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RESEARCH ARTICLE

Effect of vitamin E and zinc supplementation in pre and post partum period in crossbred cows on anti-oxidant, immunity status and performance of new born calves

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Abstract: The present study was undertaken to investigate the effect of zinc (Zn) supplementation alone and its combination with vitamin E on growth, anti oxidant status and immunity status of new born calves of crossbred cows. The cows were supplemented with Zn @ 0 ppm (T_0), Zn @ 80 ppm (T_1) and Zn @ 80 ppm + 1000 IU α-tocopheryl acetate/d (T_2) from 60 days prepartum up to 90 days postpartum. The fat, SNF, total solid, protein, Zn and Cu contents content of colostrum showed no effect of dietary treatments but α-tocopherol content was higher (P<0.01) in group T_2 as compared to other groups. Total immunoglobulin in colostrum of T_2 group was 25.87% higher than control. There was no effect of dietary treatments on plasma Zn and Cu levels of calves, however α-tocopherol concentration at day 5 was higher (P<0.05) in group T_2 as compared to other groups. Status of antioxidant enzymes, lymphocyte proliferation index, total and differential leukocyte count was not affected by the treatments. Higher birth weight (P<0.05) was obtained in group T_2 group as compared to other group. Calves from treatment groups showed lower incidence of morbidity and mortality due to calf scour and other infectious diseases compared to control.

Keywords: α-tocopherol, Body weight, Calf morbidity, Colostrum, Crossbred cow, Immunity

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Introduction

Colostrum serves as the first source of nutrients that a calf consumes after birth. It contains large amount of energy, protein, minerals and fat soluble vitamins which are required by the calves for normal metabolic functions, growth and establishment of immune system. At birth, calves exhibit a poorly developed (physiologically immature) immune system which renders them more susceptible to infectious diseases (Teixeira et al. 2014). Dairy replacement success or failure is dependent on newborn calf health and growth which can be impaired by poor maternal health, colostrum deprivation and poor calf nutrition (Teixeira et al. 2014). Maternal nutrition during late gestation and after parturition can have lasting impacts on calf health, growth and performance performance (Abuelo et al. 2019; Dunn et al.2017). Calves supplemented with Zn shows greater average daily gain and lower mortality (Kegley et al. 2001; Ahola et al. 2004). Feldman et al. (2019) found that Zn supplementation was beneficial for prevention of diarrhea in dairy calves, thus, minimizing the use of antimicrobial. Calf diarrhea is a major cause of high mortality and morbidity in the dairy industry and it has been found that Zn status in animals declined in gastrointestinal disorders (Ranjan et al. 2006). Holstein cows supplemented with combination of inorganic and organic minerals (Zn, Cu, Mn and Co) from 21 day prepartum showed greater concentration of IgG in the colostrum (Kincaid and Socha, 2004;Jaff et al.2020;Putman et al.2018). The α-tocopherol does not cross the placenta in appreciable amounts, and calf is dependent on colostrum to obtain vitamin E after birth. Weiss et al. (1998) supplemented vitamin E and found higher vitamin E content in colostrum compared to control cows. Supplementation of fat soluble vitamins E and D_3 in advance pregnant buffaloes during 30 days prepartum resulted in higher birth weight of calves (Sikka et al. 2002). The vitamin E (α-tocopherol) status of dairy cows is one the important components of immune system because of its antioxidant effects at parturition, plasma concentrations of vitamin E were found to decrease by 47% because of secretion of the vitamin into the udder during colostrogenesis, decreased DM intake at calving, and an increased need for antioxidants during this time (Kafilzadeh et al. 2014) showing that supplementation of vitamin E had positive effect on mother and young ones. Minerals and vitamin supplementation in pre and post partum period affect the immunoglobulin levels as well as the antioxidant enzyme activity of the calves (Kincaid, 2008; Gaal et al. 2006). So supplementation of certain minerals and vitamins might affect the composition of colostrum ultimately the health and performance of the calves. Therefore,the objectives of present study was to determine the effects of zinc supplementation alone and its combination with vitamin E in pre and post partum period on growth, anti oxidant status and immunity status of new born calves of crossbred cows.

Materials and Methods

Selection of animals and feeding management

Twenty four crossbred (Karan Fries) pregnant cows in their late lactation (before drying) were selected from institute herd. All the cows were free from anatomical, physiological and infectious disorders, which are ascertained by physical examination as well as from records. They were divided into three groups of eight animals each on the basis of their expected producing ability (EPA; Lush, 1945). All the experimental procedure were in compliance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2012, India) for the care and use of animal for scientific purposes. Experimental animals were fed iso-nitrogenous and iso-caloric diet as per NRC (1989). In addition to the basal level zn was not supplemented (T_0),while cows of groups T_1 and T_2 were supplemented with Zinc @ 80 mg/kg DMI and Zn @ 80 mg/kg DM intake along with vitamin E @ 1000 IU/ cow/ d respectively, in the dry period (60 days before parturition) which was continued up to 90 days of lactation. Zinc was supplied in the form of $ZnSO_4.7H_2O$ (Thomas Baker Pvt. Ltd.). The cows were moved to calving pen 15 days before the expected date of calving and provided with soft bedding during this time. Colostrum samples on day 1 and 5 were collected from cows and milk composition (Fat, protein, SNF and total solids), Zn and Cu Copper content, α-tocopherol content and total immunoglobulins estimations were carried out. Blood samples were collected from individual calf on day 1 and 5 after birth for the estimation of plasma zinc and copper, α-tocopherol, total antioxidant activity, enzymes (Superoxide dismutase, glutathione peroxidase and alkaline phosphatase), lymphocyte proliferation, Plasma total immunoglobulin, total leukocyte count and differential leukocyte count. The incidence of morbidity and mortality in calves were recorded up to the age of three months.

Chemical analysis of samples

The roughage and concentrate were ground individually, labeled and analyzed for proximate composition as per AOAC (2005) and cell wall constituents as per Goering and Van Soest (1970). Concentration of Zn and Cu in colostrum and blood samples were analysed by atomic absorption spectrophotometer (Hitachi Z-5000, Hitachi Ltd., Japan). An HPLC method for simultaneous estimation of retinol and α-tocopherol in feed, fodders, plasma and milk samples was adopted (Chawla and Harjit Kaur, 2001; Harjit Kaur et. al. 2004). In blood samples the haemoglobin (Drabkin ,1944), total leukocyte count was made by the Haemocytometer method by (Schalm,1961), total antioxidant activity in term of Ferric Reducing Antioxidant Power assay by Benzie and Strain (1999). The activity of superoxide dismutase (Marklund and Marklund ,1974), glutathione peroxidase (Hafeman et al. ,1974), Alkaline phosphatase activity (Klin,1970). Immunoglobulins in the plasma sample were estimated by Zn turbidity method (McEwan and Fisher, 1970). Colostrum samples were analysed for percentage of fat (AOAC 2005), crude protein (BIS, 1981) and total solids (AOAC, 2005). The SNF content of colostrum was calculated by subtracting percentage of fat from total solids. Fat, protein, SNF and total solids contents of milk were determined by Funke-Gerber Lactostar (Benny Impex Pvt. Ltd.).

Statistical analysis: Statistical analysis of the data was by ANOVA as per Snedecor and Cochran (1994) with the help of software package (SPSS 1998). The effect of treatments was analysed by two-way ANOVA.

$X_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$ (i= 1… I; j= 1… J)

where μ, Overall mean; α_i, Row effect; β_j, Column effect and ε_{ij}, Random error for observation X_{ij}

Results and Discussion

Effect of supplementation of Zn and vitamin E on composition of colostrum

The fat, SNF, total solid and protein content of colostrum decreased on the day of parturition to day 5 (Table 1) and was similar (P>0.05) between different groups from 1st to 5th day showing no effect of dietary treatments. The contents of fat, protein, SNF and total solids of colostrum were within the range reported earlier in crossbred cows (Panda et al. 2006; Patoo et al. 2016) and were not affected by the dietary treatment of cows.

The Zn, Cu and α-tocopherol contents of colostrum

There was no effect of dietary treatments on Zn and Cu content of colostrum on both day 1 and day 5 samples in three respective groups (P>0.05; Table 2). The α-Tocopherol content of colostrum on day 1 averaged 4.68, 4.87 and 5.52 µg/ml and decreased to 3.29, 3.38 and 4.09 µg/ml in groups T_0, T_1 and T_2, respectively and was significantly (P<0.01) higher in group T_2 on both days (P<0.01; Table 2). Higher α-tocopherol content in group T_2 might be due to supplementation of Vitamin E along with Zn in this group which affect the absorption and secretion of α-tocopherol in the colostrum (Weiss *et al*. 1997; Panda et al. 2006; Micinski et al. 2017).

Influence of supplementation of Zn and Vitamin E on total immunoglobulin concentration of colostrum

Cows supplemented with combination of Zn and vitamin E produced higher ($P<0.05$) total Ig (25.87%) in colostrum than control (Table 2). At the end of colostrum period the total Ig concentration in colostrum declined in all the groups. The values were similar in all the groups. The total immunoglobulin was significantly ($P<0.05$) higher in group T_2 indicating that cows supplemented with combination of Zn and vitamin E produced more colostral immunoglobulins and have better immunity status over other two groups (Panigrahi et al. 2005; Sikka and Lal, 2006; Dang et al. 2009; Immler et al.2022).

Plasma Zn, Cu and α-tocopherol Status of Calves

There was no effect of dietary treatments given to cows on plasma Zn and Cu levels of calves on first 5 days of their age (Table 3). The plasma α-tocopherol concentration at day 5 was higher ($P<0.05$) in group T_2 than other two groups. At the end of colostrum feeding period, plasma α-tocopherol concentration in calves increased in group T_2 which might be due to consumption of vitamin E rich colostrum from vitamin E supplemented cows (Pavlata et al. 2004;. Kincaid et al. 2004; Sikka and Lal, 2006)

Antioxidant status of Calves

The activity of FRAP and erythrocytic antioxidants like SOD and GPx was similar ($P>0.05$) in different groups on day 1 and 5. The activity of ALP was also not affected by treatments (Cusack et al. 2005; Gaal et al. 2006).

Immunity status of calves

Cell mediated immune response in calves was assessed by *in vitro* lymphocyte proliferation by using ConA as mitogen. The stimulation index (SI) after birth averaged 0.73, 0.70 and 0.75 and increased to 0.93, 0.95 and 0.94 at the end of 5 days of colostrum feeding (Table 5). However, there was no difference in lymphocyte proliferation index of calves on both days in three respective groups Though supplementation of Zn at different levels ranging from 15-360 ppm in calves resulted in improved lymphocyte proliferation response (Kegley et al. 2001; Nagalakshmi et al. 2009) but feeding of colostrum from Zn supplemented cows did not improve the cell mediated immunity of their calves (Table 5) because there was no increase in the Zn content of colostrum (Table 2) which could have stimulated the T-cell proliferation. Buffalo calves fed on vitamin E supplemented milk (1500 and 2000 IU/d) for 60 days resulted in improvement in cell mediated immune response (2.06 vs.1.89) compared to control (Panda, 2006), but no such effect was seen in the present experiment in calves born to T_2 group of cows supplemented with Zn and vitamin E probably because the experiment was restricted to only 5 days and moreover the amount of Zn and vitamin E had not increased at such extent to produce effect.

Table 1: Composition of colostrum in cows

Day	T_0	T_1	T_2	SEM
		Fat (%)		
1	6.46	6.40	6.39	0.08
5	4.88	4.78	4.83	0.08
		SNF (%)		
1	16.79	15.64	16.65	0.30
5	8.50	8.40	8.23	0.13
		Total solids (%)		
1	23.25	22.04	23.04	0.30
5	13.38	13.18	13.05	0.10
		Protein (%)		
1	11.08	11.33	11.24	0.12
5	4.19	4.18	3.99	0.06

Table 2: The Zn, Cu, α-tocopherol and total immunoglobulin concentration in colostrum of cows

Day	T_0	T_1	T_2	SEM
		Zinc (ppm)		
1	12.82	13.63	13.55	0.30
5	10.00	10.49	10.92	0.28
		Copper (ppm)		
1	0.62	0.60	0.63	0.02
5	0.49	0.53	0.51	0.03
		α-tocopherol (μg/mL)		
1	4.68^a	4.87^a	5.52^b	0.11
5	3.29^a	3.38^a	4.09^b	0.10
		α-tocopherol (μg/g) of fat		
1	72.94^a	76.37^a	86.97^b	2.29
5	67.80^a	71.20^a	85.18^b	2.34
		Total Immunoglobulin (mg/mL)		
1	23.38^a	27.18^{ab}	29.43^b	1.07
5	3.93	3.77	3.89	0.51

^{a,b} Means having different superscripts within a row differ significantly ($P<0.05$).

The total Ig concentration at birth of calves was not affected by dietary treatments (Table 5). At the end of colostrum period, the total Ig concentration was higher ($P<0.05$) in group T_1 and T_2 calves than control group. Sikka et al. (2002) also observed that calves born from vitamin E supplemented buffaloes showed higher absorbed Ig levels (51 vs. 30 mg/10 mL) in blood serum up to 45 days after birth. In a similar study, secretion of immunoglobulins in colostrum was enhanced by 80% in fat soluble vitamins injected buffaloes (Sikka and Lal, 2006).

Total and differential leukocyte count of calves

There was no difference in the TLC count at birth and at day 5 in T_0, T_1 and T_2 groups of calves (Table 6). The TLC and DLC values in the study were in normal physiological range (Mishra et al. 2005; Kapale et al. 2008).

Birth weight of calves

The birth weight of calves averaged 27.0, 23.0 and 30.2 kg in three respective groups (Table 7). Higher ($P<0.05$) birth weight was observed in group T_2 compared to other groups. Supplementation of two doses of fat soluble vitamins (vitamin A and D_3 @ 2,500,000 IU and vitamin E @ 1000 IU) in advance pregnant buffaloes during 30 days prepartum resulted in higher birth weight (29.0 vs. 27.5 kg) of calves (Sikka et al. 2002). Injection of either 1000 IU vitamin E and 10 mg Se or 2000 IU vitamin E and 20 mg Se at 4th and 2nd week prepartum to heifers resulted in

Table 3. Plasma Zn, Cu and α-tocopherol status of calves

Day	T_0	T_1	T_2	SEM
		Zinc (ppm)		
1	0.79	0.83	0.81	0.027
5	1.00	0.99	1.02	0.029
		Copper (ppm)		
1	0.61	0.58	0.59	0.026
5	0.70	0.71	0.69	0.030
		α-tocopherol (µg/mL)		
1	0.41	0.38	0.47	0.033
5	0.52^a	0.59^a	0.88^b	0.058

[a,b] Means having different superscripts within a row differ significantly($P<0.05$).

Table 4. Antioxidant status of calves

Days	T_0	T_1	T_2	SEM
		FRAP (µmol/L)		
01	2614.3	2571.4	2652.3	45.73
05	2847.6	2909.5	2957.1	46.47
		SOD (Units/g Hb)		
01	1848.2	1928.2	1804.1	44.11
05	1943.1	2129.0	2034.4	52.06
		GPx (Units/g Hb)		
01	119.87	121.65	123.39	1.34
05	122.39	122.27	125.18	0.90
		ALP (U/L)		
01	67.34	70.45	72.93	2.45
05	40.14	39.70	37.58	1.35

Table 5: Immunity status of calves

Days	T_0	T_1	T_2	SEM
		Lymphocyte Stimulation Inde x		
1	0.73	0.70	0.75	0.020
5	0.93	0.95	0.94	0.016
		Total Ig (mg/mL)		
1	7.20	8.40	7.86	0.40
5	18.95^a	22.08^b	21.59^b	0.56

[a,b] Means having different superscripts within a row differ significantly ($P<0.05$).

Table 6. Total and differential leukocyte count of calves

Days	T_0	T_1	T_2	SEM
		Total leukocyte Count (10^3 cumm)		
1	7.81	8.02	7.77	0.18
5	7.62	7.91	7.69	0.14
		Neutrophils (%)		
1	22.40	22.20	23.60	0.77
5	22.20	21.80	22.40	0.62
		Lymphocytes (%)		
1	73.60	74.20	73.40	0.84
5	75.00	75.00	74.80	0.52
		Monocytes (%)		
1	2.00	1.40	1.20	0.23
5	1.20	1.40	1.00	0.22
		Eosinophils (%)		
1	1.60	1.80	1.40	0.34
5	1.20	1.40	1.20	0.26
		Basophils (%)		
1	0.40	0.40	0.40	0.13
5	0.40	0.40	0.60	0.13

Table 7: Birth weight, morbidity and mortality rate of calves in different groups

	T_0	T_1	T_2	SEM
	Body weights (kg)			
Birth weight (kg)	27.0[ab]	23.0[a]	30.2[b]	1.40
	Morbidity and mortality			
Morbidity (%)	50.0	37.5	25.0	
Mortality (%)	37.5	25.0	25.0	

[a,b] Means having different superscripts within a row differ significantly (P<0.05).

tendency towards higher birth weight (41.10 and 42.03 vs. 39.84 kg) and ADG (516 and 529 vs. 512 g) of their calves compared to unsupplemented control (Moeini et al. 2008). Calves fed on milk from vitamin E supplemented buffaloes showed higher average body weights (47.10 vs. 43.23 kg) between 2-8 weeks of age (Panda et al. 2006). The higher birth weight of calves in the vitamin E and Zn supplemented group might be due to effect on growth and immunity status of calves.

Morbidity and mortality rate

The morbidity (due to pneumonia and calf scour) recorded in different groups of calves was 50.0, 37.5 and 25.0% (Table 7) which indicated that, the calves born from cows supplemented combination of Zn and vitamin E were in better immune status than other two groups of calves. The mortality of calves recoded in groups T_0, T_1 and T_2 was 37.5, 25.0 and 25.0%, respectively. The role of vitamin E in bovine neonatal morbidity has been established through relationships between vitamin E status and higher morbidity. It has been observed that there has been strong relationship between neonatal morbidity and vitamin E status (Panousis et al. 2001 Mee 2014). Improved humoral or cellular immunity have been reported after supplementation of vitamin E and Zn (Gaal et al. 2006; Moeini et al. 2008). Thus, supplementation of both Zn and its combination with vitamin E to cows were responsible for better immune response and performance of calves.

Conclusion

The supplementation of combination of Zn and vitamin E from 60 days prepartum up to 90 days postpartum produced more colostral immunoglobulins higher plasma α-tocopherol level and higher birth weight, lower the incidence of calf morbidity and mortality. Hence, supplementation of Zn @ 80 ppm along with vitamin E @ 1000 IU/d from 60 days drying off period to early lactation of 90 days proved beneficial for cows and calf growth performance. However more work need to be required to different levels, duration of supplementation as well as on the combination required to study the effect on growth and health status of calves.

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