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EFFECT OF ANTIOXIDANT SUPPLEMENTATION ON HAEMATOLOGICAL PARAMETERS OF TRANSITION DAIRY CATTLE

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Abstract: In the present study 24 healthy dairy cows belonging to two to four parity, from 220 days of pregnancy were selected and divided randomly into four different groups G0, G1, G2 and G3. G0 animals were kept as control animals. G1animals were supplemented with organic selenium at the rate of 0.3 ppm/kg dry matter. G2 animals were supplemented with vitamin E at the rate of 1000 IU/day. G3 animals were supplemented with organic selenium 0.3 ppm and vitamin E 1000 IU per day. Blood samples were collected on 220, 250 days of pregnancy, on the day of calving and on 30 days post-partum. Data were analysed using SPSS Version 24.0 by two factor ANOVA for repeated measures. No significant difference was observed in haemoglobin concentration and total leucocyte count between different experimental groups or between different experimental periods. But total erythrocyte count was found to be significantly higher for G3 animals on 250 days of pregnancy, day of calving and on 30 days post-partum. Volume of packed red cells were significantly higher for G3 animals on the day of calving compared to G0 animals. Improvement in haematological parameters through supplementation of organic selenium and/or vitamin E indicates alleviation of oxidative stress during transition period.
Keywords: Dairy cows, oxidative stress, transition period, vitamin E, Selenium, haematological parameters.

Introduction

Dairy cows undergo metabolic and physiological alterations during the transition from pregnancy to lactation. Pressures of intensive dairy cattle management practices and genetic selection to maximize milk production have increased the metabolic stresses associated with parturition. So management of dairy cows during the transition period from late gestation to early lactation is of great importance both with respect to the economic concerns of the farmer and also animal welfare concerns. Usually, this period is associated with several health disorders like ketosis, mastitis, metritis, retained fetal membranes, hypocalcemia. Reduction in immune responses during this period predisposes animals to infectious diseases. The major cause for this crisis is the augmented production of free radicals due to the increased metabolic rate due to rapid phase foetal growth, calving and initiation of lactation.

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Whenever the rate of production of free radicals exceed the antioxidant capacity of the animal oxidative stress results, which makes the animal susceptible to diseases.

During pregnancy and lactation, major thrust is given for foetal growth and milk production. In negative energy balance, the health of dam will be compromised for meeting the requirements for foetal growth and then later for lactation. This not only makes the animal more vulnerable to diseases but also adversely affect the reproductive capacity and milk production potential of the cow in its later life. Realising the huge economic impact of this crisis on dairy farming, in the recent past, the measures for assessing and alleviating transition stress have gained the attention of researchers all over the world. Vitamin E and Selenium are the most studied antioxidants in ruminants. Vitamin E (α- tocopherol) is a potent chain breaking, lipid soluble antioxidant which protects biological membranes from oxidative damage. Selenium (Se) is a part of different selenoproteins including glutathione peroxidise, one of the most important antioxidant enzyme. Further studies need to be conducted to confirm what benefits the antioxidant vitamins and minerals supplementation could make on cross bred cattle of Kerala when they are supplemented over and above the required levels. So, in the present study, our objective was to evaluate the health effects of supplementation of antioxidants on transition dairy cows through measurement of haematological parameters.

Materials and Methods

Twenty four crossbred dairy cattle of 220 days of gestation were selected from University Livestock Farm and Fodder Research Development Scheme, College of Veterinary and Animal Sciences, Mannuthy as the experimental animals. All animals were apparently healthy, dewormed before mating and vaccinated routinely against infectious diseases like foot and mouth disease and hemorrhagic septicemia. The experimental animals were housed in different sheds according to stage of pregnancy and lactation. All sheds were scientifically constructed with the facility for individual feeding and provision for *ad libitum* watering. The cows were divided into four groups of six each and were allocated randomly to one of the three dietary treatments, **G0** (adult cattle ration), **G1** (adult cattle ration + 0.3 ppm of organic Se), **G2** (adult cattle ration + 1000 IU Vitamin E) and **G3 (**adult cattle ration + 0.3 ppm of organic Se + 1000 IU Vitamin E). Scientific management practices were carried out throughout the experimental period.

Sample Collection

Blood samples were collected in a vaccutainer containing heparin (75 USP units) on 220 days of pregnancy from all the animals by jugular vein puncture and then on monthly interval till one month postpartum. The blood with anticoagulant was directly used for analysis of haematological parameters.

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

Results and Discussion

Table 1. The results of TEC (millions/µl)

Group	220 d	250 d	DOC	30 d pp	F value	P value
G0	4.92 ± 0.44	4.52 ± 0.31^{a}	4.15 ± 0.17^{a}	4.60 ± 0.22^{a}	1.393^{ns}	0.284
G1	5.17 ± 0.41^{AB}	4.48 ± 0.22^{aA}	4.23 ± 0.34^{aA}	5.25 ± 0.28^{aB}	3.995*	0.028
G2	4.2 ± 0.51	4.52 ± 0.44^{a}	4.52 ± 0.31^{a}	5.03 ± 0.23^{a}	0.940^{ns}	0.446
G3	5.68 ± 0.46^{AB}	5.78 ± 0.37^{bA}	5.77 ± 0.39^{bA}	6.23 ± 0.32^{bB}	4.458*	0.020
F value	1.817^{ns}	3.468*	5.715**	6.839**		
P value	0.177	0.036	0.005	0.002		

ns- non significant, *- significant at 0.05 level, **- significant at 0.01 level

Table 2. The results Hb concentration (%)

Group	220 d	250 d	DOC	30 d pp	F value	P value
G0	9.92 ± 0.393	9.52 ± 0.46	9.33 ± 0.36	9.48 ± 0.37	0.600^{ns}	0.493
G1	9.85 ± 0.69	9.67 ± 0.35	9.37 ± 0.28	9.78 ± 0.28	0.317^{ns}	0.628
G2	9.22 ± 0.52	9.15 ± 0.57	9.03 ± 0.82	9.17 ± 0.67	0.037^{ns}	0.885
G3	10.95 ± 0.49	10.75 ± 0.65	10.87 ± 0.65	10.89 ± 0.40	0.089^{ns}	0.965
F value	1.805^{ns}	1.755^{ns}	2.084^{ns}	2.72^{ns}		
P value	0.179	0.188	0.134	0.072		

Table 3. The results of TLC per µl

Group	220 d	250 d	DOC	30 d pp	F value	P value
G0	11033.3 ± 878.9	9716.67 ± 907.90	11300.0 ± 371.48	10966.7 ± 483.51	2.410^{ns}	0.108
G1	9550.0 ± 715.43	9166.67 ± 389.59	10950.0 ± 328.38	10350.0 ± 261.73	2.695^{ns}	0.083
G2	9150.0 ± 649.49	9233.33 ± 294.00	10533.3 ± 333.33	10100.0 ± 265.83	3.239^{ns}	0.097

G3	8216.67 ± 810.5	9250.0 ± 388.80	10033.3 ± 537.07	9400.0 ±393.70	2.626 ^{ns}	0.098
F value	2.326 ^{ns}	0.210 ^{ns}	1.848 ^{ns}	3.052 ^{ns}		
P value	0.106	0.888	0.171	0.054		

Table 4. The results of VPRC (%)

Group	220 d	250 d	DOC	30 d pp	F value	P value
G0	32.50 ± 1.38	30.83 ± 1.54	29.33 ±1.31^{a}	30.83 ± 1.08	1.609 ^{ns}	0.229
G1	30.67 ± 2.55	31.00 ± 1.13	31.50 ±1.20^{ab}	32.17 ± 0.95	0.271 ^{ns}	0.845
G2	29.83 ± 2.07	30.33 ± 1.89	30.67 ± 1.67^{a}	32.33 ± 1.71	0.906 ^{ns}	0.462
G3	34.83 ± 1.49	35.00 ± 1.21	35.17 ± 0.79^{b}	35.83 ± 1.01	0.337 ^{ns}	0.799
F value	1.315 ^{ns}	2.147 ^{ns}	3.801 *	3.044 ^{ns}		
P value	0.297	0.126	0.026	0.053		

The mean RBC count on day 220, 250 days of pregnancy, day of calving and on 30 day post-partum of all the four experimental groups are presented in Table 1.

When analysed within period between group, the mean TEC were significantly different on 250 days of pregnancy, day of calving and on 30 days post-partum with significantly high TEC for G3 compared to G0, G1 and G2. The results suggest a positive effect of supplementation with both vitamin E (1000 IU/day) and Se (0.3 ppm/day) on TEC of dairy cattle. It might 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 (Mills, 1957). Supporting results were reported by Surai (2006) that selenoproteins through their antioxidant properties improves membrane stability of erythrocytes. Significantly low TEC found in G0 animals during 250 day of pregnancy, day of calving and 30 day post-partum might be due to increased oxidative stress the animals were experiencing during this period. Fibach and Rachmilewitz (2008) reported that increased oxidative stress increase rate of hemolysis. 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).

On within group between period analysis, there was no significant differences was noted between different periods in G0 and G2 group. Similar results were reported by Ate *et al*.

(2009) that there was no significant difference in TEC between advanced pregnant and early lactating dairy cows. In G2, there was no significant difference in mean TEC between different periods, suggesting that vitamin E alone might not be able to reduce hemolysis significantly during periods of increased oxidative stress.

In G1 and G3, mean TEC during 30 days post-partum were significantly higher compared to 250 days of pregnancy and day of calving. These results suggest a supporting role of Se and Se + vitamin E supplementation during increased oxidative stress. According to findings of Necheles *et al.* (1968), infants genetically low in GSH-Px were having increased hemolysis compared to normal infants. According to the findings of Brownlee *et al.* (1977), lipid peroxidation and hemolysis were high in vitamin E deficient rats.

The mean Hb concentration on 220, 250 days of pregnancy, day of calving and on 30 day post-partum of all the four experimental groups are presented in Table 2.

When analysed within period between group, there was no significant difference in mean Hb concentration. This was in accordance with the findings of Ate *et al.* (2009), that there was no significant difference in Hb concentration between advanced pregnant and early lactating dairy cows. But, Patel *et al*. (2017) reported that there was significant increase in Hb concentration from 30 days pre-partum to day of calving and decreased during post-partum.

When analysed within group between period, there was no significant difference in mean Hb concentration. In the current study, 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. Similar results were reported by Abdalla and Abdelatif (2014) that there was no significant difference in mean Hb concentration between selenium and vitamin E supplemented group of dairy cows and control animals during transition period. They suggested that it could be due to increased provision of amino acids for lactogenesis during periparturient period. Since all the values were in the normal range, it can be considered that all the animals included in the study were not anaemic.

The mean TLC on 220, 250 days of pregnancy, day of calving and on 30 day post-partum of all the four experimental groups are presented in Table 3.

When analysed within period between group and within group between period, there was no significant difference in mean TLC. Similar results were reported by Ate *et al*. (2009) that there was no significant difference in TLC between advanced pregnant and early lactating cows. Calamari *et al*. (2011) observed similar results that the total WBC counts were not affected by Se supplementation at 0.31 or 0.50 mg/kg dry matter compared to

unsupplemented group of animals. In G2 and G3 animals also there was no significant difference observed in TLC between different periods. From the current study, it could found that there was no significant difference in TLC during different physiological stages of transition period. 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.

The mean VPRC on 220, 250 days of pregnancy, day of calving and on 30 day post-partum of all the four experimental groups are presented in Table no 4.

On within period between group analysis, the mean VPRC values were significantly different on day of calving. There was no significant difference between G0, G1 and G2. G3 was having significantly high mean VPRC values compared to G0 and G2. But, there was no significant difference between G1 and G3. The high VPRC on the day of calving for G3 might be due to the complementary haemopoetic activity of vitamin E and Se during this period which is evident by significantly high TEC values seen during this period. But Abdalla and Abdelatif (2014) reported that there was no significant difference in mean PCV between selenium and vitamin E supplemented group of dairy cows and control animals during transition period. They suggested that it could be due to increase catabolism of proteins during periparturient period.

On within group between period analysis, there was no significant difference in mean VPRC values between different periods in any of the treatment groups. According to Ate *et al.* (2009) there was no significant difference in mean VPRC between advanced pregnant and early lactating dairy cows. But, Nazifi *et al*. (2008) reported that mean VPRC were significantly higher in pregnant cows compared to post-partum results.

Conclusion

The present study on evaluation of effects of supplementation of organic selenium and/or vitamin E during transition period in dairy cows revealed a significant positive effect on haematological parameters indicating significant improvement in health, which will definitely help the animals counteracting increased oxidative stress during transition period.

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