Journal of Animal Physiology and Animal Nutrition

DOI: 10.1111/jpn.12190

ORIGINAL ARTICLE

Effect of additional vitamin E and zinc supplementation on immunological changes in peripartum Sahiwal cows

G. Chandra[1], A. Aggarwal[1], M. Kumar[2], A. K. Singh[1], V. K. Sharma[3] and R. C. Upadhyay[1]

1 Dairy Cattle Physiology Division, National Dairy Research Institute Karnal, Haryana, India
2 Department of Animal Nutrition, DUVASU Mathura, Uttar Pradesh, India, and
3 Dairy Cattle Nutrition Division, National Dairy Research Institute Karnal, Haryana, India

Summary

This study was conducted to exploit ameliorative effect of additional vitamin E and/or zinc supplementation on immune response of peripartum Sahiwal cows. Thirty-two pregnant dry Sahiwal cows were blocked into four treatment groups (n = 8), namely control, zinc (Zn), vitamin E (Vit E) and zinc + vitamin E (Zn + Vit E). Feeding regimen was same in all the groups except that the Sahiwal cows in the zinc-, vitamin E- and zinc + vitamin E-fed groups were additionally supplemented with 60 mg Zn/kg DM, 1000 IU vitamin E and 60 mg/kg + 1000 IU Zn + vitamin E, respectively, from day 60 pre-partum to day 90 post-partum. Blood samples were collected on days −60, −45, −30, −15, −7, −3, 0, 3, 7, 15, 30, 45, 60, 90 and 120 with respect to day of parturition and analysed for total immunoglobulin (TIG), immunoglobulin G (IgG), interleukin-2 (IL-2), vitamin E (Vit E) and zinc (Zn) status. Before calving, cows showed a decrease in plasma TIG, IgG, IL-2, Vit E and Zn levels. However, increased levels of plasma TIG, IgG, IL-2, Vit E and Zn were observed after calving. After calving, Sahiwal cows supplemented with Zn + Vit E had higher plasma TIG, IgG and IL-2 in comparison with cows of control and Zn + Vit E-fed groups. In the present study, plasma vitamin E level was higher in Vit E-fed and Zn + Vit E-fed cows; however, zinc level was higher in Zn- and Zn + Vit E-supplemented cows. In conclusion, a reduced immune response during peripartum period in Sahiwal cows was ameliorated by dietary vitamin E and zinc supplementation.

Keywords peripartum, dairy cows, vitamin E, zinc

Correspondence G. Chandra, Department of Veterinary Physiology and Biochemistry, College of Veterinary and Animal Sciences, SVBPUAT, Modipuram, Meerut-250110, Uttar Pradesh, India. Tel:+91 805 2968634; Fax: +91 184 2254751; E-mail: gulabdrvet@gmail.com

Received: 23 June 2013; accepted: 13 March 2014

Introduction

The peripartum period is crucial for cow health and milk production (Grummer, 1995). The immune system of dairy cows is challenged around parturition, resulting in an increased susceptibility to infectious diseases (Kehrli et al., 1998; Maurya, 2011). The postpartum period of dairy cattle is known to be a time when important host immune responses such as lymphocyte proliferation, antibody development and cytokine production are reduced (Sordillo, 2005). This may partly be due to a decrease in plasma concentrations of antioxidant micronutrients such as vitamin A, vitamin E and Zn observed at this time (Johnston and Chew, 1984; Goff and Stabel, 1990; Meglia et al., 2004; Chandra and Aggarwal, 2009; Maurya, 2011). Micronutrient deficiencies around calving have been associated with diseases such as retained foetal membranes, endometritis and mastitis (Panda et al., 2006).

Various supplements and additives are used in periparturient dairy animal rations to minimize stress due to gestation, calving and early lactation. Among these, vitamin E and Zn supplementation has been used during pregnancy, lactation and other times of stress. Vitamin E and Zn are essential nutrients and are required for a well-functioning immune system (Weiss, 2002). When supplemented during pre- and post-partum period, the protective effects of vitamin E on animal health may be due to its role in the reduction of glucocorticoids, which are immunosuppressive (Tengerdy, 1980). Immunity boost-up effects of vitamin E may be elicited through its effect on cell membrane stability and regulatory role in biosynthesis of various inflammatory mediators (Smith et al., 1997). Dietary vitamin E supplementation increased IgM and interleukin production by bovine peripheral mononuclear cells (Stabel et al., 1992) and also modulated prostaglandin release from

activated macrophages during infections (Likoff et al., 1981). Prostaglandins inhibit the functional activity of lymphocytes and macrophages by decreasing the susceptibility of cells to mitogen stimulation and subsequent cellular differentiation (Meydani and Hafek, 1992). Zinc supplementation enhances cow's immunity by increasing resistance to infections and by decreasing severity of infection (Goff and Stabel, 1990; Campbell and Miller, 1998). Zinc deficiencies can result in atrophy of the thymus and other lymphoid organs (Underwood and Suttle, 1999). Fraker et al. (1986) found a depression of T-lymphocyte functions in Zn-deficient animals.

Considering these facts, the present study was therefore designed to investigate the effect of an additional vitamin E and zinc supplementation on immune response in peripartum Sahiwal cows under Indian conditions.

Materials and methods

The experiment was conducted in the cattle yard of National Dairy Research Institute (NDRI), Karnal, Haryana. It is situated on an altitude of 250 metres above mean sea level, latitude and longitude position being 29° 42″N and 79° 54″E respectively. Minimum ambient temperature is recorded near 0 °C in winter, and maximum temperature goes up to 45 °C in summer with annual rainfall of 700 mm. All protocols, approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the article number 13 of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) rules, laid down by the Government of India were followed.

Experiment design and feeding

Thirty-two medium-producing, clinically healthy prepartum Sahiwal cows were selected from cattle yard of NDRI, Karnal. Selected cows were randomly assigned to four treatments ($n = 8$) with having minimum possible intra-herd variation in terms of body weight (409 ± 9.0 vs. 403 ± 9.2 vs. 406 ± 7.9 vs. 398 ± 7.1 kg) and parity (2.8 ± 0.5 vs. 3.2 ± 0.5 vs. 3.0 ± 0.4 vs. 3.2 ± 0.3). Experimental Sahiwal cows were monitored from 2 months before (−60 days) to 4 months after (+120 days) calving.

The nutrient requirements of cows were met by feeding total mixed ration (TMR) at 0800 and 2000 hours *ad libitum* (NRC, 2001). The ingredient and nutrient compositions of TMR fed during experimental period are presented in Table 1. The experimental animals were supplemented individually with 60 mg Zn/kg DM in the form of feed-grade zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$; Luancheng Terife Agricultural Materials, Shijiazhuang, Hebei Province, China), 1000 IU Vit E in the form of DL-alpha-tocopheryl acetate (Xi'an Healthful Biotechnology, Hi-New Zone, Xi'an, China) and a combination of 60 mg/kg Zn and 1000 IU Vit E (Zn + Vit E).

Table 1 Ingredient and nutrient compositions of diets fed during the experimental period

	Control	Zn	Vit E	Zn + Vit E
Ingredients (%, as DM)				
Berseem fodder	19.8	19.8	19.8	19.8
Corn silage	14.2	14.2	14.2	14.2
Ground yellow maize	28.9	28.7	28.5	28.3
Groundnut cake	15.7	15.7	15.7	15.7
De-oiled Mustard cake	5.2	5.2	5.2	5.2
Wheat bran	6.4	6.4	6.4	6.4
Rice bran	8.3	8.3	8.3	8.3
Dicalcium phosphate	0.6	0.6	0.6	0.6
Salt	0.3	0.3	0.3	0.3
Trace minerals and vitamins premix*	0.6	0.6	0.6	0.6
Zinc sulphate heptahydrate†	–	0.2	–	0.2
DL-alpha-tocopheryl acetate†	–	–	0.4	0.4
Chemical composition (as DM)				
Dry matter, % (as fed)/ (wet weight)	75.6	75.6	75.6	75.6
Organic matter, %	78.3	78.3	78.3	78.3
Crude protein, %	17.5	17.5	17.5	17.5
NDF, %	38.6	38.6	38.6	38.6
ADF, %	25.9	25.9	25.9	25.9
Calcium, %	1.00	1.00	1.00	1.00
Phosphorus, %	0.41	0.41	0.41	0.41
Magnesium, %	0.2	0.2	0.2	0.2
Sodium, %	0.20	0.20	0.20	0.20
Chloride, %	0.41	0.41	0.41	0.41
Manganese, mg/kg	63.83	63.83	63.83	63.83
Copper, mg/kg	23.2	23.2	23.2	23.2
Zinc, mg/kg	49.54	109.54	49.54	109.54
DL-alpha-tocopheryl acetate, IU (total intake per animal and day)	211	211	1,211	1,211

Zn, zinc-treated group; Vit E, vitamin E-treated group; Zn + Vit E, zinc + vitamin E-treated group; ADF, acid detergent fibre; DM, dry matter; NDF, neutral detergent fibre.

*Premix composition per kilogram: vitamin A, 700 000 IU; vitamin D3, 70 000 IU; vitamin E, 250 mg; nicotinamide, 3000 mg; Ca, 190 000; P, 90 000; Na, 50 000; Cu, 1200 mg; Zn, 9600 mg; Fe, 1500 mg; Mn, 6000 mg; I, 325 mg; Co, 150 mg; Se, 10 mg; Mg, 19 000 mg.

†Zinc sulphate heptahydrate (0.20%) and DL-alpha-tocopheryl acetate (0.40%) were substituted for ground yellow maize to provide 60 mg/kg Zn and 1000 IU vitamin E.

The animals that received a basal diet devoid of additional supplemental Zn and Vit E acted as control. Zn and Vit E contents of the basal diet were 49 mg/kg diet DM and 211 IU (in total)/per cow and day.

Blood collection

Peripheral blood samples were collected at 0700 hours in heparinized vacutainer tubes (Becton Drive, Franklin Lakes, NJ, USA) by jugular vein puncture, posing minimum stress to buffalo calves, on days −60, −45, −30, −15, −7, −3, 0, 3, 7, 15, 30, 45, 60, 90 and 120 in relation to the expected date of calving. The samples were brought to the laboratory in chilled ice boxes soon after collection and centrifuged at 1200 *g* at 4 °C for 20 min to separate the plasma for the analysis of TIG, IgG, IL-2, Vit E and Zn.

Laboratory analysis

Feed intake after weighing feed refusals was recorded, and dry matter (DM) intake was calculated daily. Feed and fodder offered to animals were analysed for DM, crude protein (CP), ether extract (EE), crude fibre (CF) and total ash (AOAC, 1990). Detergent method was used for the estimation of neutral detergent fibre (NDF) and acid detergent fibre (ADF) in feed and fodder offered to cows during experimental period (Van Soest et al., 1991).

Total immunoglobulin (TIG) in the plasma sample was estimated by zinc turbidity method (McEvan and Fisher, 1970). IgG was determined in plasma of cows by 'Bovine IgG ELISA Kit' from Cusabio Biotech, Wuhan, Hubei, China. The detection level of the assay was 4.68 to 600 μg/ml. Intra- and interassay coefficients of variation were 6.74% and 9.95% respectively. IL-2 was determined in plasma of cows by 'Bovine IL-2 ELISA Kit' from Cusabio Biotech. The minimum detectable level of bovine IL-2 was 40 pg/ml. Intra- and interassay coefficients of variation were 5.74% and 8.95% respectively.

Vit E was measured in feed and plasma on a Waters HPLC Model 510 (Milford, MA, USA) fitted with a tunable absorbance detector model 486, a μ-Bondapak C-18 column of 3.9 × 300 mm size (Waters). The solvent system consisted of acetonitrile and HPLC water in the ratio of 95:5 at the flow rate of 1.7 ml/min (Chawla and Kaur, 2000). Tocopherol was detected at 290 nm. Zn was assayed in feed and plasma using an atomic absorption spectrophotometer (Model Z-5000, Polarized Zeeman Atomic Absorption Spectrophotometer; Hitachi High-Technologies Corporation, Tokyo, Japan) (AAS, 1988).

Statistical analysis

Data for all measured variables were analysed as repeated measures using the MIXED procedure of statistical software package SPSS version 19 (SPSS for Windows, V19.0; SPSS, Chicago, IL, USA). Two models were used.

The first model was used to estimate sampling day effect, treatment effect (Vit E and Zn supplementation) and their interaction:

$$Y_{ijk} = \mu + T_i + D_j + (T \times D)_{ij} + e_{ijk}$$

where Y_{ijk} = dependent variable; μ = overall mean of the population; T_i = mean effect of the treatment (Vit E and Zn supplementation) ($i = 1\ldots4$); D_j = mean effect of day of sampling ($j = 1\ldots15$) with day as a repeated factor; $(T \times D)_{ij}$ = effect of the interaction between effects of treatment group and day of sampling; and e_{ijk} = unexplained residual element assumed to be independent and normally distributed.

The second model was used to estimate the effect of physiological phase (pre- and post-partum), treatment effect (Vit E and Zn supplementation) and their interaction:

$$Y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + e_{ijk}$$

where Y_{ijk} = dependent variable; μ = overall mean of the population; P_i = mean effect of physiological phase (i = pre-partum, post-partum) with physiological phase as a repeated factor; T_i = mean effect of the treatment (Vit E and Zn supplementation) ($i = 1\ldots4$); $(T \times P)_{ij}$ = effect of the interaction between effect of treatment group and physiological phase; and e_{ij} = unexplained residual element assumed to be independent and normally distributed.

Data were analysed across sampling days relative to day of calving, with day 0 representing the day of calving. Day of calving was considered as a post-partum variable.

Briefly, the model included group, day (or physiological phase) and their interaction as fixed effects, and animal within group as the random effect. The pairwise comparison of means was made using 'Fisher's least significant difference test'. Different parameters (IgG, IL-2, Vit E and Zn) were correlated with Pearson's correlation method.

Results

The DMI decreased during pre-partum period and was lowest on the day of calving in all the four groups

Table 2 Feed intake as well as total immunoglobulin, immunoglobulin G, interleukin-2, vitamin E and zinc during pre- and post-calving along with p-values of vitamin E- and zinc-supplemented cows and non-supplemented control cows

	Groups					p-Value		
Parameters	Control	Zn	Vit E	Zn + Vit E	SEM	Group	Day	Groups × days
Total immunoglobulin (mg/ml)								
Pre-calving	32.55^{aA}	34.54^{abA}	34.35^{abA}	36.10^{bA}	0.85	0.030	<0.001	1.000
Post-calving	27.88^{aB}	31.20^{bB}	31.10^{bB}	33.57^{cB}	0.77			
Immunoglobulin G (mg/ml)								
Pre-calving	27.03^{aA}	28.61^{bA}	28.72^{bA}	30.02^{cA}	0.63	<0.001	<0.001	0.140
Post-calving	23.11^{aB}	25.45^{bB}	27.30^{cB}	29.61^{dA}	0.57			
Interleukin-2 (pg/ml)								
Pre-calving	62.14^{aA}	63.49^{aA}	63.77^{aA}	67.03^{bA}	1.41	<0.001	0.012	0.992
Post-calving	49.43^{aB}	52.93^{bB}	53.35^{bB}	60.19^{cB}	1.14			
Vitamin E (μg/ml)								
Pre-calving	2.69^{aA}	2.70^{aA}	2.92^{bA}	2.90^{b}	0.08	<0.001	<0.001	0.662
Post-calving	2.09^{aB}	2.08^{aB}	2.63^{bB}	2.74^{b}	0.06			
Zinc (μg/ml)								
Pre-calving	1.25^{aA}	1.42^{bA}	1.28^{aA}	1.43^{bA}	0.05	<0.001	<0.001	1.000
Post-calving	0.89^{aB}	1.18^{bB}	0.93^{aB}	1.20^{bB}	0.05			

SEM, standard error of the mean; Zn, zinc-treated group; Vit E, vitamin E-treated group; Zn + Vit E, zinc + vitamin E-treated group.
Means with different superscripts in small letters in a row and capital letters in column differ significantly ($p < 0.05$).

(Table 2). DMI started increasing after parturition due to the need of higher energy for production of milk (Table 2).

In the present experiment, plasma TIG levels steadily declined towards calving and found to be lowest on 3 days post-calving and then started to increase in all four groups (Fig. 1). Decline in plasma TIG concentration towards calving was found to be lower in Sahiwal cows receiving both Zn and Vit E. Plasma TIG showed higher ($p < 0.05$) values pre-calving compared with the level registered at post-calving in all four groups. During post-partum, concentration of TIG was recorded significantly higher ($p < 0.05$) in Zn + Vit E-supplemented cows in relation to control, Zn- and Vit E-fed groups (Table 2).

Plasma IgG level showed higher values pre-calving compared with the level observed at post-calving in all the four groups (Fig. 2). But post-calving plasma IgG levels were higher ($p < 0.05$) in Zn + Vit E group than in all other groups (Table 2). Lowest concentration of IgG was found at 3 days post-calving in control and Zn-fed groups and on day of calving in Vit E and

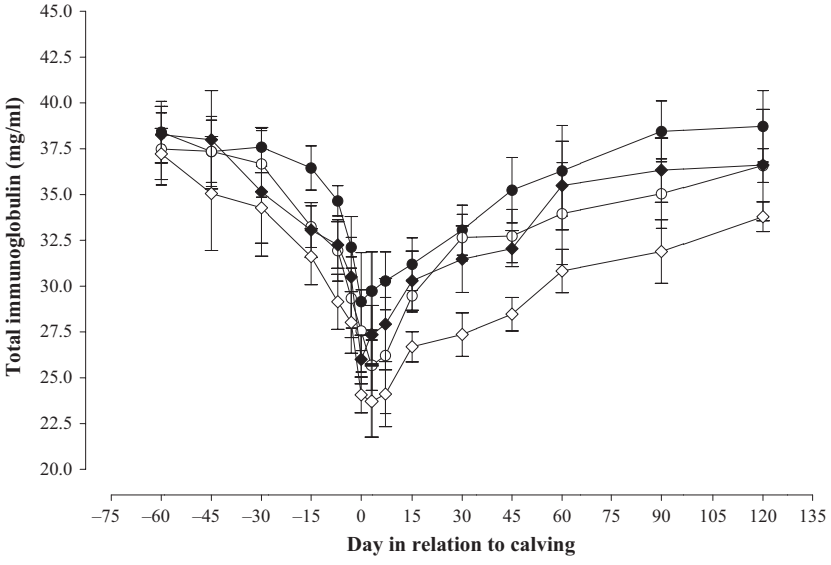

Fig. 1 Changes in plasma total immunoglobulin levels in control (◇), Zn (♦), Vit E (○) and Zn + E (●) groups of peripartum Sahiwal cows.

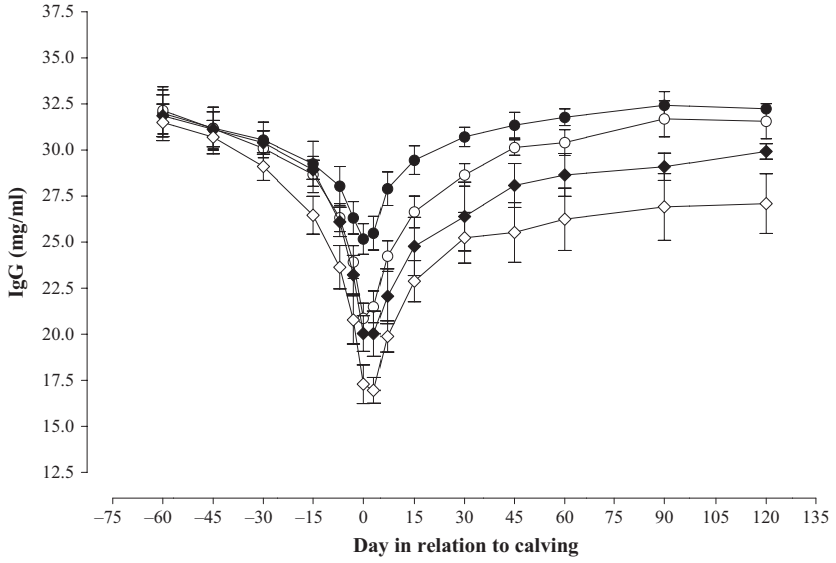

Fig. 2 Changes in plasma immunoglobulin G levels in control (◇), Zn (♦), Vit E (○) and Zn + E (●) groups of peripartum Sahiwal cows.

Zn + Vit E groups. In Zn + Vit E-fed group, IgG decreasing level was lower towards calving followed by Vit E, Zn and control groups. IgG was positively correlated with Zn ($p < 0.01$, $r = 0.559$) and Vit E ($p < 0.01$, $r = 0.652$).

In all four groups, pre-calving plasma IL-2 level was higher ($p < 0.05$) than at post-calving. However, in Zn + Vit E group, pre- and post-calving plasma IL-2 level was higher than in control, Zn and Vit E groups (Table 2). Plasma IL-2 began to decrease steadily from 30 days pre-partum and reached lowest on 3 days post-calving in all four respective groups (Fig. 3). Decreasing plasma IL-2 level was lower ($p < 0.05$) in Zn + Vit E group cows with respect to other three groups (Fig. 3). Interleukin-2 was positively correlated with IgG ($p < 0.01$, $r = 0.664$), Zn ($p < 0.01$, $r = 0.587$) and Vit E ($p < 0.01$, $r = 0.599$).

Pre-calving plasma Vit E showed significant ($p < 0.05$) different values than ones observed at post-calving in control, Zn and Vit E groups, whereas pre- and post-calving plasma Vit E was nearly similar in Zn + Vit E group (Table 2). Plasma Vit E concentrations were higher ($p < 0.05$) in Vit E and Zn + Vit E groups than in non-supplemented or only Zn-fed cows on all days of peripartum except days −60 and −45. The plasma Vit E level

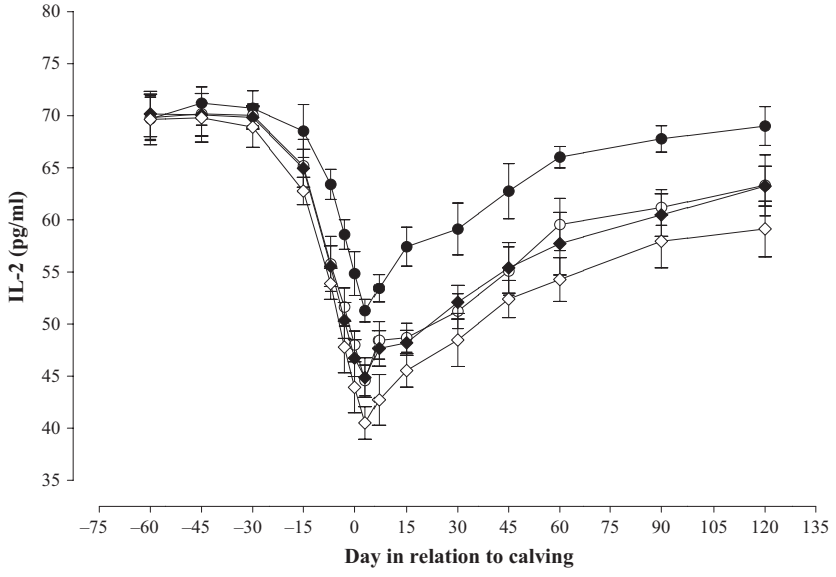

Fig. 3 Changes in plasma interleukin-2 levels in control (◇), Zn (♦), Vit E (○) and Zn + E (●) groups of peripartum Sahiwal cows.

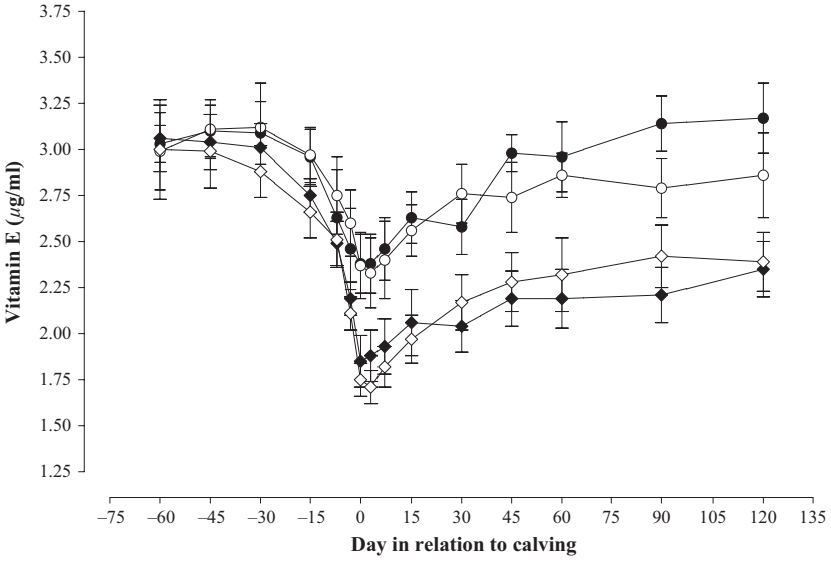

Fig. 4 Changes in plasma vitamin E levels in control (◇), Zn (♦), Vit E (○) and Zn + E (●) groups of peripartum Sahiwal cows.

continued to decrease substantially till calving (day 0) in all the groups. However, this decline was lower ($p < 0.05$) towards parturition in Vit E and Zn + Vit E groups (Fig. 4).

Similar to Vit E, plasma Zn levels were also decreased towards calving and they were lowest ($p < 0.05$) on 3 days post-partum in all the four groups (Fig. 5). Pattern of decreasing Zn plasma level was lower ($p < 0.05$) towards calving in Zn and Zn + Vit E groups in comparison with control and Vit E groups. Pre- and post-calving plasma Zn levels were higher in Zn and Zn + Vit E groups than in control and Vit E-fed animals (Table 2).

The overall value of milk yield was observed higher ($p < 0.05$) in Zn + Vit E group (8.57 ± 0.67 kg) in comparison with its level in control (6.86 ± 0.69 kg), Zn (7.75 ± 0.63 kg) and Vit E (7.78 ± 0.69 kg) groups. Overall milk yield increased by 13%, 13.4% and 24.9% in Zn, Vit E and Zn + Vit E groups, respectively, in comparison with control.

We observed symptoms of diseases in the control and experimental animals, that is, mastitis, retained foetal membrane and post-partum metritis. The number of mastitis cases was 4, 1, 0 and 0; retained foetal membrane was 4, 1, 1 and 0; and post-partum metritis cases was 4, 1, 1 and 0 in the control, zinc (Zn),

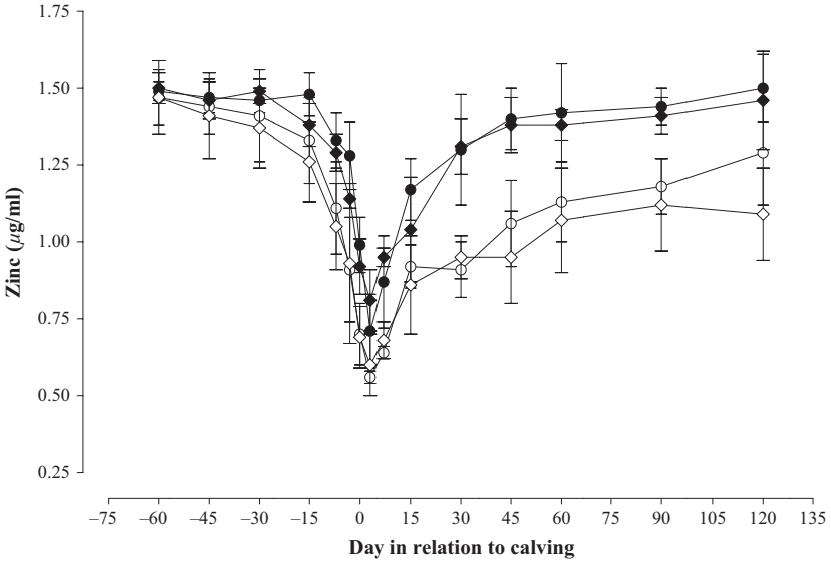

Fig. 5 Changes in plasma zinc levels in control (◇), Zn (♦), Vit E (○) and Zn + E (●) groups of peripartum Sahiwal cows.

vitamin E (Vit E) and zinc + vitamin E (Zn + Vit E) groups respectively.

Discussion

In the present experiment, DMI decreased around parturition due to significant ($p < 0.05$) increase in oxidative stress around calving as also observed by Castillo et al. (2005) and Grummer et al. (2004). DMI increased ($p < 0.05$) in Vit E-supplemented group in comparison with non-supplemented group during periparturient period (Chandra and Aggarwal, 2009).

In the present study, investigated immunological parameters (total plasma immunoglobulin, IgG and interleukin-2) decreased towards calving, but this decline was lower in Zn- and Vit E-supplemented Sahiwal cows. Decreased plasma TIG concentrations observed in this study were in agreement with previous reports in dairy cattle (Chandra and Aggarwal, 2009; Maurya, 2011). Conversely, Politis et al. (2004) reported that 3000 IU pre-partum and 1000 post-partum Vit E supplementation improved bovine leukocyte function and stimulated the production of immunoglobulin. Suppressed immune response during peripartum period is responsible for increased susceptibility of cows to infectious diseases (Chandra and Aggarwal, 2009; Maurya, 2011). This may partly be due to a decrease in plasma concentrations of antioxidants nutrients, that is, vitamins A and E, and Zn during transition period (Johnston and Chew, 1984; Goff and Stabel, 1990; Meglia et al., 2004; Chandra and Aggarwal, 2009).

Results of present findings on decreased plasma IgG concentration around calving are in consistent with findings of Herr et al. (2011) and of Maurya (2011). Decreased plasma IgG levels might be due to reduced antibody secretion by B cells (Detilleux et al., 1995) and increased lacteal IgG excretion in colostrum (Van Kampen and Mallard, 1997). In the present findings, we documented a significant decrease in plasma IgG in the early post-calving period, which might be due to decreased circulating plasma Zn and Vit E concentrations. This result is in line with those from previous studies (Chatterjee et al., 2003; Maurya, 2011).

There were no earlier reports regarding changes in circulating IL-2 levels in dairy cows during peripartum period. In the present study, post-calving plasma IL-2 concentration was lower in comparison with pre-calving IL-2 concentration. IL-2 production decreased towards parturition, which might be due to the decreased production by Th1 cytokines (Delassus et al., 1994). Along with other factors, reduced circulating plasma concentration of IL-2 is responsible for lowered cell-mediated immunity. The changes in immune status develop in parallel with increased plasma corticosteroid levels as well as increases in oestrogen and progesterone. Corticosteroids are also known to suppress cell-mediated immune responses by suppressing the production of Th1 cytokines, IFN-γ and IL-2 (Vacca et al., 1992). Reduced production of IFN-γ and IL-2 during peripartum period is also responsible for suppressed Th1 immune responses (Sordillo et al., 1991; Shafer-Weaver and Sordillo, 1997).

In the present experiment, findings on increased plasma concentration of IL-2 in Zn-supplemented cows are in agreement with findings of Prasad et al. (2002); Kaltenberg et al. (2010) in humans. Zn is also needed for the maintenance of lymphocyte replication and antibody production. Therefore, insufficient Zn plasma concentrations during periparturient period impaired immune response by suppressing lymphocyte proliferation and IL-2 secretion (Pinna et al., 2002). Similar to the increased level of IL-2 in Zn-fed cows, Vit E-supplemented groups also have increased plasma IL-2 levels, which is in consistent with the findings of Meydani et al. (1990) and Moriguchi and Itoh (1997) in humans. They concluded that dietary Vit E supplementation stimulates the production of IL-2 by thymocytes, which might be due to inhibiting production prostaglandin E2 (PGE2).

Findings of the present study show positive correlations between plasma IgG, Zn and Vit E concentrations. A similar correlation between the concentration of plasma IgG and plasma Zn was found by Prasad et al. (1993). Zn supplementation restored the normal level of Zn in lymphocytes and neutrophils but also improved numbers of circulating T lymphocytes and IgG (Cossack, 1989; Prasad et al., 1993) in humans. Dietary Vit E supplementation increased lymphocyte proliferation (Garber et al., 1996) and IgG production (Cao et al., 1992). Similar to IgG, plasma IL-2 concentration was also positively correlated with plasma Vit E and Zn levels. This is in agreement with the findings of Meydani et al. (1990) and Kaltenberg et al. (2010) in humans. This might be due to the increased production of IL-2 in dietary Zn- and Vit E-fed peripartum Sahiwal cows.

In accordance with other studies (Goff and Stabel, 1990; Chandra and Aggarwal, 2009; Bouwstra et al., 2010; Maurya, 2011), plasma Vit E decreases as parturition approaches and remains low for several days after parturition. Decreased plasma Vit E concentration after calving is mainly due to reduced dietary Vit E intake and their losses via colostrum (Goff and Stabel, 1990). However, the decline at calving may

also be explained by a decreased plasma lipoprotein concentration (Herdt and Stowe, 1991). Similar to the present findings, various other workers (Politis et al., 1996; Weiss, 2002) also reported beneficial effect of dietary Vit E supplementation on plasma Vit E levels and enhanced immune function during parturition.

The significant drop in plasma Zn concentration at calving (Meglia et al., 2001, 2004) is most likely a consequence of colostrum formation (Goff and Stabel, 1990), parturition stress and acute phase response due to inflammatory reactions in the uterus.

Stress induces synthesis of metallothionein, a protein associated with Zn distribution. As a consequence, Zn is redistributed from blood to other tissues (Xin et al., 1993). In support to other studies (Campbell and Miller, 1998; Maurya, 2011), the concentrations of plasma Zn were higher in Zn and Zn + Vit E groups than in non-supplemented control and Vit E groups.

In the present experiment, the milk yield was found to be higher in Zn + Vit E group followed by Vit E, Zn and control groups, indicating that Vit E and Zn supplementation increased the milk yield. This was attributed to an increase in DMI in Vit E- and Zn-supplemented groups (Maurya, 2011). As Griffiths et al. (2007) showed, supplementing cows with 529 CTM (providing daily 360 mg Zn, 200 mg Mn, 125 mg Cu as amino acid complexes and 12 mg cobalt (Co) from Co glucoheptonate) resulted in a 6.3% increase in milk production. Increase in milk production might be partially attributed to the role of Zn in the cell division and protein synthesis, as increasing Zn can improve integrity of epithelial tissue, such as teats and udder tissue (Sobhanirad et al., 2010).

In conclusion, plasma levels of TIG, IgG, IL-2, Vit E and Zn decreased as the day of calving advances. Dietary supplementation of vitamin E and Zn improved immune response in peripartum Sahiwal cows by increasing the production of TIG, IgG and IL-2. Dietary Zn and vitamin E supplementation maintained plasma concentration of Vit E and Zn. It also reduces the number of mastitis, retained foetal membrane and post-partum metritis cases in experimental animals as compared to control. Therefore, dietary supplementation of Zn and vitamin E during peripartum period helps to improve immune and health status of animals.

Acknowledgements

The authors express sincere thanks to the Director, NDRI, Karnal, for providing necessary facilities for this work. The financial assistance for the research was provided by Department of Biotechnology (DBT), New Delhi, India, and National Initiative on Climate Resilient Agriculture (NICRA) project of Indian Council of Agricultural Research (ICAR), New Delhi.

References

AAS, 1988: *Phillips Scientific Atomic Absorption Data Book*, 5th edn. Phillips Scientific, Cambridge, UK.

AOAC, 1990: *Official Methods of Analysis*, 15th edn. Association of Official Analytical Chemists, Washington, DC.

Bouwstra, R. J.; Nielen, M. J.; Newbold, R.; Jansen, E. H. J. M.; Jelinek, H. F.; van Werven, T., 2010: Vitamin E supplementation during the dry period in dairy cattle. Part II: oxidative stress following vitamin E supplementation may increase clinical mastitis incidence postpartum. *Journal of Dairy Science* **93**, 5696–5706.

Campbell, M. H.; Miller, J. K., 1998: Effect of supplemental dietary vitamin E and zinc on reproductive performance of dairy cows and heifers fed excess iron. *Journal of Dairy Science* **81**, 2693–2699.

Cao, Y.; Maddox, J. F.; Mastro, A. M.; Scholz, R. W.; Hildenbrandt, G.; Reddy, C. C., 1992: Selenium deficiency alters the lipoxygenase pathway and mitogenic response of bovine lymphocytes. *Journal of Nutrition* **122**, 2121–2127.

Castillo, C.; Hernandez, J.; Bravo, A.; Lopez-Alonso, M.; Pereira, V.; Benedito, J. L., 2005: Oxidative status during late pregnancy and early lactation in dairy cows. *The Veterinary Journal* **169**, 286–292.

Chandra, G.; Aggarwal, A., 2009: Effect of DL-α-tocopherol acetate on calving induced oxidative stress in periparturient crossbred cows during summer and winter seasons. *Indian Journal of Animal Nutrition* **26**, 204–210.

Chatterjee, P. N.; Kaur, H.; Panda, N., 2003: Effect of vitamin E supplementation on plasma antioxidant vitamins and immunity status of crossbred cows. *Asian-Australasian Journal Animal Sciences* **16**, 1614–1618.

Chawla, R.; Kaur, H., 2000: A new HPLC technique developed for simultaneous estimation of β-carotene, α-tocopherol and retinol in blood plasma and feed sample. *NDRI News* **4**, 2.

Cossack, Z. T., 1989: T-lymphocyte dysfunction in the elderly associated with zinc deficiency and subnormal nucleoside phosphorylase activity: effect of zinc supplementation. *European Journal of Cancer and Clinical Oncology* **25**, 973–976.

Delassus, S.; Coutinho, G. C.; Saucier, C.; Darche, S.; Kourilsky, P., 1994: Differential cytokine expression in maternal blood and placenta during gestation. *Journal of Immunology* **152**, 2411–2420.

Detilleux, J. C.; Kehrli, M. E. Jr; Stabel, J. R.; Freeman, A. E.; Kelley, D. H., 1995: Study of immunological dysfunction in periparturient Holstein cattle selected for high and average milk production. *Veterinary Immunology and Immunopathology* **44**, 251–267.

Fraker, P. J.; Gershwin, M. E.; Good, R. A.; Prasad, A., 1986: Interrelationships between zinc and immune function. *Federation Proceeding* **45**, 1474–1479.

Garber, M. J.; Roeder, R. A.; Davidson, P. M.; Pumfrey, W. M.; Schelling, G. T., 1996: Dose-response effects of vitamin E

supplementation on growth performance and meat characteristics in beef and dairy steers. *Canadian Journal of Animal Science* **16**, 63–72.

Goff, J. P.; Stabel, J. R., 1990: Decreased plasma retinol, α-tocopherol, and zinc concentration during the periparturient period. Effect of milk fever. *Journal of Dairy Science* **73**, 3195–3199.

Griffiths, L. M.; Loeffler, L. H.; Socha, M. T.; Tomlinson, D. J.; Johnson, A. B., 2007: Effects of supplementing complexed zinc, manganese, copper and cobalt on lactation and reproductive performance of intensively grazed lactating dairy cattle on the south island of New Zealand. *Animal Feed Science and Technology* **137**, 69–83.

Grummer, R. R., 1995: Impact in changes in organic nutrient metabolism on feeding the transition cow. *Journal of Animal Science* **73**, 2820–2833.

Grummer, R. R.; Mashek, D. G.; Hayırlı, A., 2004: Dry matter intake and energy balance in the transition period. *Veterinary Clinics of North America: Food Animal Practice* **20**, 447–470.

Herdt, T. H.; Stowe, H. D., 1991: Fat-soluble vitamin nutrition for dairy cattle. *Veterinary Clinics of North America: Food Animal Practice* **7**, 391–415.

Herr, M.; Bostedt, H.; Failing, K., 2011: IgG and IgM levels in dairy cows during the periparturient period. *Theriogenology* **75**, 377–385.

Johnston, L. A.; Chew, B. P., 1984: Peripartum changes of plasma and milk vitamin A and β-carotene among dairy cows with or without mastitis. *Journal of Dairy Science* **67**, 1832–1840.

Kaltenberg, J.; Plum, L. M.; Ober-Blöbaum, J. L.; Hönscheid, A.; Rink, L.; Haase, H., 2010: Zinc signals promote IL-2-dependent proliferation of T cells. *European Journal of Immunology* **40**, 1496–1503.

Kehrli, M. E. Jr; Kimura, K.; Goff, J. P.; Stabel, J. R.; Nonnecke, B. J., 1998: Periparturient immunosuppression in dairy cows: nutrition and lactation effects. In: T. H. Wensing (ed.), *Production Diseases in Farm Animals. 10th International Conference on Production Diseases*. Wageningen Academic Publishers, Utrecht, the Netherlands, pp. 41–55.

Likoff, R. O.; Guptill, D. R.; Lawrence, L. M.; McKay, C. C.; Mathias, M. M.; Nockels, C. F.; Tengerdy, R. P., 1981: Vitamin E and aspirin depress prostaglandins in protection of chickens against *E. coli* infection. *American Journal of Clinical Nutrition* **34**, 245–251.

Maurya, P., 2011: Leptin level in relation to immunity, energy metabolites and cellular adaptations during dry period and early lactation in crossbred cows. MVSc thesis, NDRI (deemed University), Karnal, India.

McEvan, A. D.; Fisher, E. W., 1970: A turbidity test for the estimation of immunoglobulins levels in neonatal calf plasma. *Clinica Chemica Acta* **17**, 155–165.

Meglia, G. E.; Johannisson, A.; Petersson, L.; Persson Waller, K., 2001: Changes in some blood micronutrients, leukocyte and neutrophil expression of adhesion molecules in periparturient dairy cows. *Acta Veterinaria Scandinavica* **42**, 109–120.

Meglia, G. E.; Holtenius, K.; Petersson, L.; Ohagen, P.; Persson Waller, K., 2004: Prediction of vitamin A, vitamin E, selenium and zinc status of periparturient dairy cows using blood sampling during the mid dry period. *Acta Veterinaria Scandinavica* **45**, 119–128.

Meydani, S. M.; Hafek, M., 1992: Vitamin E and the immune response in nutrition and immunology. In: R. K. Chandra (ed.), *Nutrition and Immunology*. Arts Biomedical Publ. & Distributors, New Foundland, pp. 105–128.

Meydani, S. N.; Barklund, M. P.; Liu, S.; Miller, R. A.; Cannon, J. G.; Morow, F. D.; Rocklin, R.; Blumberg, J. B., 1990: Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *American Journal of Clinical Nutrition* **53**, 557–563.

Moriguchi, S.; Itoh, T., 1997: Vitamin E enhances T cell-differentiation through increased epithelial cell function in rat thymus. *Nutrition Research* **17**, 873–883.

NRC (National Research Council), 2001: *Nutrient Requirements of Dairy Cattle*, 7th revised edn. National Academy, Washington, DC.

Panda, N.; Kaur, H.; Mohanty, T. K., 2006: Reproductive performance of dairy buffaloes supplemented with varying levels of vitamin E. *Asian-Australasian Journal of Animal Sciences* **19**, 19–25.

Pinna, K.; Kelley, D. S.; Taylor, P. C.; King, J. C., 2002: Immune functions are maintained in healthy men with low zinc intake. *Journal of Nutrition* **132**, 2033–2036.

Politis, I.; Hidiroglou, N.; White, J. H.; Gilmore, J. A.; Williams, S. N.; Scherf, H., 1996: Effects of vitamin E on mammary and blood leukocyte function, with emphasis on chemotoxis, in periparturient dairy cows. *American Journal of Veterinary Research* **57**, 468–471.

Politis, I.; Bizelis, I.; Tsiaras, A.; Baldi, A., 2004: Effect of vitamin E supplementation on neutrophil function, milk composition and plasmin activity in dairy cows in a commercial herd. *Journal of Dairy Science* **71**, 273–278.

Prasad, A. S.; Fitzgerald, J. T.; Hess, J. W.; Kaplan, J.; Pelen, F.; Dardenne, M., 1993: Zinc deficiency in elderly patients. *Nutrition* **9**, 218–224.

Prasad, A. S.; Bao, B.; Beck, F. W. J.; Sarkar, F. H., 2002: Zinc enhances the expression of interleukin-2 and interleukin-2 receptors in HUT-78 cells by way of NF-κB activation. *Journal of Laboratory and Clinical Medicine* **140**, 272–289.

Shafer-Weaver, K. A.; Sordillo, L. M., 1997: Bovine CD8 + suppressor lymphocytes alter immune responsiveness during the postpartum period. *Veterinary Immunology and Immunopathology* **56**, 53–64.

Smith, K. L.; Hogan, J. S.; Weiss, W. P., 1997: Dietary vitamin E and selenium affect mastitis and milk quality. *Journal of Animal Science* **75**, 1659–1665.

Sobhanirad, S.; Carlson, D.; Kashani, R. B., 2010: Effect of zinc methionine or zinc sulfate supplementation on milk production and composition of milk in lactating dairy cows. *Biological Trace Element Research* **136**, 48–54.

Sordillo, L. M., 2005: Factors affecting mammary gland immunity and mastitis susceptibility. *Livestock Production Science* **98**, 89–99.

Sordillo, L. M.; Redmond, M. J.; Campos, M.; Warren, L.; Babiuk, L. A., 1991: Cytokine activity in bovine mammary gland secretions during the periparturient period. *Canadian Journal of Veterinary Research* **55**, 298–301.

Stabel, J. R.; Reinhardt, T. A.; Stevens, M. A.; Kehrli, M. E. Jr; Nonnecke, B. J., 1992: Vitamin E effects on in vitro immunoglobulin M and interleukin-1 beta production and transcription in dairy cattle. *Journal of Dairy Science* **75**, 2190–2198.

Tengerdy, R. P., 1980: Disease resistance: immune response. In: L. J. Machlin (ed.), *Vitamin E: A Comprehensive Treatise*. Marcel Dekker, New York, NY, pp. 429–443.

Underwood, E. J.; Suttle, N. F., 1999: *The Mineral Nutrition of Livestock*, 3rd edn. CABI Publishing, CAB International, Wallingford, Oxon, UK.

Vacca, A.; Felli, M. P.; Farina, A. R.; Martinotti, S. S.; Maroder, M.; Screpanti, I. U.; Meco, D.; Petrangeli, E.; Frati, L.; Gulino, A., 1992: Glucocorticoid receptor-mediated suppression of the interleukin 2 gene expression through impairment of the cooperativity between nuclear factor of activated T cells and AP-1 enhancer elements. *Journal of Experimental Medicine* **175**, 637–646.

Van Kampen, C.; Mallard, B. M., 1997: Effects of peripartum stress and health on circulating bovine lymphocyte subsets. *Veterinary Immunology and Immunopathology* **59**, 79–91.

Van Soest, P. J.; Robertson, J. B.; Lewis, B. A., 1991: Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597.

Weiss, W. P., 2002: Relationship of mineral and vitamin supplementation with mastitis and milk quality. In: *Proceedings of the 41st Annual Meeting of the National Mastitis Council*. NMC, Orlando, FL, pp. 37–44.

Xin, Z.; Waterman, D. F.; Hemken, R. W.; Harmon, R. J., 1993: Copper status and requirement during the dry period and early lactation in multiparous Holstein cows. *Journal of Dairy Science* **76**, 2711–2716.