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

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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, G0 (control), G1 (selenium @ 0.3 ppm/kg DM/day), G2 (vitamin E @ 1000 IU/day) and G3 (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-TEC, total erythrocyte count-TLC and serum biochemical parameters (total protein, albumin, globulin and albumin: globulin ratio) were estimated. We observed oxidative stress in G0 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) [11]. 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 suffers from oxidative stress (Abuelo et al., 2015) [11]. 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 G₀ (control), G₁ (selenium @ 0.3 ppm/kg DM/day), G₂ (vitamin E @ 1000 IU/day) and G₃ (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°C 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 -50°C until analysis. Glutathione peroxidase activity was determined photometrically in semiautomatic biochemical 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 -50°C until analysis. Blood superoxide dismutase activity was determined photometrically in semiautomatic biochemical 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 -50°C 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) [32].

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 G₁ (0.3 ppm Se) had significantly (P<0.05) higher GSH-Px activity compared to G₀ (control) and G₃ (1000 IU vitamin E). G₃ (selenium @ 0.3 ppm/kg DM + vitamin E @ 1000 IU per day) significantly differed from G₂ (1000 IU vitamin E) but not from G₁ (0.3 ppm Se).

On 30 day post-partum, blood GSH-Px values were significantly (P<0.05) high in G₃ animals compared to G₀ and G₂ animals and there was no significant difference between G₁ and G₃ animals.

When analysed within group between periods, significant difference in blood GSH-Px activity was found only on G₀ (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 G₀ (control) and G₁ (Se @ 0.3 ppm + vitamin E @ 1000 IU/day) animals. In G₁ and G₂ the average blood SOD activities were high in all the experimental periods compared to G₀. In G₂ (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)**

<table>
<thead>
<tr>
<th>Group</th>
<th>220th day</th>
<th>250th day</th>
<th>Day of calving</th>
<th>30 day post-partum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₀</td>
<td>314.33 ± 57.29</td>
<td>360.33 ± 20.99</td>
<td>409.43 ± 38.13</td>
<td>413.07 ± 72.81</td>
<td>0.447</td>
</tr>
<tr>
<td>G₁</td>
<td>431.85 ± 76.16</td>
<td>388.53 ± 46.73</td>
<td>415.68 ± 70.84</td>
<td>459.90 ± 60.82</td>
<td>0.878</td>
</tr>
<tr>
<td>G₂</td>
<td>291.2 ± 45.02</td>
<td>399.23 ± 40.92</td>
<td>429.78 ± 79.33</td>
<td>331.80 ± 35.56</td>
<td>0.090</td>
</tr>
<tr>
<td>G₃</td>
<td>466.83 ± 90.43</td>
<td>502.70 ± 68.96</td>
<td>528.67 ± 54.09</td>
<td>567.73 ± 92.34</td>
<td>0.554</td>
</tr>
<tr>
<td>P value</td>
<td>0.234</td>
<td>0.197</td>
<td>0.511</td>
<td>0.137</td>
<td></td>
</tr>
</tbody>
</table>

G₀- Control, G₁- Se supplemented group, G₂- vitamin E supplemented group, G₃- 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 G₀ having significantly high serum catalase activity compared to G₁ and G₂. There was no significant difference between G₀ and G₁ and G₁ was significantly high compared to G₃. 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)**

<table>
<thead>
<tr>
<th>Group</th>
<th>220th day</th>
<th>250th day</th>
<th>Day of calving</th>
<th>30 day post-partum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₀</td>
<td>11.196 ± 2.599</td>
<td>9.129 ± 1.809</td>
<td>6.406 ± 0.760</td>
<td>9.149 ± 1.795</td>
<td>0.073</td>
</tr>
<tr>
<td>G₁</td>
<td>7.752 ± 2.843</td>
<td>5.605 ± 1.028</td>
<td>6.852 ± 0.948</td>
<td>7.98 ± 0.836abc</td>
<td>0.636</td>
</tr>
<tr>
<td>G₂</td>
<td>10.815 ± 3.985</td>
<td>8.364 ± 1.482</td>
<td>5.927 ± 1.251</td>
<td>4.618 ± 0.781bcd</td>
<td>0.054</td>
</tr>
<tr>
<td>G₃</td>
<td>6.073 ± 1.546</td>
<td>5.803 ± 1.734</td>
<td>3.565 ± 0.487</td>
<td>4.013 ± 1.030abc</td>
<td>0.295</td>
</tr>
<tr>
<td>P value</td>
<td>0.445</td>
<td>0.290</td>
<td>0.079</td>
<td>0.014</td>
<td></td>
</tr>
</tbody>
</table>

G₀- Control, G₁- Se supplemented group, G₂- vitamin E supplemented group, G₃- 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 G₀ (control) compared to G₁ (0.3 ppm Se) and G₂ (1000 IU vitamin E). The mean serum MDA of G₁ (0.3 ppm Se) was significantly high compared to G₂ (1000 IU vitamin E).

When analysed within group between periods, there was significant difference in serum MDA values in G₀ (control) and G₁ (p<0.05). In G₀, 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 G₀. In G₁ animals, similar pattern was observed except that the 30th day results were not significantly high from day of calving results. In G₁ and G₂ 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)**

<table>
<thead>
<tr>
<th>Group</th>
<th>220th day</th>
<th>250th day</th>
<th>Day of calving</th>
<th>30 day post-partum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₀</td>
<td>1.90 ± 0.31A</td>
<td>5.41 ± 1.4AB</td>
<td>6.45 ± 0.80B</td>
<td>13.33 ± 1.82AB</td>
<td>0.032</td>
</tr>
<tr>
<td>G₁</td>
<td>3.16 ± 0.99</td>
<td>7.8 ± 3.01</td>
<td>8.15 ± 2.69</td>
<td>9.06 ± 1.74B</td>
<td>0.053</td>
</tr>
<tr>
<td>G₂</td>
<td>1.65 ± 0.34</td>
<td>4.26 ± 2.13</td>
<td>3.00 ± 0.48</td>
<td>4.00 ± 0.52B</td>
<td>0.370</td>
</tr>
<tr>
<td>G₃</td>
<td>1.45 ± 0.32A</td>
<td>3.55 ± 1.13AB</td>
<td>3.63 ± 0.48B</td>
<td>5.46 ± 0.84ABC</td>
<td>0.016</td>
</tr>
<tr>
<td>P value</td>
<td>0.173</td>
<td>0.497</td>
<td>0.067</td>
<td>0.008</td>
<td></td>
</tr>
</tbody>
</table>

G₀- Control, G₁- Se supplemented group, G₂- vitamin E supplemented group, G₃- Se and vitamin E supplemented group

**A**, **B**, **C**, **D**, **E**- 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.
G₀: Control, G₁: Se supplemented group, G₂: vitamin E supplemented group, G₃: 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. G₃ animals were having significantly high mean VPRC values compared to G₀ and G₂. However, there was no significant difference between G₁ and G₃. No significant difference was observed between G₀, G₁ and G₂. 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)

<table>
<thead>
<tr>
<th>Group</th>
<th>220th day</th>
<th>250th day</th>
<th>Day of calving</th>
<th>30day post-partum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₀</td>
<td>32.50 ± 1.38</td>
<td>30.83 ± 1.54</td>
<td>29.33 ± 1.31</td>
<td>30.83 ± 1.08</td>
<td>0.229</td>
</tr>
<tr>
<td>G₁</td>
<td>30.67 ± 2.55</td>
<td>31.00 ± 1.13</td>
<td>31.50 ± 1.20</td>
<td>32.17 ± 0.95</td>
<td>0.845</td>
</tr>
<tr>
<td>G₂</td>
<td>29.83 ± 2.07</td>
<td>30.33 ± 1.89</td>
<td>30.67 ± 1.67</td>
<td>32.33 ± 1.71</td>
<td>0.462</td>
</tr>
<tr>
<td>G₃</td>
<td>34.83 ± 1.49</td>
<td>35.00 ± 1.21</td>
<td>35.17 ± 0.79</td>
<td>35.83 ± 1.01</td>
<td>0.799</td>
</tr>
</tbody>
</table>

P value:
- **: significant at 0.01 level,
- *: significant at 0.05 level,
- #: significant at 0.1 level,
- ~ : significant at 0.1 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, G₃ were having significantly (p<0.05) high TEC compared to G₀, G₁ and G₂. There was no significant difference between G₀, G₁ and G₂. On day of calving and on 30 days post-partum, G₃ was having significantly (p<0.01) high TEC compared to G₀, G₁ and G₂. No significant difference was noted between G₀, G₁ and G₂.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>220th day</th>
<th>250th day</th>
<th>Day of calving</th>
<th>30day post-partum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₀</td>
<td>4.92 ± 0.44</td>
<td>4.52 ± 0.31</td>
<td>4.15 ± 0.17</td>
<td>4.60 ± 0.22</td>
<td>0.284</td>
</tr>
<tr>
<td>G₁</td>
<td>5.17 ± 0.41</td>
<td>4.48 ± 0.22</td>
<td>4.23 ± 0.34</td>
<td>5.25 ± 0.28</td>
<td>0.028</td>
</tr>
<tr>
<td>G₂</td>
<td>4.2 ± 0.51</td>
<td>4.52 ± 0.44</td>
<td>4.52 ± 0.31</td>
<td>5.03 ± 0.23</td>
<td>0.446</td>
</tr>
<tr>
<td>G₃</td>
<td>5.68 ± 0.46</td>
<td>5.78 ± 0.37</td>
<td>5.77 ± 0.39</td>
<td>6.23 ± 0.32</td>
<td>0.020</td>
</tr>
</tbody>
</table>

P value:
- **: significant at 1% level,
- *: significant at 5% level,
- #: significant at 10% level, and means with same lower case as superscripts have no significant difference between the groups;
- #: significant at 1% level, and 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 G₀ and G₂. In G₁, mean TEC during 30th day post-partum were significantly high compared to 250th day of pregnancy and day of calving results. TEC on 220th 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 G₃, 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.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>220th day</th>
<th>250th day</th>
<th>Day of calving</th>
<th>30day post-partum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₀</td>
<td>9.92 ± 0.39</td>
<td>9.52 ± 0.46</td>
<td>9.33 ± 0.36</td>
<td>9.48 ± 0.37</td>
<td>0.493</td>
</tr>
<tr>
<td>G₁</td>
<td>9.85 ± 0.69</td>
<td>9.67 ± 0.35</td>
<td>9.37 ± 0.28</td>
<td>9.78 ± 0.28</td>
<td>0.628</td>
</tr>
<tr>
<td>G₂</td>
<td>9.22 ± 0.52</td>
<td>9.15 ± 0.57</td>
<td>9.03 ± 0.82</td>
<td>9.17 ± 0.67</td>
<td>0.885</td>
</tr>
<tr>
<td>G₃</td>
<td>10.95 ± 0.49</td>
<td>10.75 ± 0.65</td>
<td>10.87 ± 0.65</td>
<td>10.89 ± 0.40</td>
<td>0.965</td>
</tr>
<tr>
<td>P value</td>
<td>0.179</td>
<td>0.188</td>
<td>0.134</td>
<td>0.072</td>
<td></td>
</tr>
</tbody>
</table>

G₀- Control, G₁- Se supplemented group, G₂- vitamin E supplemented group, G₃- Se and vitamin E supplemented group

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 8: Total leucocyte count (mean ± SE, n=6) G₀- Control, G₁- Se supplemented group, G₂- vitamin E supplemented group, G₃- Se and vitamin E supplemented group

<table>
<thead>
<tr>
<th>Group</th>
<th>220th day</th>
<th>250th day</th>
<th>Day of calving</th>
<th>30day post-partum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₀</td>
<td>11033.3 ± 878.9</td>
<td>9716.67 ± 907.9</td>
<td>11300.0 ± 371.48</td>
<td>10966.7 ± 483.51</td>
<td>0.108</td>
</tr>
<tr>
<td>G₁</td>
<td>9530.0 ± 715.43</td>
<td>9166.67 ± 389.39</td>
<td>10930.0 ± 328.38</td>
<td>10350.0 ± 261.73</td>
<td>0.083</td>
</tr>
<tr>
<td>G₂</td>
<td>9130.0 ± 649.49</td>
<td>9233.33 ± 294.00</td>
<td>10533.3 ± 333.33</td>
<td>10100.0 ± 265.83</td>
<td>0.097</td>
</tr>
<tr>
<td>G₃</td>
<td>8216.67 ± 810.5</td>
<td>9250.0 ± 388.80</td>
<td>10033.3 ± 537.07</td>
<td>9400.0 ± 933.70</td>
<td>0.098</td>
</tr>
<tr>
<td>P value</td>
<td>0.106</td>
<td>0.888</td>
<td>0.171</td>
<td>0.054</td>
<td></td>
</tr>
</tbody>
</table>

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. G₀ and G₁ animals were having significantly high total protein concentration compared to G₂ animals. Total protein concentration of G₃ 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)

<table>
<thead>
<tr>
<th>Group</th>
<th>220th day</th>
<th>250th day</th>
<th>Day of calving</th>
<th>30day post-partum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₀</td>
<td>7.95 ± 0.57</td>
<td>8.57 ± 0.39</td>
<td>8.59* ± 0.33</td>
<td>8.29 ± 0.44</td>
<td>0.510</td>
</tr>
<tr>
<td>G₁</td>
<td>8.25 ± 0.42</td>
<td>7.79 ± 0.35</td>
<td>8.51* ± 0.19</td>
<td>8.12 ± 0.48</td>
<td>0.618</td>
</tr>
<tr>
<td>G₂</td>
<td>7.88 ± 0.17</td>
<td>8.78 ± 0.46</td>
<td>8.02* ± 0.26</td>
<td>7.84 ± 0.33</td>
<td>0.173</td>
</tr>
<tr>
<td>G₃</td>
<td>8.12 ± 0.27</td>
<td>8.01 ± 0.36</td>
<td>7.18* ± 0.35</td>
<td>7.41 ± 0.33</td>
<td>0.215</td>
</tr>
<tr>
<td>P value</td>
<td>0.902</td>
<td>0.271</td>
<td>0.009*</td>
<td>0.457</td>
<td></td>
</tr>
</tbody>
</table>

G₀- Control, G₁- Se supplemented group, G₂- vitamin E supplemented group, G₃- 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)

<table>
<thead>
<tr>
<th>Group</th>
<th>220th day</th>
<th>250th day</th>
<th>Day of calving</th>
<th>30day post-partum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₀</td>
<td>3.76 ± 0.225</td>
<td>3.809 ± 0.125</td>
<td>3.767 ± 0.168</td>
<td>3.807 ± 0.216</td>
<td>0.959</td>
</tr>
<tr>
<td>G₁</td>
<td>3.913 ± 0.121</td>
<td>3.752 ± 0.110</td>
<td>4.258 ± 0.178</td>
<td>3.885 ± 0.235</td>
<td>0.300</td>
</tr>
<tr>
<td>G₂</td>
<td>3.768 ± 0.227</td>
<td>3.827 ± 0.084</td>
<td>3.92 ± 0.152</td>
<td>3.836 ± 0.123</td>
<td>0.924</td>
</tr>
<tr>
<td>G₃</td>
<td>3.88 ± 0.085</td>
<td>4.119 ± 0.105</td>
<td>4.219 ± 0.231</td>
<td>3.699 ± 0.121</td>
<td>0.134</td>
</tr>
<tr>
<td>P value</td>
<td>0.897</td>
<td>0.101</td>
<td>0.212</td>
<td>0.904</td>
<td></td>
</tr>
</tbody>
</table>

G₀- Control, G₁- Se supplemented group, G₂- vitamin E supplemented group, G₃- 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. G₁ animals were having significantly (p<0.01) low concentration of serum globulin compared to G₀, G₁ and G₂. No significant difference could be found between G₀, G₁ and G₂. No significant difference in mean serum globulin values could be observed on within group between period analysis.
3.3.4 Albumin Globulin ratio
The mean A:G ratio on 220\textsuperscript{th} & 250\textsuperscript{th} day of pregnancy, the day of calving and on the 30\textsuperscript{th} 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>
<thead>
<tr>
<th>Group</th>
<th>220\textsuperscript{th} day</th>
<th>250\textsuperscript{th} day</th>
<th>Day of calving</th>
<th>30 day post-partum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G\textsubscript{0}</td>
<td>4.189 ± 0.463</td>
<td>4.761 ± 0.419</td>
<td>4.83 ± 0.417</td>
<td>4.48 ± 0.517</td>
<td>0.467</td>
</tr>
<tr>
<td>G\textsubscript{1}</td>
<td>4.337 ± 0.386</td>
<td>4.039 ± 0.266</td>
<td>4.252 ± 0.174</td>
<td>4.23 ± 0.387</td>
<td>0.917</td>
</tr>
<tr>
<td>G\textsubscript{2}</td>
<td>4.11 ± 0.291</td>
<td>4.949 ± 0.441</td>
<td>4.093 ± 0.342</td>
<td>4.01 ± 0.377</td>
<td>0.232</td>
</tr>
<tr>
<td>G\textsubscript{3}</td>
<td>4.24 ± 0.288</td>
<td>3.889 ± 0.402</td>
<td>2.963 ± 0.409</td>
<td>3.714 ± 0.282</td>
<td>0.164</td>
</tr>
</tbody>
</table>

P value: 0.009
g-

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; Konvicina 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 30\textsuperscript{th} day post-partum. This indicated that initiation of lactation and tissue repair related to purpuraemum 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\textsubscript{1} and G\textsubscript{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\textsubscript{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\textsubscript{0} animals on 30\textsuperscript{th} 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\textsubscript{2}\textsuperscript{−} (superoxide anion) to O\textsubscript{2} and to the less reactive H\textsubscript{2}O\textsubscript{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) \[33\].
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) [21] reported that there was an 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) [30].

In the current study, when analysed between groups, significant difference in serum catalase activity could be observed only on 30 days post-partum. G0 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) [34]. 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 malonaldehyde (MDA) levels on 30 days post-partum were significantly low for G2 (vitamin E @ 1000 IU/day) animals compared to G0 and G1 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 Konvienca 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, Nazif et al. (2008) [23] 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 G3 animals was significantly (p<0.05) high compared to G0 and G2 animals. The high VPRC on the day of calving for G3 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, G3 animals were having significantly high TEC compared to G0, G1 and G2 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 G3. 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 G1 and G3 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, 3]. 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) [40] 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) [41] 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 G3 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 make any significant difference in serum albumin levels in dairy cows (Sobhaniarad 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 supranutritional 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
G3 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) [23] 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
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7. References


