



Effect of vitamin E supplementation on antioxidant status and selective humoral and cellular immune responses in periparturient buffaloes

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ABSTRACT

This study was aimed at assessing the anti oxidant status and selective humoral and cellular immune response mediators in periparturient buffaloes supplemented with vitamin E in the feed regularly. Murrah buffaloes (12) were selected during their late gestation from NDRI herd and divided randomly into 2 groups, comprising 6 each. Buffaloes of group 1 were given only the control diet, while group 2 was supplemented with 2,000 IU/day/head vitamin E along with control feed. Blood sample was drawn from each buffalo at weekly interval from day –56 to day +56 relative to parturition by jugular vein- puncture. Nitric oxide (NO) level was quantified using modified Griess reaction whereas IL-6, total antioxidant activity (TAA) and IgG levels were estimated in blood plasma using ELISA kits. TAA and IgG levels increased significantly upon vitamin E supplementation. However, levels of cellular immune response mediators (NO and IL-6) were significantly lowered. Except for plasma NO, the levels of all other mediators declined significantly on the day of calving as compared to prepartum levels in both the groups. TAA was also significantly reduced. The magnitude of decline was significantly greater in group 1. It could be concluded that peripartum supplementation of vitamin E to buffaloes not only improved humoral and cellular immune responses but also enhanced total antioxidant activity.

Key words: IgG, IL-6, Murrah buffaloes, Nitric oxide, Peripartum period, Total antioxidant activity

The periparturient period, between late pregnancy and early lactation is certainly the most crucial stage for the postpartum health. This period poses tremendous physiological stress to the animal due to intense mammary gland growth, accompanied by a high energy demand by growing fetus and an increased oxygen requirement to the dam (Gitto *et al.* 2002). The increased oxygen demand augments the production of reactive oxygen species (ROS) which is counteracted by the natural antioxidant defense system of the body. But near to parturition, the reduced availability of antioxidant defense (Gitto *et al.* 2002) increases oxidative stress along with gradual changes in cellular and humoral immune responses which may contribute to periparturient disorders.

Vitamin E, a fat-soluble membrane antioxidant, protects the dairy cows from oxidative damage (Herdt and Stowe 1991), and is also important for optimum functioning of both the cellular and humoral immune systems. Tengerdy (1980) reported the stimulation of serum antibody synthesis particularly IgG in response to vitamin E supplementation in cattle. This study was planned with an objective of assessing the effect of peripartum vitamin E supplementation on antioxidant status and selective cellular and humoral immune responses in buffaloes.

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MATERIALS AND METHODS

Selection and feeding management: Healthy peripartum Murrah buffaloes (12) were selected from the NDRI herd for experimentation. All these buffaloes were maintained under general managemental practices as followed for the herd. These buffaloes were randomly divided into control-group 1 and treatment-group 2, consisting of 6 animals each. Feeding was done as per Kearn (1982) standards based on changes in fortnightly body weight to group 1 (control feed). Group 2 buffaloes were supplemented with 2,000 IU α -tocopheryl acetate/day/head from 56 days prepartum to 21 days postpartum in addition to the control feed. The feed grade DL α -tocopheryl acetate was weighed accurately and mixed with concentrate for feeding. The water was provided *ad lib.* to all the buffaloes.

Blood sampling: Blood sample (15 ml) from each buffalo was drawn in sterile heparinised vacutainer tube at 6.00 AM in the morning by jugular venipuncture on –56, –49, –42, –35, –28, –21, –14, –7, 0, +7, +14, +21, +28, +35, +42, +49, +56 days relative to parturition. Immediately after collection, the blood was transported to the laboratory in an icebox for further processing. The heparinised tubes were centrifuged at 3,000 rpm for 15 min, plasma was aliquoted into different fractions and stored at –20°C until analysis of selected cellular and humoral immune response mediators.

Quantification of immune response mediators: Humoral

responses were measured in terms of plasma IgG and cellular responses by plasma IL-6 and NO. TAA was measured to assess the antioxidant status of these buffaloes. TAA, IL-6 and IgG were quantified by ELISA kits and NO was quantified using modified Griess reaction as described by Shoker *et al.* (1997).

Statistical analysis: The data were statistically analyzed using 3-way analysis of variance (ANOVA) with variables as treatment, stage (prepartum vs postpartum) and periods (days with in pre-and post-partum period) by SAS software considering $P < 0.05$ as the level of significance. In order to detect the changes in immune profile at calving with respect to prepartum period in both the groups, student's *t*-test was employed by Graphpad prism 5 software.

RESULTS AND DISCUSSION

Total antioxidant activity is a single measure that aims to describe the dynamic equilibrium between pro-oxidants and antioxidants in the plasma compartment (Ghiselli *et al.* 2000). This investigation revealed significant increase in TAA in vitamin E supplemented group than that of control (Fig. 4), thereby depicting better oxidative tolerance in supplemented buffaloes. Although the declining pattern of TAA till calving was similar to that of cows as reported by Chatterjee *et al.* (2003), the differences between periods or stages in both the groups were not statistically significant. At calving TAA was significantly reduced as compared to pre- or post-partum stage. The TAA values could provide complementary information about the homeostasis of the animal than conventional metabolic parameters especially glucose or NEFA alone whose values could be modified by

many factors such as nutritional or the hormonal status.

Vitamin E as an immunopotentiator is reported to enhance humoral immune responses in several laboratory and domestic animals. Tengerdy (1980) found stimulatory effect of vitamin E supplementation on serum antibody synthesis, particularly IgG. We also observed a significant ($P < 0.05$) rise in plasma IgG concentration upon vitamin E supplementation to periparturient buffaloes (24.68 ± 1.06 vs 23.45 ± 0.68 mg/ml). The variations were also significant between periods and stages in both the groups. The levels declined significantly ($P < 0.05$) at calving (Fig. 1) followed by a gradual rise in post partum period. Mean levels declined from 27.73 ± 1.60 mg/ml 56 days before parturition to 17.42 ± 1.54 mg/ml on the day of calving in group 1 and from 28.07 ± 1.72 mg/ml to 18.96 ± 1.72 mg/ml in group 2. The pattern of declining IgG during periparturient period in this investigation (Fig. 1) was similar to that of cows as reported in previous studies (Brenner *et al.* 1995, Herr *et al.* 2011). Brenner *et al.* (1995) reported a significant drop in IgG concentration ($P < 0.05$) in prepartum cows (11–7 days before parturition), from an average of 37.6 ± 3.7 mg/ml for 5 consecutive days to 28.0 ± 5.5 mg/ml on the day of parturition. Herr *et al.* (2011) reported the concentration to be declining significantly between the eighth weeks up to 1 day antepartum in cows. The decrease of serum IgG continued until the expulsion of fetus. After expulsion of the fetus till day 7 postpartum, the IgG concentrations in cows remained at a low level (15–20 mg/ml). From then on and until the 4th week postpartum a significant increase of IgG values was observed with values reaching to 36.2 ± 9.9 mg/ml. Total reduction in IgG until expulsion of

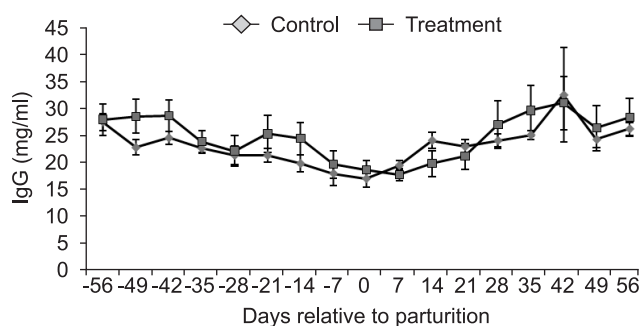


Fig. 1. Effect of peripartum vitamin E on plasma IgG concentration of buffaloes.

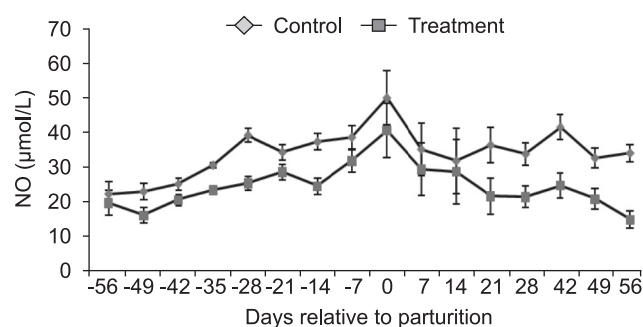


Fig. 2. Effect of peripartum vitamin E on plasma nitric oxide concentration of buffaloes.

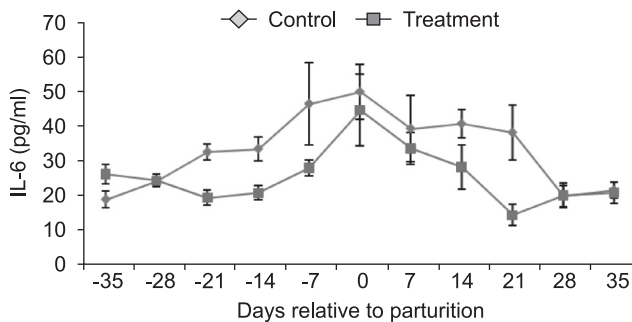


Fig. 3. Effect of peripartum vitamin E on plasma IL-6 concentration of buffaloes.

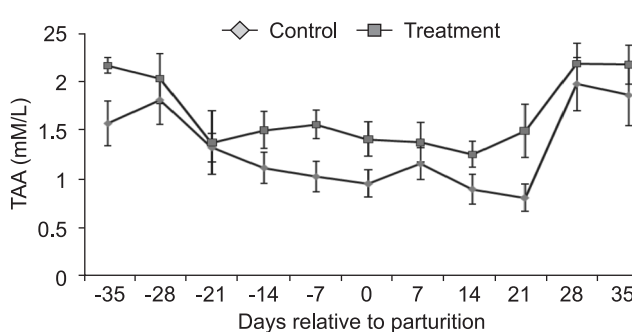


Fig. 4. Effect of peripartum vitamin E on plasma TAA activity of buffaloes.

fetus in our study was 37.94% in normal and 36.82% in supplemented buffaloes, where as it was 59.2% in cows (Herr *et al.* 2011).

Nitric oxide was significantly lower in treatment group ($P < 0.05$) of buffaloes. The level enhanced significantly around calving in relation to prepartum level in both the groups ($P < 0.05$) of buffaloes (Fig.2), declining gradually during postpartum period. The difference was significant between periods but not between stages. Our data is in agreement with previous studies in several mammalian species including cattle, buffaloes, sheep, rats and humans (Kimberly *et al.* 2005, Houzha *et al.* 2010) demonstrating drastic change in nitric oxide during transition period. Christen *et al.* (2007) opined that nitric oxide axis activation was required during the transition period. Kimberly *et al.* (2005) opined that increased nitric oxide could potentially aid in remodeling of the vascular bed prior to meet the increased demand for blood flow during pregnancy. There are reports indicating that the inducible nitric oxide synthase (iNOS) derived nitric oxide contribute to the majority of changes in nitric oxide production during pregnancy and labor and can synthesize cytokines (Moilanen and Vapaatalo 1995). Pattern of decline during post calving period as observed in this investigation was similar to that reported for ovines (Kimberly *et al.* 2005) and buffaloes (Houzha *et al.* 2010).

The cytokines IL-1, IL-6 and TNF- α , were produced in significant quantities during the periparturient period (Singh *et al.* 2008). We also observed, significantly elevated IL-6 levels ($P < 0.05$) in vitamin E supplemented buffaloes throughout the experimental period. The level enhanced significantly around calving in relation to prepartum level in both the groups ($P < 0.05$) of buffaloes (Fig. 3), declining during postpartum period. The difference however, was not significant between pre- and post- partum stage in both the groups. This indicated that the Th₂ cell dominant immune system during pregnancy was shifted to a Th₁ cell dominant immune system around calving. Similar results were obtained in earlier studies (Makhseed *et al.* 2000, Ishikawa *et al.* 2004) in cattle. Ishikawa *et al.* (2004) recorded higher concentration of serum IL-6 at parturition followed by a decline until 1 week postpartum. The average concentration before calving was higher than those after calving. Makhseed *et al.* (2000) related elevated concentration at parturition to uterine contraction and the propagation of labour. Thus, IL-6 monitoring around parturition in cows could possibly be used to know the status of pregnancy and maternal adaptability to pregnancy and parturition (Ishikawa *et al.* 2004).

It was concluded that alteration in humoral and cellular immune responses coupled with low antioxidant defense system as monitored by IgG, IL-6, NO levels and total antioxidant activity towards the day of parturition in buffaloes reflected a physiological phenomenon that could eventually lead to reduced immunity but before hand supplementation of vitamin E to buffaloes antepartum improved the humoral as well as cellular immune responses

with higher total antioxidant activity around parturition and afterwards which could combat production related diseases.

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