Shahat and Abdel-Monem, 2011) [11]. Antioxidant supplementation (zinc) didn't made any significant difference in serum albumin levels in dairy cows (Sobhanirad and Naserian, 2012) [36]. Contradictory results were reported by Hall *et al*. (2014) [17] that serum albumin concentration in dairy cows were improved significantly due to supra-nutritional Se supplementation (0.3 ppm) during transition period. But in a study by El-Shahat and Abdel-Monem, (2011) [11] serum albumin was significantly high in vitamin E supplemented group of ewes.

4.3.3 Serum Globulin

G_3 animals were having significantly low globulin concentration on the day of calving compared to other experimental groups. Sikka *et al*. (2002, 2006) [35, 36] reported that there was up to 80 per cent increase in secreted immunoglobulin levels in colostrum in buffaloes supplemented with antioxidant vitamins one month pre-partum when compared to control. This could be the reason for low serum globulin in dairy cows on the day of calving. No significant difference in mean serum Globulin concentration between different periods in any of the experimental groups.

4.3.4 Albumin Globulin Ratio

In the present study there was no significant difference in albumin: globulin ratio between different groups or between different periods. This was in accordance with Kumar *et al*. (2009) [21] that there was no significant difference in albumin : globulin ratio between Se supplemented and non supplemented lambs.

5. Conclusion

Oxidative stress is an inevitable condition during transition period. Antioxidant supplementation is the most widely used and studied method to alleviate it. In our study, we selected most studied antioxidants and their dosages to find their effect on our dairy cows. Results found to reduce oxidative stress in terms of MDA, but hard to conclude as antioxidant enzymes showed variations in values suggestive of various other factors might have affected their concentrations in physiological conditions. In our opinion, studies need to be extended to seasonal/regional variations in antioxidant concentrations in transition dairy cows and standardisation of local factors (physiological and environmental) for developing regional strategies for antioxidant supplementation.

6. Acknowledgements

We acknowledge Kerala Veterinary and Animal Sciences University, Pookode, Wayanad for the financial support and the infrastructure provided for the research work.

7. References

1. Abuelo A, Hernandez J, Benedito JL, Castillo C. The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. J Anim. Physiol. Anim. Nutr. 2015; 99:1003-1016.
2. Ate IU, Rekwot PI, Nok AJ, Tekdek LB. Haematological values of cows during third trimester of pregnancy and early lactation in settled cattle herds in Zaria, Northern Nigeria. Afr. J Biomed. Res. 2009; 12:225-231.
3. Bernabucci U, Ronchi B, Lacetera N, Nardone A. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J Dairy Sci. 2005; 88:2017-2026.

4. Bicalho MLS, Lima FS, Ganda EK, Foditsch C, Meira EBS, Machado VS *et al*. Effect of trace mineral supplementation on selected minerals, energy metabolites, oxidative stress and immune parameters and its association with uterine diseases in dairy cattle. J Dairy Sci. 2014; 97:1-15.
5. Brownlee NR, Huttner JJ, Panganamala RV, Cornwell DG. Role of vitamin E in glutathione-induced oxidant stress: methemoglobin, lipid peroxidation, and hemolysis. J Lipid Res. 1977; 18:635-644.
6. Calamari L, Petrera F, Abeni F, Bertin G. Effects of either sodium selenite or Se yeast (Sc CNCM 1-3060) supplementation on selenium status and milk characteristics in dairy cows. Livest. Sci. 2010; 128:154-165.
7. Calamari L, Petrera F, Abeni F, Bertin G. Metabolic and haematological profiles in heat stressed lactating dairy cows fed diets supplemented with different selenium sources and doses. Livest. Sci. 2011; 142:128-137.
8. Castillo C, Hernandez J, Bravo A, Lopez-Alonso M, Pereira V, Benedito JL. Oxidative status during late pregnancy and early lactation in dairy cows. Vet. J. 2005; 169:286-292.
9. Castillo C, Hernandez J, Valverde I, Pereira V, Sotillo J, Lopez-Alonzo M *et al*. Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Res. Vet. Sci. 2006; 80:133-139.
10. Doni MG, Falanga A, Delaini F, Vicenzi E, Tomasiak M, Donati MB. The effect of vitamin E or selenium on the oxidant antioxidant balance in rats. Br. J Exp. Path. 1984; 65:75-80.
11. El-Shahat KH, Abdel-Monem UM. Effect of dietary supplementation with vitamin E and/or selenium on metabolic and reproductive performance of Egyptian Baladi ewes under subtropical conditions. World Appl. Sci. J. 2011; 12:1492-1499.
12. Esposito G, Irons PC, Webb EC, Chapwanya A. Interaction between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim. Reprod. Sci. 2014; 144:60-71.
13. Fibach E, Rachmilewits E. The role of oxidative stress in hemolytic anemia. Curr. Mol. Med. 2008; 7:609-619.
14. Flohe L, Gunzler WA, Schock HH. Glutathione peroxidise: A selenoenzyme. FEBS. J. 1973; 32:132-134.
15. Fridovich I. Superoxide radical and superoxide dismutases. Annu. Rev. Biochem. 1995; 64:97-112.
16. Gong J, Ni J, Wang D, Shi B, Yan S. Effect of dietary organic selenium on milk selenium concentration and antioxidant and immune status in mid lactation dairy cows. Livest. Sci. 2014; 170:84-90.
17. Hall JA, Bobe G, Vorachek WR, Kasper K, Traber MG, Mosher WD *et al*. Effect of supranutritional organic selenium supplementation on post-partum blood micronutrients, antioxidants, metabolites and inflammation biomarkers in selenium replete dairy cows. Biol. Trace Elem. Res. 2014; 161:272-287.
18. House WA, Bell AW. Sulfur and selenium accretion in the gravid uterus during late gestation in Holstein cows. J Dairy Sci. 1994; 77:1860-1869.
19. Kendall NR, Mackenzie AM, Telfer SB. The trace element and humoral immune response of lambs administered a zinc, cobalt and selenium soluble glass bolus. Livest. Sci. 2012; 148:81-86.
20. Konvicna J, Vargova M, Paulikova I, Kovac G, Kostecka Z. Oxidative stress and antioxidant status in dairy cows during prepartal and postpartal periods. Acta. vet. BRNO. 2015; 84:133-140.
21. Kumar N, Garg AK, Dass RS, Chaturvedi VK, Mudgal V, Varshney VP. Selenium supplementation influences growth performance, antioxidant status and immune response in lambs. Anim. Feed Sci. Technol. 2009; 153:77-87.
22. Kumaraguruparan R, Subapriya R, Kabalimoorthy J, Nagini S. Antioxidant profile in the circulation of patient with fibroadenoma and adenocarcinoma of the breast. Clin. Biochem. 2002; 35:275-279.
23. Machado VS, Oikonomou G, Lima SF, Bicalho MLS, Kacar C, Foditsch C *et al*. The effect of injectable trace minerals (selenium, copper, zinc and manganese) on peripheral blood leukocyte activity and serum superoxide dismutase activity of lactating Holstein cows. Vet. J. 2014; 200:299-304.
24. Mates JM, Gomez CP, De Castro IN. Antioxidant enzymes and human diseases. J Clin. Biochem. 1999, 2014; 32:595-603.
25. Michiels C, Raes M, Toussaint O, Remacle J. Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free Radic. Biol. Med. 1994; 17:235-248.
26. Mills GC. Hemoglobin catabolism I: glutathione peroxidase an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. J Biol. Chem. 1957; 229:189-197.
27. Nazifi S, Ahmadi MR, Gheisari HR. Haematological changes of dairy cows in post partum period and early pregnancy. Comp. Clin. Pathol. 2008; 17:157-163.
28. Necheles TF, Boles TA, Allen DM. Erythrocyte glutathione-peroxidase deficiency and hemolytic disease of the new born infant. J Pedstr. 1968; 72:319-324.
29. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab. Clin. Med. 1967; 70:158-169.
30. Patel B, Kumar N, Jain V, Kumar F, Ajithkumar HM, Naik MA *et al*. Haematological status of Karan Fries cows during transition period in hot humid condition. Int. J Sci. Environ. Technol. 2017; 6:793-797.
31. Saikumar P, Jaya MR, Renuka MRD. Oxidative stress in pregnancy. IOSR-JDMS. 2013; 3:12-13.
32. Schalm OW, Jain NC, Carroll EJ. Veterinary Haematology. (4th Ed.). Lea and Febiger, Philadelphia, 1986, 45-48.
33. Sharma N, Singh NK, Singh OP, Pandey V, Verma PK. Oxidative stress and antioxidant status during transition period in dairy cows. Asian-Aust. J Anim. Sci. 2011; 24:479-484.
34. Sikka P, Lal D. Studies on vitamin mineral interactions in relation to passive transfer of immunoglobulins in buffalo calves. Asian-Aust. J Anim. Sci. 2006; 19:825-830.
35. Sikka P, Lal D, Arora U, Sethi RK. Growth and passive immunity in response to micronutrient supplementation in new born calves of Murrah buffaloes given fat soluble vitamins during late pregnancy. Livest. Prod. Sci. 2002; 75:301-311.
36. Sobhanirad S, Naserian AA. Effects of high dietary zinc concentration and zinc sources on hematology and biochemistry of blood serum on Holstein dairy cows. Anim. Feed Sci. Technol. 2012; 177:242-246.

37. Sordillo LM, Aitken SL. Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. And Immunopathol. 2009; 128:104-109.
38. Sunde RA, Hoekstra WG. Structure, synthesis and function of glutathione peroxidase. Nutr. Rev. 1980; 38:265-273.
39. Teixeira AGV, Lima FS, Bicalho MLS, Kussler A, Lima SF, Felippe MJ, Bicalho RC. Effect of an injectable trace mineral supplement containing selenium, copper, zinc and manganese on immunity, health and growth of dairy calves. J Dairy Sci. 2014; 97:1-11.
40. Umesh D, Rakesh R, Mahesh CS, Varshney VP. Effect of vitamin E and selenium supplementation on oxidative stress indices and cortisol level in blood in water buffaloes during pregnancy and early postpartum period. Trop. Anim. Health Prod. 2010; 42:405-410.
41. Wichtel JJ. A review of selenium deficiency in grazing ruminants, part 1: new roles for selenium in ruminant metabolism. N. Z. Vet. J. 1998; 46:47-52.
42. Yagi K. Assay for blood plasma or serum. Methods Enzymol. 1984; 105:328-331.