EFFECTS OF ANTIOXIDANTS ON GROWTH AND TOTAL ANTIOXIDANT CAPACITY OF NEONATE CALVES

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Abstract
Micronutrients support immune functions and are essential for optimized health and production of animals. This study was conducted to evaluate the effect of vitamin E and vitamin C, alone or in combinations, on growth and antioxidant capacity of neonate dairy calves. For this purpose, eighteen female Holstein Friesian calves were selected. Experimental treatments were; T0 or control (milk without vitamin supplementation), T1 (milk supplemented with 250mg of vitamin E), T2 (milk supplemented with 500mg of vitamin E), T3 (milk supplemented with 1000mg of vitamin C), T4 (milk supplemented with 250mg of vitamin E and 1000mg of vitamin C) and T5 (milk supplemented with 500mg of vitamin E and 1000mg of vitamin C). Experimental treatments were started from the third day till 60th days of the calf’s life. Blood was collected in clot-activator tubes for TAC at day 01, 30 and 60. Body measurements were noted during 1st, 3rd, 6th and 9th week of this trial. Results showed that the TAC was significantly high in the T1 group during the first month. At the end of the trial (day 60), TAC were highest in T0 and T1 comparatively. The wither height, heart girth and body length were significant in supplemented groups compared to control. In conclusion, it is asserted that the supplementations of vitamin E and vitamin C have beneficial effects on growth and antioxidant capacity of neonate calves.

Keywords: Antioxidants, Micronutrients, Neonate calves, Vitamin C, Vitamin E

INTRODUCTION
Oxidative stress is one of the major factors which compromise the immune cell capabilities in dairy calves in the first month of their life (1). Oxidation and the production of free radicals are an integral part of aerobic metabolism. A variety of reactive oxygen species (ROS) are produced by normal metabolic processes and by certain leukocyte populations during defense against disease (2). An imbalance between increased production of ROS and the availability of antioxidant defenses needed to reduce ROS accumulation may expose cows and neonatal calves to increased oxidative stress (3). Antioxidant molecules used to capture these ROS. Micronutrients can maintain immune system functions and nutritive interventions are highly suitable to improve animal health (4).

Ascorbic acid (vitamin C) is an antioxidant which has many diverse biological activities since it influences enzyme activities and numerous cellular functions. It is significantly found in endocrine tissues and leukocytes where it acts as free radical scavenger (5). Vitamin E (α-tocopherol) functions as an essential lipid-soluble antioxidant, scavenging hydroperoxyl radicals in a lipid milieu. Its antioxidant properties play a major role in protecting erythrocyte membranes and nervous tissues (6). Vitamin E has been reported as an...
excellent biological chain-breaking antioxidant that protects cells and tissue from lipoperoxidative damage induced by free radicals. Vitamin C limits the metabolic signs of stress and alleviates the physiological consequences of stress, resulting in improved performance, immunological competence and behavior in animals. The antioxidative property of vitamin E in animal is suggested to have significant role in the development of immune response through protection of the cells, such as lymphocytes, macrophages, and plasma cells from oxidative damages, and enhances the function and proliferation of these cells in face the oxidative stress (7).

Both antioxidant vitamins (vitamin C and vitamin E) have been studied for their effects on plasma level of these vitamins and immunity of dairy calves (8). Although various studies have been conducted on vitamin E and vitamin C for their efficacy on calves’ health, there are no recent studies which have determined the effect of vitamin E and vitamin C on growth and antioxidants capacity of dairy neonates. Therefore, this study was designed to analyze the effect of vitamin E and vitamin C on growth and total antioxidant capacity of neonate calves.

MATERIALS AND METHODS

ANIMALS’ MANAGEMENT

This study was accomplished at Military Dairy Farm, Quetta, Balochistan. The herd was kept in stall-shed housing and had Holstein Friesian cattle. The cows were provided with corn silage, wheat straw, alfalfa and concentrates. After parturition, the calf was weighed and moved to pens. Two kilograms (kg) of dam’s colostrum was fed by bottle during the first hour (h) of calf’s life which was followed by every 12 hours (hr) for the next two days. After that, the herd milk was fed (2kg twice a day) till 60th day of the calves’ life.

EXPERIMENTAL DESIGN

Eighteen neonatal female Holstein Friesian calves were recruited for this study and were distributed randomly into six groups, each group having (n=3) calves. All groups were homogenous for age, sex, month of birth and all calves were healthy. Experimental treatments were T0 or control (milk without vitamin supplementation), T1 (milk supplemented with 250mg of vitamin E), T2 (milk supplemented with 500 mg of vitamin E), T3 (milk supplemented with 1000mg of vitamin C), T4 (milk supplemented with 250 mg of vitamin E and 1000 mg of vitamin C) and T5 (milk supplemented with 500mg of vitamin E and 1000 mg of vitamin C). Experimental treatments were started from the third day till 60th day of the calf’s life. For the determination of total antioxidant capacity, 5 milliliters (ml) blood from jugular were collected in clot-activator tubes, from all calves in the first day of life as day 1 (before any treatment), 30 and 60 and the serum was kept at –20˚C until further analyses. All the experimental procedures were approved by the Ethical Review Committee of the Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan.

EVALUATION OF TOTAL ANTIOXIDANT CAPACITY

Total antioxidant capacity (TAC) was measured by commercial kit “Bovine Total Antioxidant ELISA Kit (lot no. 202008019), Bioassay Technology Lab, England, United Kingdom with BioTek, ELX800 ELISA reader at 450 nm wavelength in u/l.

EVALUATION OF GROWTH PERFORMANCE

Body measurements including wither height, heart girth and body length were noted with measuring tape at day 1 (1st week) then 3rd, 6th and 9th weeks of calf’s life in all experimental calves.

STATISTICAL ANALYSES

All the data were analyzed using SPSS (version 20.0, IBM Corp, Armonk, NY). The differences of TAC and body measurement values between the groups were analyzed by ANOVA. Post hoc Duncan’s multiple range tests was used to compare the measured variables at each sampling time between the
groups. All the data expressed as mean standard error of mean (±SEM) and P value of ≤0.05 was considered as significant.

RESULTS

GROWTH PERFORMANCE

The body measurements of calves were much improved when vitamin E and C provided either alone or in combinations (Table 1). Wither height (wither height, heart girth, body length, back height and waist height) were found significant (P≤0.05) in treatments either supplemented with vitamin E and C alone or in combinations as compared to control from third week onward till end of the trial (9th week). Heart girth was significantly larger in supplemented groups at the end of 3rd week; afterwards at the end of 6th and 9th week, it was found significant in T5 group compared to control. Body length remained unaffected for the first three weeks but significantly improved in vitamins supplemented groups at the end of 6th and 9th weeks of the study as compared to control.

Table I. Effect of dietary supplementation of vitamin E and C, alone or in combinations, on body measurements of dairy calves

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Measurement (week)</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wither height (cm)</td>
<td>01</td>
<td>25.33±1.45</td>
<td>26.67±0.33</td>
<td>25.23±0.39</td>
<td>24.67±0.67</td>
<td>25.30±0.35</td>
<td>26.67±0.33</td>
</tr>
<tr>
<td></td>
<td>03</td>
<td>26.60±1.30</td>
<td>28.60±0.10</td>
<td>25.76±0.12</td>
<td>26.90±0.36</td>
<td>27.30±0.55</td>
<td>28.73±0.17</td>
</tr>
<tr>
<td></td>
<td>06</td>
<td>28.70±1.51</td>
<td>31.23±0.37</td>
<td>30.77±0.09</td>
<td>30.37±0.18</td>
<td>30.50±0.44</td>
<td>32.30±0.12</td>
</tr>
<tr>
<td></td>
<td>09</td>
<td>30.67±1.50</td>
<td>33.72±0.12</td>
<td>33.47±0.03</td>
<td>33.60±0.17</td>
<td>33.53±0.47</td>
<td>33.23±0.09</td>
</tr>
<tr>
<td></td>
<td>01</td>
<td>26.13±0.68</td>
<td>27±0.00</td>
<td>25.83±0.44</td>
<td>25.90±0.38</td>
<td>25.87±0.95</td>
<td>27.33±0.33</td>
</tr>
<tr>
<td>Heart girth (cm)</td>
<td>03</td>
<td>27.53±0.67</td>
<td>28.97±0.12</td>
<td>27.73±0.17</td>
<td>28.10±0.29</td>
<td>27.73±0.83</td>
<td>29.23±0.18</td>
</tr>
<tr>
<td></td>
<td>06</td>
<td>29.43±0.72</td>
<td>31.72±0.23</td>
<td>30.77±0.15</td>
<td>31.20±0.30</td>
<td>31.50±0.71</td>
<td>33.17±0.15</td>
</tr>
<tr>
<td></td>
<td>09</td>
<td>31.47±0.69</td>
<td>34.57±0.20</td>
<td>35.50±0.45</td>
<td>34.00±0.30</td>
<td>34.47±0.62</td>
<td>36.00±0.2</td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>03</td>
<td>27.47±0.79</td>
<td>28.50±0.17</td>
<td>28.40±0.1</td>
<td>29.17±0.7</td>
<td>28.87±0.58</td>
<td>28.43±0.73</td>
</tr>
<tr>
<td></td>
<td>09</td>
<td>31.63±0.87</td>
<td>34.47±0.28</td>
<td>34.47±0.28</td>
<td>35.17±0.37</td>
<td>35.07±0.63</td>
<td>35.47±0.30</td>
</tr>
</tbody>
</table>

T0 (Control), T1 (milk supplemented with 250mg of vitamin E), T2 (milk supplemented with 500mg of vitamin E), T3 (milk supplemented with 1000mg of vitamin C), T4 (milk supplemented with 250mg of vitamin E and 1000mg of vitamin C) and T5 (milk supplemented with 250mg of vitamin E and 2000mg of vitamin C). Different superscripts show significant difference between experimental groups.

TOTAL ANTIOXIDANT CAPACITY

This study finds a positive impact of antioxidant vitamins supplementation on oxidative status of new born cattle calves (Table 2). Total antioxidant capacity (TAC) was high (P≤0.05) in T1 at the age of one month as compared to other treatments and control. During the first month of the trial, TAC was found lowest (P≤0.05) in T5 compared to other treatments and control. In the final sampling (day 60), TAC was significant in T0 as well as in T1, T2 and T5 groups comparatively.

Table II. Effect of dietary supplementation of vitamin E and C, alone or in combinations, on total antioxidant capacity of dairy calves

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sampling (day)</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>01</td>
<td>7.22±0.37</td>
<td>7.23±0.61</td>
<td>6.21±0.14</td>
<td>7.95±1.53</td>
<td>7.33±0.12</td>
<td>6.18±0.14</td>
</tr>
<tr>
<td>antioxidant</td>
<td>03</td>
<td>6.26±0.26</td>
<td>7.48±0.59</td>
<td>5.76±0.31</td>
<td>5.89±0.18</td>
<td>5.62±0.31</td>
<td>4.96±0.03</td>
</tr>
<tr>
<td>capacity</td>
<td>06</td>
<td>6.47±0.24</td>
<td>6.5±0.58</td>
<td>5.87±0.16</td>
<td>5.18±0.03</td>
<td>5.14±0.33</td>
<td>5.43±0.4</td>
</tr>
</tbody>
</table>

T0 (Control), T1 (milk supplemented with 250mg of vitamin E), T2 (milk supplemented with 500mg of vitamin E), T3 (milk supplemented with 1000mg of vitamin C), T4 (milk supplemented with 250mg of vitamin E and 1000mg of vitamin C) and T5 (milk supplemented with 250mg of vitamin E and 2000mg of vitamin C). Different superscripts show significant difference between experimental groups.

DISCUSSION

This study was aimed to evaluate the effect of vitamin E and vitamin C supplementation, alone or in combinations, on growth performance and total-antioxidant capacity of neonatal dairy calves. The combined supplemental vitamin E and C have a better effect on calves in terms of total growth performance and antioxidant capacity.
Dairy calves were unable to mount an effective immune response during their first weeks of life, which contributes to increased disease susceptibility during this period. Oxidative stress (OS) diminishes the immune cell capabilities of humans and adult cows, and dairy calves also experience OS during their first month of life (1). Redox balance reflects the equilibrium between the concentration of pro-oxidants and the availability of antioxidant defenses. Excessive accumulation of pro-oxidants such as reactive oxygen and nitrogen species (RONS) can lead to disruption of cell membrane and damage to proteins, lipids, and DNA in a process known as oxidative stress (OS). There is also evidence in transition dairy cows indicating a negative effect of OS on immune responses (9). Parenteral supplementation of minerals and vitamins with antioxidant effects in a low-stress weaning system prevented the decrease in TAC and GPx activity, improved antibody response and had positive effects on body weight of animals (10).

Vitamin E and vitamin C supplemented groups showed significantly lowered malondialdehyde (MDA) level, higher activities of superoxide dismutase (SOD), catalase (CAT) enzymes and higher Ferric Reducing Antioxidant Power (FRAP) activities in erythrocytes than control in chicken. However, supplementation with a combination of vitamins proved to be better than individual supplementation (7). Vitamin C supplementation lowers the MDA level and increases the TAC and SODS levels in newborn calves (11). Vitamin E improved the antioxidative capacity in goat kids (12). These results are in line with this current study which showed that the total antioxidant capacity was improved by supplementation of vitamin E comparatively to other treatments. Another study suggested that vitamin C treatment had a positive effect on dairy cows and their calves by improving oxidative parameters (11).

This study finds that the vitamin E and vitamin C supplementation have a positive effect on body conformations of new born cattle calves. The wither height, heart girth and body length were much better in vitamin supplemented groups as compared to control. Previous research suggests that vitamin E had a positive effect on body growth of dairy calves (13). Other studies also showed that vitamin C supplementation had improved the weight gain and body growth of cattle calves (14, 15). Similarly, vitamin C supplementation to new born calves improved the health status and growth performance by improving the oxidative status through increasing total antioxidants capacity and lowering the MDA level (11).

**CONCLUSION**

This study concluded that the supplementation of vitamin E and vitamin C have a beneficial effect on newborn dairy calves’ health by improving antioxidants system and body growth. Moreover, the combined supplementation of both antioxidant vitamins has a significant effect on calves’ health and growth performance.

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**Conflict of Interest:**

All the authors report no conflict of interest on this research.

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