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ORIGINAL ARTICLE

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Effects of preweaning parenteral supplementation of vitamin E and selenium on hematology, serum proteins, and weight gain in dairy calves

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Abstract The beneficial effects of nutritional or parenteral administration of vitamin E (vit E) and selenium (Se) have not been universal among studies, and potentially confounding factors, such as Se or vit E status at the time of administration, duration and dose, and preexisting incidence of disease within a herd before administration, can all affect the outcome of treatment. Forty calves were used in the present study. The animals were divided into two groups (test, n=20; control, n=20). The two groups of calves were homogeneous for parity of dams, sex, and month of birth. In the test group, vit E and Se were injected at a dose of 300 U vit E (α-tocopherol acetate) plus 6 mg Se (sodium selenite) per 45 kg body weight (vit E + Se, BASF, Spain) at 24–48 h and 14 days after birth. Sampling was conducted from the jugular vein between 24 and 48 h after birth (before drug administration in the test group) and days 7, 14, 21, and 28, and erythrogram, leukogram, total serum protein, and fibrinogen amounts were determined. The amounts of beta and gamma globulins and the osmotic fragility of red blood cells were measured only at the end of the trial. Calves were weighed after birth, at days 7, 14, 21, and 28, and after 3 months. A general linear model showed significant changes for packed cell volume (PCV), fibrinogen, neutrophil, lymphocyte, and monocyte levels in the control group and total protein and lymphocyte in the test group, but interaction between sampling time and group for all parameters was not significant. White blood cells and hemoglobin levels at the third week and PCV and beta globulin amounts at the fourth week of study showed significant differences between groups (p<0.05). There were no significant differences for other measured parameters and weight gain between groups.

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Introduction

The best understood function of vitamin E (vit E) is as a lipid-soluble cellular antioxidant (Hogan et al. 1993). Via this function and perhaps other functions, vit E is involved in maintenance of cellular membrane, arachidonic acid metabolism, immunity, and reproductive function.

Selenium (Se) is a component of the enzyme glutathione peroxidase (GSH-PX), which is an important component of the cellular antioxidant system. More recently, Se was identified as a component of type I iodothyronine-5-deiodinase, the enzyme that converts T4 to T3 (National Research Council 2001).

An important interrelationship exists between Se, vit E, and the sulfur-containing amino acids in preventing some of the nutritional diseases caused by their deficiency (Koller and Exone 1986). Factors that influence metabolism in animals increase the occurrence of free radicals. Free radical disturbances have been associated with deficiencies of natural protective substances such as vit E and Se (Miller et al. 1991).

There has been considerable research effort to improve understanding of the role of Se and vit E in the health and performance of cows and their offspring. However, beneficial effects of nutritional or parenteral administration of vit E and Se have not been universal among studies, and potentially confounding factors, such as Se or vit E status at the time of administration, duration and dose, and preexisting incidence of disease within a herd before administration, can all affect the outcome of treatment (Weiss et al. 1983; Stowe et al. 1988; Nemec et al. 1990; Lacetera et al. 1996; Ndiweni and Finch 1996; Erskine et al. 1997; Arechiga et al. 1998).

Iran is generally Se deficient (Izadyar 1987), and all mineral premix for farm animals have been supplemented with Se in the form of sodium selenite. More recent studies have shown that Se from sodium selenite is poorly trans-

Table 1 Ingredient composition of concentrate mix fed to calves (% dry matter)

Corn	50
Barley	15
Soybean meal	22
Beet pulp	3
Wheat bran	3
Molasses	5.5
DCP	0.2
Limestone	0.9
Mineral and vitamin supplement[a]	0.4

DCP Dicalcium phosphate
[a]Vitamin A 50,000 IU; vitamin E 0.1 g; vitamin D3 10,000 IU; Ca 196 g; P 96 g; Na 71 g; Mg 19 g; Fe 3 g; Cu 0.3 g; Mn 2 g; Zn 3 g; Co 0.1 g; I 0.1 g; Se 0.0001 g

ferred to milk and that it is unable to maintain the Se status of nursing calves (Pehrson et al. 1999; Gunter et al. 2003). Ruminants poorly absorb sodium selenite partially due to the strong reducing environment in the rumen, which partly converts the Se to insoluble forms and as a result, transfer of Se to calves via placenta and milk could be decreased (Pehrson et al. 1999). Thus, neonatal calves can be Se deficient due to low levels of Se in milk at a few weeks after birth.

The objective of this study was to evaluate the effects of injected Se and vit E on hematology, serum proteins, and performance of neonatal dairy calves during an Se- and/or vit E-deficient period.

Materials and methods

The study was conducted in a dairy herd with approximately 400 calves per year at Mashhad suburb (northeast of Iran). This herd consisted of purebred animals of Holstein breed. The herd was totally confined in free-stall housing without access to pasture. Cows were fed with alfalfa hay, concentrate (Table 1), and corn silage.

Cows were dried 2 months before the expected time of parturition and transferred to a separate stall. As the time of parturition approached, the cows were moved to straw-bedded maternity pens. Prompt assistance was given to cows with dystocia. Following parturition, the umbilicus of

Table 2 Percentiles of serum total protein, fibrinogen, WBC, leukocyte types, and statistical comparisons between trial groups

Age	Total protein (g/l)		Fibrinogen (g/l)		WBC (10^9/l)		Neutrophils (10^9/l)		Lymphocytes (10^9/l)		Monocytes (10^9/l)		Eosinophils (10^9/l)	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
24–48 h														
25	61	62	3	3	6.650	5.738	3.127	2.948	1.79	1.95	0.0	0.09		
50	66[a]	66	4	3.5[a]	8.100	7.250	4.720	4.200	2.63[a]	2.64[a]	0.17	0.18[a,b]	0.0	0.0
75	72	70	5	4.3	8.850	9.175	6.524	6.536	3.84	3.16	0.41	0.32		
P value	NS		NS		NS		NS		NS		NS		NS	
First week														
25	64	60	4	4	7.000	7.350	2.264	1.979	3.48	2.36	0.14	0.2		
50	68[a]	65	6	5[b]	9.400*	8.100	2.916	3.900	4.65[b]	3.68[a,b]	0.22	0.26[a]	0.0	0.0
75	71	69	6	7	10.800	9.775	4.820	5.851	6.06	5.27	0.49	0.49		
P value	NS		NS		NS		NS		NS		NS		NS	
Second week														
25	60	58	4	4	6.700	5.525	1.190	1.281	4.02	3.36	0.1	0.0		
50	64[b]	63	5	5[a,b]	7.600	7.450	2.240	1.996	4.82[b]	4.97[b]	0.28	0.16[b]	0.0	0.0
75	68	67	8	6	8.925	8.600	3.443	3.300	5.88	6.07	0.65	0.5		
P value	NS		NS		NS		NS		NS		NS		NS	
Third week														
25	62	60	4	3	7.600	6.525	1.748	1.070	4.49	4.58	0.08	0.16		
50	64[a,b]	62	6	4.5[a,b]	8.700	7.750	2.139	2.025	5.95[b]	5.27[b]	0.24	0.24[a,b]	0.0	0.0
75	68	66	7	6.3	10.000	8.500	3.696	3.007	7.22	5.88	0.41	0.43		
P value	NS		NS		S		NS		NS		NS		NS	
Fourth week														
25	61	60	3	4	6.900	7.500	1.726	2.022	4.5	3.76	0.06	0.12		
50	63[b]	64	5	5[a,b]	8.350	9.100	2.830	3.168	7.13[b]	4.42[b]	0.22	0.25[a,b]	0.0	0.0
75	66	69	6	6.5	12.000	9.500	4.563	4.868	8.47	6.24	0.5	0.56		
P value	NS		NS		NS		NS		NS		NS		NS	

Means in columns with unlike superscripts ([a,b]) are significantly different ($p \leq 0.05$)
NS Nonsignificant difference, *S* significant difference ($p \leq 0.05$)

each calf was treated with povidone iodine, and the calf was weighed and transferred to an individual pen. Within the first 6 h of life, 2.5 kg of the dam's colostrums was bottle-fed to the calf, and colostrum feeding was continued every 12 h for 48 h. After 48 h, herd milk was used for feeding twice daily (2 kg every 12 h) until the calf was 10 days old. After this time, concentrate (Table 1), high-quality alfalfa, and water were allowed ad libitum. The calves were weaned at 45 days of age. The heifer calves were mainly used as herd replacements.

Forty calves were used in the present study. The animals were divided into two groups (test, n=20; control, n=20). The two groups of calves were homogeneous for parity of dams, sex, and month of birth. In the test group, vit E and Se injected at a dose of 300 U vit E (α-tocopherol acetate) plus 6 mg Se (sodium selenite) per 45 kg body weight (BW) (vit E + Se, BASF) at 24–48 h and 14 days after birth. Sampling was conducted from the jugular vein between 24 and 48 h after birth (before drug administration in the test group) and repeated at days 7, 14, 21, and 28. The blood was added to EDTA-containing tubes for hematological measurements and plain tubes for serum extraction and measurement of total protein and serum protein electrophoresis. The serum was harvested after centrifugation at 3,000 rpm for 10 min and stored at −20°C until required for analysis. Body weight was also recorded after birth, at days 7, 14, 21, and 28, and after 3 months.

The hematocrit (packed cell volume, PCV) levels were obtained by microhematocrit method. Red (RBC) and white blood cell (WBC) measurements were conducted using manual standard method (Dacie and Lewis 1984). Hemoglobin (Hb) amount was determined by cyanmethemoglobin method (Dacie and Lewis 1984). Differential leukocyte counts and fibrinogen measurement were performed on routinely prepared Giemsa-stained blood films using the cross-sectional technique and heat-precipitating method, respectively (Jain 1986). One hundred leukocytes were identified. Serum total protein (tp) was determined by biuret colorimetric method using a spectrophotometer (Jenway 6105, Jenway Ltd., Felsted, England, UK). The levels of beta and gamma globulins and osmotic fragility of RBC were determined by cellulose acetate electrophoresis (Helena system) and the method described by Chanarin (1989) only at the end of the trial.

Statistical analyses were performed with SPSS package (version 9). For each parameter, the effects of sampling time on test results were examined with a general linear model (GLM) that included sampling time, group of

Table 3 Percentiles of RBC parameters and indices and statistical comparisons between trial groups

Age	PCV (%)		RBC (10^{12}/l)		Hb (g/l)		MCV (fl)		MCHC (%)	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
24–48 h										
25	27	28	5	4.5	8.3	8	55	51	28	26
50	31	30[a,b]	5.5	5	9.6	9.2	60	55	30	31
75	37	36	6.08	5.8	11.5	9.8	72	71	32	33
P value	NS		NS		NS		NS		NS	
First week										
25	27	29	4.5	4.58	8.82	8.8	57	54	28	28
50	34	31.5[b]	5.15	5.5	10.25	9.25	61	57	29	29
75	39.5	35	6.41	6.11	11.5	10.18	64	66	31	31
P value	NS		NS		NS		NS		NS	
Second week										
25	29	27	4.85	4.5	7.95	7.8	52	55	26	28
50	33	31[b]	5.5	5	8.9	8.8	60	59	28	29
75	36.5	32.25	5.8	5.62	10.4	10.4	72	66	30	31
P value	NS		NS		NS		NS		NS	
Third week										
25	29	27.25	5	4.9	8.5	7.9	51	54	28	26
50	33	31[b]	5.7	5.1	9.9	8.8	57	57	29	29
75	35	34	6.2	6	10.7	9.7	66	62	31	30
P value	NS		NS		S		NS		NS	
Fourth week										
25	30	26	5.5	5	8.43	7.7	48	46	27	28
50	33	29.5[a]	6	5.5	9.65	9.4	53	53	29	30
75	37	33	6.5	6.5	10.13	10.2	61	58	30	33
P value	S		NS		NS		NS		NS	

Means in columns with unlike superscripts ([a,b]) are significantly different ($p \leq 0.05$)
NS Nonsignificant difference, *S* significant difference ($p \leq 0.05$)

Table 4 Percentiles of globulins and osmotic fragility and statistical comparisons

	β-Globulin (g/l)	γ-Globulin (g/l)	Osmotic fragility (min %)	Osmotic fragility (max %)
Test				
25	10.98	11.24	0.75	0.35
50	12.37	15.34	0.9	0.45
75	13.73	17.74	0.9	0.50
Control				
25	7.62	9.80	0.75	0.34
50	10.2	15.30	0.77	0.43
75	12.37	17.72	0.90	0.50
P value	S	NS	NS	NS

NS Nonsignificant difference, *S* significant difference ($p \leq 0.05$)

study, and interaction between them as fixed factors and calf as a random factor. In each group, differences between sampling times were determined by Bonferroni test as a pairwise comparison. Nonparametric independent *t* test (Mann–Whitney) was used to investigate significant difference at various sampling times between groups. $P \leq 0.05$ was considered as significant.

Results

The results are shown in Tables 2, 3, 4, and 5.

GLM showed significant changes for PCV, fibrinogen, neutrophil, lymphocyte, and monocyte levels in the control group and total protein and lymphocyte in the test group, but interaction between sampling time and group for all parameters was not significant. There were significant differences between groups for WBC and Hb levels at the third week and PCV and beta globulin amounts at the fourth week of study ($p<0.05$). There were no significant differences for other measured parameters and performance between groups.

Table 5 Effects of vit E and Se on weight gain (kg) of trial groups

	Gain during 1 month	Gain during 3 months	ADG during 1 month	ADG during 3 months
Test				
25	−1.13	36.75	−0.04	0.408
50	2.5	42.5	0.08	0.473
75	6.5	45.75	0.217	0.508
Control				
25	.63	37.75	0.02	0.419
50	5.25	41	0.175	0.456
75	8.38	45.5	0.285	0.506
P value	NS	NS	NS	NS

ADG Average daily gain, *NS* nonsignificant difference

Discussion

Several biochemical systems exist in cells and extracellular fluid to remove reactive oxygen and other oxidants. Antioxidant systems include molecules such as vit E and Se-containing enzyme, which act as membrane antioxidants to maintain the integrity of phospholipids against oxidative damage and peroxidation (McCay and King 1980; Di Mascio et al. 1991).

Beneficial effects of nutritional or parenteral administration of vit E and Se have not been universal among studies, and potentially confounding factors, such as Se or vit E status at the time of administration, duration and dose, and preexisting incidence of disease within a herd before administration, can all affect the outcome of treatment.

Bednarek et al. (1996) suggested higher blood leukocyte count and gamma globulin level in parenterally vit E- and Se-supplemented calves than in the control, but the differences for erythrocyte count, hemoglobin concentration, and haematocrit were not significant. In another study, WBC counts were significantly higher in vit E- and Se-injected groups of rats than in the control, but the RBC count and the hemoglobin, PCV, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and MCH concentration (MCHC) values were apparently not influenced by the injection of vit E and Se (Cay and Naziroglu 1999). An erythrocyte metalloenzyme of clinical significance is Se-containing glutathione peroxide (GSH-PX). There is a direct correlation between metal deficiency and enzyme activity. Although erythrocytic GSH-PX is directly related to a muscle disorder in ruminants, white muscle disease, it is inconsistently accompanied by methemoglobinemia. Hemolytic anemia is not a routinely reported clinical feature of Se deficiency in ruminants. Heinz bodies in Se-deficient Florida cattle are reported to be resolved by Se treatment. Whether the cause of the methemoglobin was Se is not clear (Kramer 2000). The effects of vit E supplementation were studied by Reddy et al. (1987). They showed that hematological response at 4 and 8 weeks of age consisted of lower MCH in calves given 500 IU vit E than other groups. In the present study, the higher levels of WBC, PCV, and Hb at the third and fourth weeks of life in the test group could be related to the protection of cell membrane and intracellular organelles by the antioxidant effects of vit E and Se and thus increase the life span of RBC and leukocytes.

Hidiroglou et al. (1992) reported no significant differences in the concentrations of immunoglobin (Ig) G1 and IgG2 among vit E-supplemented and control calves, but IgM was significantly higher for calves supplemented with 2,700 IU vit E than for control. In another study, Hidiroglou et al. (1995) indicated that differences among vit E-supplemented and control calves were not significant for IgG1, IgG2, and IgM concentrations. Similar results were also obtained for overall study weight gain and daily weight gain. The effect of vit E on immune responses of Holstein calves was investigated by Reddy et al. (1986). They reported no significant differences in the concentrations of IgG1 and IgG2 among treatments, but IgM was

significantly higher at week 6 in calves given the high amount of oral supplementation than in all other calves. Nemec et al. (1990) reported no significant differences in anti-*Brucella abortus* strain 19 IgG1, IgG2, or IgM antibody levels due to Se, vit E or Se/vit E treatments. Rivera et al. (2002) reported the increase in circulating antibodies to a foreign antigen with supplementation of 1,140 IU vit E/day. In the present study, the significantly higher beta globulin level in the test group could be attributed to higher IgM amounts probably due to positive effects of vit E and Se supplementation as previously reported (Reddy et al. 1986; Hidiroglou et al. 1992).

Reddy et al. (1985) suggested no significant difference in weekly weight gain or serum total protein between vit E-supplemented and control calves. They reported that calves may not get enough vit E with conventional calf starters, and supplementation may be essential to obtain maximum performance. Reddy et al. (1987) suggested that overall weight gains at 24 weeks were higher with 125 and 250 IU and intermediate with 500 IU supplementation compared with no supplementation in calves.

In a study of response of beef calves to parenteral administration of Se, Castellan et al. (1999) suggested better average daily gain in calves receiving four multiple injections of Se than in control calves, but average daily gain for calves given only one injection was not significantly different from controls. Ullrey et al. (1977), Swecker et al. (1989), and Lacetera et al. (1996) all have reported no significant impact of Se and/or vit E supplementation of dams on body weight gain of their calves. In another report, injection of one or two vit E and Se supplements had no effect on body weight, total gain, and average daily gain of neonatal calves (Weiss et al. 1983). According to Rivera et al. (2002), supplemental vit E had limited effects on performance of beef calves. Gunter et al. (2003) indicated no significant differences for body weight, total gain, and average daily gain between calves nursing cows supplemented with Se and calves nursing cows supplemented with no Se, or between calves nursing cows supplemented with sodium selenite and seleno-yeast. Wright et al. (1997) used 80 crossbred calves to evaluate Se and vit E combinations. The authors suggested that preweaning vit E and/or Se supplementation did not influence postweaning performance, stress responses, or vaccination responses in beef calves with adequate vit E status.

It seems that sufficient dam supplementation with vit E and Se prepartum could protect calves against deficiencies in the first few weeks of life and the benefits of further supplementation depend on the efficiency of vit E and Se transfer from dam to calf. Intramuscular injection of vit E caused elevated amounts in serum for at least 28 days, whereas injection of Se increased concentrations of Se in whole blood and serum for 28 days and increased whole-blood glutathione peroxidase activity for at least 84 days (Maas et al. 1993). Another report suggested that prepartum Se supplementation of the dam elevated Se of blood serum in the calf at birth. This supplementation caused the increased level of Se in serum and reduced the amount of Se taken up in serum of calves injected with additional Se (Weiss et al. 1983).

Enjalbert et al. (1999) indicated parenteral supplementation of newborn beef calves with 1.38 mg of Se did not result in normal Se status 1 month later when dams were Se-deficient and remained unsupplemented postpartum. They revealed adding 13–45.5 mg of dietary Se daily to beef cows for 15 days in late pregnancy produced satisfactory Se status in cows and their calves even 3 months after the end of supplementation.

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