

Original Research Article

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Influence of Antioxidant Administration during Periparturient Period on Total Antioxidant Status in Surti Buffaloes

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ABSTRACT

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The animals were divided into two groups comprising of ten animals in each group as: Group-I: Treatment group of Surti buffaloes treated with Inj. Vitamin E and Selenium (E-CARE Se) on 60th, 45th, 30th and 15th day before expected date of parturition and after parturition on 15th, 30th day and Group-II: Control group given Inj. Normal Saline (IM) as placebo treatment. The mean plasma Total Antioxidant Status (TAS) concentration did not differ significantly ($p>0.05$) at different days interval in between treatment and control groups. The pooled mean plasma TAS level was found significantly ($p<0.05$) decreased in trend from 60th day to 45th day, 30th day and 15th day pre partum and on the day of calving and there after significantly ($p<0.05$) increased in trend at 15th day, 30th day and non-significantly ($p>0.05$) increased at 45th day and thereafter significantly ($p<0.05$) increased at 60th day post partum in Surti buffaloes. The mean plasma TAS concentration did not differ significantly ($p>0.05$) in between pregnant and non-pregnant groups at different days interval except at 60th days prepartum it was significantly ($p<0.05$) higher in pregnant group as compared to non-pregnant group.

Introduction

The transition or periparturient period, from 3 weeks before to 3 weeks after parturition, is a stressful time for dairy cows (Drackley, 1999). An imbalance between increased production of ROS and reduced availability of antioxidant defenses near the time of parturition increases oxidative stress and may contribute to

periparturient disorders in dairy cows (Gitto *et al.*, 2002). During gestation oxidative stress plays a role in the initiation of pre-term labor (Pressman *et al.*, 2003) and during normal parturition (Fainaru *et al.*, 2002) assuring ovulation, ovarian steroidogenesis, oocyte maturation, blastocyst formation, luteolysis and luteal maintenance in pregnancy (Sugino *et al.*, 2000). Total Antioxidative Capacity

(TAC) is defined as a measure of overall free-radical scavenging potential (Paszowski and Clarke, 1996). Quantification of a single antioxidant often tells little about whole body defense, making it necessary to evaluate multiple indicators of redox status. Although, measuring multiple antioxidants or biomarkers of stress is helpful, measuring a myriad of variables also tends to be impractical. Consequently, total antioxidant capacity assays are being employed on a routine basis. Vitamin E is an important antioxidant that has been shown to play an important role in immuno responsiveness and health in dairy cows (Weiss and Spears, 2006). Moreover, vitamin E is involved in the formation of leukotrienes, prostaglandin and prostacyclin and has got a role in the duration of postpartum interval. In Vitamin E and Selenium deficiency condition, free radicals accumulate and not only damage cell membranes, but also disrupt several processes linked to the synthesis of steroids (Seagerson and Libby, 1982) and prostaglandins (Harrison and Conrad, 1984).

Materials and Methods

The present research work was undertaken on twenty (20) Surti buffaloes during their transient period i.e. two month before their expected date of parturition to two month after parturition, dividing into treatment (n=10) & control (n=10) groups, at Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat, over a period from May, 2014 to April, 2015. The animals were fed green fodder, hay and compounded concentrate, as per the standard feeding schedule followed on the farm. The animals had free access to drinking water. The animals were also washed and sprinkled with water twice daily or were allowed to wallow in the pond during hot noon hours of summer season to reduce heat stress and to improve oestrus expression in them. In control group of 10

animals to which 10 ml normal saline injected IM on 60th, 45th, 30th and 15th day before expected date of parturition and after parturition on 15th, 30th day. Treatment Group of 10 animals to which the injectable product E-CARE Se (DL- α Tocopheryl Acetate I.P. equivalent to Tocopherol (Vitamin E) Base - 50mg, Sodium Selenite U.S.P. equivalent to Selenium Base -1.5mg in each ml) was administered IM on 60th, 45th, 30th and 15th day before expected date of parturition and after parturition on 15th, 30th day at the dose rate of 10 ml (500 mg vit. E and 15 mg Se.). Pregnancy diagnosis was carried out per rectally at 90 days post breeding. Again the group was made from all 20 animals irrespective of treatment and control group on the base of its conception in pregnant (n=13) and non-pregnant (n=7) groups.

Blood collection and laboratory examination

Blood samples were collected from all those selected animals on approximate day 60, 45, 30, 15 before the expected date of parturition, on the day of parturition and 15, 30, 45 and 60 day after parturition in EDTA vacutainer for plasma. The plasma was separated from vacutainers containing 5 ml blood samples immediately after its collection and stored at -20°C in deep freezer until analysis. Plasma total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1999).

Statistical analysis

The tests of significance for pregnant vs. non-pregnant and treatment vs. control groups were made by Standard Student's paired 't' test. The fortnight-wise variation within the group was tested for each trait by using completely randomized design as well as the mean differences between and within the groups were tested using Duncan's New

Multiple Range Test (DNMRT) at 1 per cent and 5 per cent level of significance.

Results and Discussion

The mean plasma TAS concentrations varied non-significantly ($p>0.05$) between treatment and control groups at different peripartum intervals. The overall mean plasma TAS levels in treatment and control groups were non-significantly ($p>0.05$) higher before parturition as compared to after parturition, and thereby the overall prepartum pooled mean plasma was also non-significantly ($p>0.05$) higher as compared to overall postpartum pooled mean (1945.833 ± 28.787 vs. 1911.979 ± 25.741 $\mu\text{m/L}$). Similarly the overall mean plasma TAS value was non-significantly ($p>0.05$) higher in the treatment group as compared to control group at prepartum phase (1969.792 ± 41.773 vs. 1921.875 ± 39.786 $\mu\text{m/L}$) and lower at postpartum phase (1905.208 ± 40.639 vs. 1918.750 ± 32.100 $\mu\text{m/L}$), and thereby the overall pooled mean concentration was also non-significantly ($p>0.05$) higher in treatment group as compared to control group (1883.333 ± 31.973 vs. 1863.426 ± 29.422 $\mu\text{m/L}$) (Table 1).

The prepartum TAS level was non-significantly ($p>0.05$) higher when compared between prepartum and postpartum at various intervals, viz., at 60th day prepartum than that of 60th day postpartum; 45th day prepartum than that of 45th day postpartum; 30th day prepartum than that of 30th day postpartum and 15th day prepartum than that of 15th day postpartum in treatment group, whereas in control group it was found non-significantly ($p>0.05$) lower at 60th day prepartum than that of 60th day postpartum; non-significantly ($p>0.05$) higher at 45th day prepartum than that of 45th day postpartum; 30th day prepartum than that of 30th day postpartum and non-significantly ($p>0.05$) lower at 15th day

prepartum than that of 15th day postpartum (Table 1). The pooled mean plasma TAS level was found to be significantly ($p<0.05$) decreased from 60th day to 45th day, 30th day and 15th day prepartum and on the day of calving and thereafter significantly ($p<0.05$) increased at 15th day, 30th day and at 60th day postpartum in Surti buffaloes. Almost same trend was found for treatment and control groups over peripartum intervals (Table 1).

The mean plasma TAS level was found non-significantly ($p>0.05$) higher on the day of calving (1480.769 ± 76.309 vs. 1333.333 ± 72.739 $\mu\text{m/L}$) in pregnant group as compared to non-pregnant group (Table 1).

The mean plasma TAS level decreased significantly ($p<0.05$) from 60th day prepartum (2256.410 ± 60.573 $\mu\text{m/L}$; 2005.952 ± 101.015 $\mu\text{m/L}$) to the day of calving (1480.769 ± 76.309 $\mu\text{m/L}$; 1333.333 ± 72.739 $\mu\text{m/L}$) and increased significantly ($p<0.05$) up to 30th day postpartum (1871.795 ± 49.360 $\mu\text{m/L}$) from day of parturition in the pregnant group and up to 15th day postpartum (1702.381 ± 71.263 $\mu\text{m/L}$) in the non-pregnant group, and thereafter non-significantly ($p>0.05$) increased at 60th day postpartum (2125.000 ± 36.847 $\mu\text{m/L}$; 2136.905 ± 21.735 $\mu\text{m/L}$) in both pregnant and non-pregnant groups of buffaloes (Table 1).

In the present study plasma total antioxidant activity (FRAP Values) declined from 60th day prepartum till calving in both the groups, TAS was lowest on the day of calving in both the groups, whereas it was non-significantly ($p>0.05$) higher in treatment group as compared to control group of Surti buffaloes and increased significantly ($p<0.05$) to reach the highest values at 60th day postpartum. Higher total antioxidant status found in the treatment group as compared to control group in the present study might be ascribed to vitamin E and Se treatment in that group.

Table.1 Mean plasma Total Antioxidant Status (TAS) levels ($\mu\text{m/L}$) at different fortnightly intervals peripartum in antioxidant treated and control groups as well as pregnant and non-pregnant groups of Surti buffaloes (Mean \pm SE)

Peripartum Phases	Days	Total Antioxidant Status (TAS) $\mu\text{m/L}$						
		Treatment (n=10)	Control (n=10)	't' - Value	Pooled (n=20)	Pregnant (n=13)	Non- pregnant (n=7)	't'- value
Prepartum	60	2254.167 \pm 50.633 ^e	2083.333 \pm 100.539 ^d	1.518	2168.750 \pm 58.182 ^f	2256.410 \pm 60.573 ^e	2005.952 \pm 101.015 ^{x,cd}	2.266 [*]
	45	2037.500 \pm 38.011 ^{cd}	2008.333 \pm 39.675 ^{cd}	0.531	2022.917 \pm 26.948 ^{de}	2032.051 \pm 36.519 ^{cd}	2005.952 \pm 39.032 ^{cd}	0.452
	30	1887.500 \pm 50.861 ^c	1879.167 \pm 49.865 ^{bc}	0.117	1883.333 \pm 34.677 ^c	1887.821 \pm 48.432 ^c	1875.000 \pm 46.362 ^{bc}	0.172
	15	1700.000 \pm 72.913 ^b	1716.667 \pm 68.268 ^b	0.167	1708.333 \pm 48.648 ^b	1717.949 \pm 63.323 ^b	1690.476 \pm 80.228 ^b	0.263
	Overall	1969.792 \pm 41.773	1921.875 \pm 39.786	0.831	1945.833 \pm 28.787	1973.558 \pm 37.864	1894.345 \pm 41.803	1.319
Day of Parturition	0	1450.000 \pm 87.797 ^a	1408.333 \pm 76.528 ^a	0.358	1429.167 \pm 56.882 ^a	1480.769 \pm 76.309 ^a	1333.333 \pm 72.739 ^a	1.255
Postpartum	15	1620.833 \pm 65.278 ^b	1741.667 \pm 50.766 ^b	1.461	1681.250 \pm 42.564 ^b	1669.872 \pm 54.910 ^b	1702.381 \pm 71.263 ^b	0.356
	30	1875.000 \pm 58.267 ^c	1850.000 \pm 51.670 ^{bc}	0.321	1862.500 \pm 38.008 ^c	1871.795 \pm 49.360 ^c	1845.238 \pm 62.806 ^{bc}	0.325
	45	1975.000 \pm 56.656 ^{cd}	1975.000 \pm 56.995 ^{cd}	0.000	1975.000 \pm 39.110 ^{cd}	1977.564 \pm 49.394 ^{cd}	1970.238 \pm 69.075 ^{cd}	0.087
	60	2150.000 \pm 38.390 ^{de}	2108.333 \pm 31.793 ^d	0.836	2129.167 \pm 24.724 ^{e,f}	2125.000 \pm 36.847 ^{de}	2136.905 \pm 21.735 ^d	0.224
	Overall	1905.208 \pm 40.639	1918.750 \pm 32.100	0.261	1911.979 \pm 25.741	1911.058 \pm 32.906	1913.691 \pm 41.718	0.048
Overall	't' -Value	1.108	0.061	--	0.877	1.246	-0.328	--
	P-Value	0.271	0.951	--	0.382	0.216	0.745	--
	Pooled	1883.333 \pm 31.973	1863.426 \pm 29.422	0.458	1873.380 \pm 21.677	1891.026 \pm 27.341	1840.609 \pm 35.385	1.110

Means bearing different superscripts (a, b, c) within a column (between phase intervals) differ significantly ($p < 0.05$), while means bearing common subscripts (x, y, z) within a row (between the groups) do not differ significantly ($p > 0.05$).

Miller *et al.*, (1993) reported that feeding 0 and 1,000 IU vitamin E/head/day during dry period to cows led to significant ($p < 0.05$) increase in the plasma total antioxidant activity at parturition. They also reported that the decrease of oxidative stress before parturition might be due to the increase of antioxidant protection that occurs in that particular physiological stage. This means that when the risk of oxidative damage increases, endogenous antioxidant protection increases too. On the other hand, Tanha *et al.*, (2011) did not observe significant ($p > 0.05$) variation in total antioxidant status on day of calving and 7th, 14th, 21st day before calving among glutamine supplemented Holstein cows. However, the TAS was found increased from prepartum to the day of calving.

Similar to present findings, Brezezinska *et al.*, (1994) reported decrease in total antioxidant activity with approaching parturition in cows and on the day of parturition, FRAP values were non-significantly ($p > 0.05$) higher in treatment group than that of control group. Further they reported, daily supplementation of vitamin E @ 1000 IU/cow/day resulted in 33, 43 and 61 per cent increase in plasma antioxidant capacity within 2, 4 and 6 weeks of supplementation during late gestation in cows.

According to Rajiv (2001), supplementation of 1500 IU/day of vitamin E during 60 days pre-partum was sufficient to maintain optimum level of total antioxidant activity at parturition in buffaloes. Chatterjee *et al.*, (2003) also found higher antioxidant status during parturition in the cows supplemented with vitamin E. They also found the values continued to decline till calving. Similarly, Chandra *et al.*, (2013) reported significant ($p < 0.05$) lower plasma TBARS values during prepartum compared with those during parturition and postpartum period in control and vitamin E treated groups, but postpartum plasma TBARS level was significant ($p < 0.05$)

lower in treatment group as compared to control group.

Panda *et al.*, (2006) reported decrease in plasma antioxidant status at calving in Murrah buffaloes. Though the plasma total antioxidant activity (FRAP Values) continued to decline from 30th days prepartum till calving. The status was non-significantly ($p > 0.05$) higher on the day of parturition in vitamin E supplemented group as compared to control group of buffaloes. The FRAP values started increasing after parturition and reached normal range after 30 days of parturition. Wullepit *et al.*, (2012) stated that plasma antioxidative status at the time of parturition showed a clear trend to be lower after calving in Holstein Friesian cows and Kankofera *et al.*, (2010) also found highest total antioxidant capacity at 2 and 1 week ante-partum with a drop towards parturition suggesting the presence of oxidative stress during this time period. Total antioxidative capacity (TAC) increased in the prepartum period with a sharp decrease at parturition. During postpartum period, the values increased again with another decrease after 3 weeks postpartum in cows. Similarly, Hayajneh (2014) reported highest antioxidative capacity 3 to 5 weeks postpartum and the lowest at 3 to 0 weeks prepartum in dairy cattle. Moreover, retention of placenta was associated with oxidative stress as shown by the significantly lower value of total antioxidant capacity (TAC) as compared to non-retention group of buffaloes (Ahmed *et al.*, 2009). Cigliano *et al.*, (2014) found significantly ($p < 0.05$) lower plasma concentration of TAC in early lactating than mid-late lactating buffaloes.

Even though the temporal pattern for the various antioxidants differed slightly, an overall trend could be noticed in that the highest values were observed at 60th day prepartum with a drop towards parturition. This may be considered as the reaction of the macro

organism to an increase in the contents of products of lipid and protein peroxidative damage appearing at similar time points. Total antioxidative capacity (TAC) increased in the prepartum period with a sharp decrease at parturition and the values again increased during postpartum. Similar time patterns were observed by Castillo *et al.*, (2006) and concluded that the antioxidative system at that time period might be efficient for the protection against oxidative stress.

Measuring TAC is considered a valuable tool for determining the overall antioxidative potential of cells or the whole organism, provided repeated sampling during certain time periods. Nonetheless, due to the wide range of chemical properties of different antioxidants, it is not possible to cover all members of the antioxidative system against ROS with a single method. Several methods of TAC determination are in use, which differ in the range of antioxidants included.

Therefore, the measurement of single antioxidants might be useful for a more sophisticated interpretation of antioxidative/oxidative profiles.

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Conflict of interest statement

Authors declare that they have no conflict of interest.

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