

Effects of supplemental parenteral administration of vitamin E and selenium to Jerseys and Holsteins during the nonlactating period

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Objective—To determine effects of breed and supplemental administration of vitamin E and selenium (Se) during late gestation on circulating concentrations of these micronutrients in periparturient Jerseys and Holsteins.

Design—Randomized controlled clinical study.

Animals—16 Jersey and 36 Holstein cows.

Procedure—Cows were allotted to blocks on the basis of breed and expected parturition date. Cows within blocks were randomly assigned to be given vitamin E or Se parenterally 3 to 4 weeks prior to anticipated parturition in a 2 X 2 factorial design.

Results—Results of ANOVA indicated Jerseys had higher blood concentrations of Se and lower serum concentrations of vitamin E than Holsteins at the end of lactation. Jerseys had higher blood concentrations of Se than Holsteins 3 to 4 weeks prior to parturition and at parturition. Selenium administration increased blood concentrations of Se at parturition. Administration of nutrients did not affect serum concentrations of vitamin E at parturition or 2 to 3 weeks after parturition or blood concentrations of Se 2 to 3 weeks after parturition.

Conclusions and Clinical Relevance— Jerseys and Holsteins consuming rations of comparable Se content differ in blood concentrations of Se during the nonlactating period, suggesting breed-related differences in Se metabolism during late lactation and the nonlactating period. Parenteral administration of Se 3 to 4 weeks prior to anticipated parturition increased blood concentrations of Se at parturition; however, Se concentrations of both groups at parturition were considered within the reference range for clinically normal cattle. (*Am J Vet Res* 2000;61:1052–1056)

Supplemental administration of vitamin E and selenium (Se) commonly is associated with a decreased incidence of retained fetal membranes,^{1,3} metritis,^{2,4} cystic ovaries,^{4,5} and periparturient mastitis⁶⁻⁸ in dairy cows. However, such benefits have not been observed in another study.⁹ Supplemental amounts of these nutrients often are added to rations formulated for lactating cows. Supplemental administration of vitamin E and Se commonly is recommended for nonlactating cows,^{4,5} but it is not performed in some dairies. Vitamin

E and Se can be administered parenterally during the nonlactating period.¹⁰

Dietary concentrations of Se and vitamin E in rations formulated for nonlactating cows vary among dairies because of differing concentrates, types of forage, and quality of forage fed during the nonlactating period. Weiss et al⁶ reported lower dietary concentrations of Se in rations formulated for nonlactating cows than for rations formulated for the early lactation period in 9 dairies in Ohio. Some dairy producers feed a ration to nonlactating cows that consists primarily, if not exclusively, of hay. This and other stored forages typically have reduced vitamin E content, compared with vegetative good-quality pasture.¹¹⁻¹³

Minimum requirements for vitamin E and Se are established for lactating dairy cows.¹⁴ However, data are limited on supplemental provision of vitamin E and Se to nonlactating cows. Dietary vitamin E recommendations for nonlactating cows vary, ranging from 200 to > 1,000 U/cow/d.¹⁴⁻¹⁸ Furthermore, there are only 2 reports^{19,20} of potential breed-related differences in serum concentrations of vitamin E in cattle, and to our knowledge, there have not been any reports of breed-related differences of Se in cattle. Lastly, studies in which investigators used parenteral supplementation of these nutrients during the nonlactating period yielded varied results. Therefore, objectives of the study reported here were to determine whether parenteral administration of supplemental vitamin E and Se during lactation would maintain adequate circulating concentrations of these nutrients during the nonlactating period, whether differences exist in circulating concentrations of vitamin E and Se between Holsteins and Jerseys during the nonlactating period, and whether parenteral administration of vitamin E and Se affects circulating concentrations of these nutrients during the nonlactating and early lactation periods.

Materials and Methods

Study design—Sixteen multiparous Jerseys and 36 multiparous Holsteins bred so that they would give birth between July and September of 1995 were included in the study. Cows were assigned to blocks on the basis of breed and expected date of parturition, and they were randomly assigned within blocks to receive vitamin E or Se treatment, using a 2 X 2 factorial design. All cows were maintained, treatments were administered, and samples were obtained in accordance with principles established in the *Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*.

Vitamin E and Se were administered parenterally 3 to 4 weeks prior to anticipated parturition date. The dose used was empirically determined on the basis of estimated mean body weights for the 2 breeds and was in accordance with guidelines

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listed on product labels. Mean weight of Holsteins and Jerseys was 591 and 409 kg, respectively. Holsteins were administered 3,000 units of vitamin E in the form of D- α -tocopherol,³ and Jerseys were administered 2,400 units. Each dose was given as a single SC injection administered behind the right shoulder. Selenium in the form of sodium selenite⁵ was administered at a dose of 32.5 mg of Se for Holsteins and 22.5 mg of Se for Jerseys via a single SC injection given on the right side of the neck. Vitamin E was administered in combination with Se, because a pure Se product was not available. Therefore, Holsteins and Jerseys given Se also received an additional 442 and 306 units of vitamin E as D- α -tocopheryl acetate, respectively.

All cows were similarly housed, managed, and fed for the duration of the study, which began in May and was completed at the end of October. During the nonlactating period, cows had unlimited access to pasture consisting primarily of tall fescue, orchard grass, and a small amount of white clover. Selenium concentrations of pasture forages ranged from 30 to 60 μ g of Se/kg of pasture forage (dry-matter [DM] basis). Analysis of pasture forages for vitamin E content was not performed, but concentrations were estimated at a mean of 50 mg of α -tocopherol/kg of pasture forage (DM basis).¹¹ Cows were fed a transition ration, which contained 0.3 mg of Se/kg of ration (DM basis), beginning approximately 1 week prior to parturition. Rations fed during early lactation were similar in feed composition and nutrient content, and they also contained 0.3 mg of Se/kg of ration (DM basis). Rations fed during the transition and early lactation periods were not analyzed for vitamin E content but were estimated to contain approximately 40.5 and 32 mg of α -tocopherol/kg of ration (DM basis), respectively.

Collection and analysis of samples—Blood samples were collected via coccygeal venipuncture into evacuated glass tubes.⁶ Four samples were obtained from each cow: end of lactation, 3 to 4 weeks prior to anticipated parturition, within 24 hours after parturition, and 2 to 3 weeks after parturition. At each collection, 10 ml of blood was collected into tubes containing EDTA for determination of Se concentration, and 10 ml of blood was collected into tubes without additives for determination of serum concentrations of vitamin E. All samples obtained for assessment of vitamin E concentrations

were protected from light and heat during handling, storage, and analysis. Serum was harvested by allowing blood samples to clot for 30 to 60 minutes, followed by centrifugation at $3,433 \times g$ for 5 minutes. Separated serum was harvested with a pipette and stored frozen at -20°C in polypropylene tubes until the time of analysis for vitamin E content. Blood samples in tubes containing EDTA were stored refrigerated until analyzed for Se concentration. Serum samples were analyzed for vitamin E content by use of high-pressure liquid chromatography (HPLC),⁴ and blood samples were analyzed for concentrations of Se by atomic absorption spectrophotometry,⁶ using a previously reported technique.²¹

Vitamin E analysis was modified from a previously reported technique.²² Extraction of vitamin E was performed by adding the following to a 1-ml aliquot of serum: 100 μ l of 1,000 μ g of α -tocopheryl acetate (ie, internal standard)/ml, 2 ml of 95% ethanol, and 3 ml of HPLC-grade cyclohexane. Between additions, the solution was vortexed for 10, 20, and 45 seconds, respectively. After centrifugation at $3,064 \times g$ for 10 minutes, the top (cyclohexane) layer of the supernatant was pipetted into a 5-ml conical vial and fully evaporated under nitrogen gas on a heating block at 60°C . One hundred microliters of 95% ethanol was added to the vial, which was vortexed intermittently for 1 minute to reconstitute the vitamin E. The entire extraction procedure for vitamin E was performed in subdued light to minimize photodegradation of vitamin E.

Samples were subjected to HPLC analysis, using a reverse-phase HPLC column,⁴ guard column, and 10- μ l injection loop. Pure methanol (100%) was used as the mobile phase at a flow rate of 2 ml/min. Ultraviolet detection was performed at 294 nm. The concentration of α -tocopherol in each sample was determined by comparing the peak area with that of known standards and using peak area of the α -tocopheryl acetate internal standard to correct for percentage recovery.

Data analysis—Data were analyzed, using a commercially available statistical program.⁸ The ANOVA model used included the main effects of vitamin E treatment, Se treatment, and breed and all 2- and 3-way interactions. Values of $P \leq 0.05$ were considered significant for all analyses.

Table 1—Effect of breed on serum concentrations of vitamin E and blood concentrations of selenium in periparturient Jerseys and Holsteins

Sample collection	Vitamin E (μ g/ml)			Selenium (ng/ml)		
	Jersey	Holstein	P value	Jersey	Holstein	P value
End of lactation	3.6 \pm 0.2	5.5 \pm 0.4	0.005	177 \pm 5	141 \pm 4	< 0.001
3 to 4 weeks prior to parturition	4.8 \pm 0.3	4.7 \pm 0.2	0.76	145 \pm 5	108 \pm 4	< 0.001
Within 24 hours after parturition	2.6 \pm 0.2	2.9 \pm 0.1	0.16	155 \pm 4	124 \pm 5	< 0.001
2 to 3 weeks after parturition	4.1 \pm 0.3	4.1 \pm 0.2	0.98	167 \pm 4	157 \pm 7	0.36

Values are expressed as least-squares mean \pm SEM of observations for 16 Jerseys and 36 Holsteins.

Table 2—Effect of parenteral administration of vitamin E or selenium during late gestation on serum concentrations of vitamin E or blood concentrations of selenium in periparturient Jerseys and Holsteins

Sample collection	Vitamin E (μ g/ml)			Selenium (ng/ml)		
	Control	Vitamin E treatment*	P value	Control	Selenium treatment*	P value
3 to 4 weeks prior to parturition	4.4 \pm 0.2	5.1 \pm 0.3	0.07	127 \pm 6	126 \pm 6	0.84
Within 24 hours after parturition	2.5 \pm 0.2	2.9 \pm 0.2	0.15	131 \pm 7	148 \pm 5	0.04
2 to 3 weeks after parturition	4.3 \pm 0.3	4.0 \pm 0.2	0.49	159 \pm 7	165 \pm 7	0.61

Values are expressed as least-squares mean \pm SEM and are based on 26 observations for the control and treatment groups, respectively.

*Jerseys were given 2,400 units of vitamin E and 22.5 mg of selenium, and Holsteins were given 3,000 units of vitamin E and 32.5 mg of Se, which were administered as SC injections immediately after we obtained the sample 3 to 4 weeks prior to parturition.

Results

Holsteins had significantly lower Se ($P < 0.001$) and higher vitamin E ($P = 0.005$) concentrations than Jerseys at the end of lactation (Table 1). Breed-related differences in Se concentrations persisted throughout the time at which the immediate postparturient sample was obtained. Jerseys had significantly higher Se concentrations than Holsteins at 3 to 4 weeks prior to anticipated parturition ($P < 0.001$) and immediately after parturition ($P < 0.001$). We did not detect other differences in serum concentrations of vitamin E between breeds during the study period.

Treatment with vitamin E did not significantly affect serum concentrations of vitamin E at parturition ($P = 0.15$) or after parturition, even though the treatment group had a higher, but not significantly so ($P = 0.07$), serum concentration of vitamin E immediately prior to vitamin E administration at 3 to 4 weeks prior to anticipated parturition (Table 2). Treatment with Se significantly ($P = 0.04$) increased blood concentrations of Se at parturition. Selenium treatment did not significantly affect postparturient blood concentrations of Se. We did not detect significant 2- or 3-way interactions.

Discussion

A decrease in serum concentrations of vitamin E was observed during the preparturient period in both breeds irrespective of treatment group. This finding has been reported in several other studies²³⁻²⁵ and is correlated with a decrease in circulating lipoprotein concentrations.²⁶ Lipoproteins transport vitamin E in the bloodstream and, therefore, affect vitamin E concentrations in circulation.²⁶⁻²⁹

Higher serum vitamin E concentrations of Holsteins at the end of lactation in the study reported here could have resulted from higher serum concentrations of lipoprotein or a higher degree of vitamin E saturation per lipoprotein particle,^{25,28,30} compared with that for Jerseys. Serum concentrations of lipids or lipoproteins, however, were not measured in the study.

Plasma or serum concentrations of vitamin E commonly are used as a practical means of evaluating vitamin E status in cattle.^{4,10,26} Serum concentrations of vitamin E $> 4.0 \mu\text{g/ml}$ are considered adequate,³¹ whereas those between 2.0 and 4.0 $\mu\text{g/ml}$ are marginal, and those $< 2.0 \mu\text{g/ml}$ are deficient, according to our reference laboratory. Regardless of breed, all groups had adequate mean concentrations of vitamin E in serum in all samples, except those obtained at parturition. Mean serum concentrations of vitamin E at parturition were in the marginal range. The periparturient decrease in serum concentrations of vitamin E can be attributed, at least in part, to a concurrent decrease in serum concentrations of lipoproteins.^{26,28,32,33} Other contributing factors may have included a periparturient decrease in vitamin E intake (as related to a periparturient decrease in DM intake)¹⁴ and removal of vitamin E from the bloodstream for colostrum synthesis.^{23,34}

Blood concentrations of Se often are used as a measure of Se status.^{10,35} Blood concentrations of Se $> 100 \text{ ng/ml}$ generally are considered adequate, whereas those between 50 and 100 ng/ml are marginal, and

those $< 50 \text{ ng/ml}$ are deficient in our region of the United States.^{35,36} Blood concentrations of Se were adequate at each sample period irrespective of breed or treatment group.

In the study reported here, parenteral administration of Se (approx 0.055 mg of Se/kg of body weight) increased blood concentrations of Se 3 to 4 weeks after injection. Maas et al³⁷ reported similar results in weaned Hereford heifers maintained on a Se-deficient diet that were given a single IM injection of Se at a rate of 0.05 mg of Se/kg. In another study,³⁸ pregnant Holstein cows given a single IM injection of 25 mg of Se had 2-fold higher mean blood concentrations of Se (41 ng/ml) than cows in the control group (19 ng/ml) for 13 weeks following Se administration. The authors of that study attributed the duration of the treatment response to the low Se status of the cows. Cows in the study reported here maintained adequate Se concentrations for the study period.

Blood concentrations of Se differed between Jerseys and Holsteins at the end of lactation. This difference persisted through the sample collection period in the middle of the nonlactating period and was still evident at parturition, despite supplemental administration of 0.3 mg of Se/kg of ration (DM basis) to all cows during approximately the last week of gestation. Comparable Se concentrations between breeds at the sample collected 2 to 3 weeks after parturition can be attributed to provision (3 to 4 weeks) of dietary Se^{21,34} prior to sample collection.

To our knowledge, breed-related differences in blood concentrations of Se observed in the study reported here have not been observed in other studies. This finding suggests a breed-related difference between Holsteins and Jerseys in Se metabolism, similar to that reported for copper, iron, and zinc.³⁹ Breed-related differences in hepatic concentrations of certain trace minerals also have been reported in beef cattle.⁴⁰

The reason for higher blood concentrations of Se in Jerseys is unknown. Because approximately 73% of blood Se exists in its cellular component,⁴¹ higher PCV could have accounted for the difference. However, neither PCV nor hemoglobin concentrations were measured in the study.

Serum concentrations of vitamin E were lower at the end of lactation for Jerseys than for Holsteins, even though all cows were consuming the same late-lactation ration (data not shown). As was anticipated, Jersey cows had an increase in serum concentrations of vitamin E early in the nonlactating period. This increase was expected for all cows, because ad libitum provision of pasture throughout the nonlactating period should have increased serum concentrations of vitamin E as a result of its presumably high vitamin E content.^{12,13,42} Similar to the situation that was evident for Se, the breed-related differences in serum concentrations of vitamin E at the end of lactation and changes throughout the nonlactating period might suggest possible breed-related differences in serum concentrations of lipoproteins.^{26,28} Limited evidence exists for cattle that would suggest breed-related differences in vitamin E metabolism. Authors of 1 study¹⁹ reported differences between beef and dairy steers in

serum concentrations of vitamin E, response to supplemental administration, and metabolism of this nutrient. Authors of another study²⁰ suggested a breed-related difference in serum concentrations of vitamin E between nonlactating dairy cows and pregnant mixed-breed beef heifers.²⁰

In the study reported here, all treatment groups maintained mean blood concentrations of Se within the adequate range for the duration of the nonlactating period, despite the fact that the forages consumed were deficient in Se and supplemental Se was not provided (except for cows in the treatment group) until the transition ration was fed beginning approximately 1 week prior to parturition. Maintenance of adequate blood concentrations of Se during the nonlactating period depends in part on blood concentrations of Se attained during late lactation⁶ and also reflects Se intake⁴³ during the nonlactating period.^{6,34}

Mean serum concentrations of vitamin E remained adequate throughout most of the nonlactating period but decreased into the marginal range at parturition. Similar to the fact that dietary content of Se influences blood concentrations of Se,^{21,34} adequate serum concentrations of vitamin E observed in the study were attributable, at least in part, to the presumably high concentration of vitamin E in the pasture forages.¹⁰⁻¹² Cows entering the nonlactating period during seasons of the year with low availability of pasture forages or otherwise managed such that fresh forage intake is limited would likely have lower serum concentrations of vitamin E at parturition as well as during most of the nonlactating period.^{10,44} Cows managed in that manner might not maintain adequate serum concentrations of vitamin E during the periparturient period, possibly resulting in suboptimal immune function⁷ and an increased incidence of disease.^{1,2,4,18}

^aVITAL E - 300, 300 U of D- α tocopherol/ml, Schering-Plough Animal Health, Kenilworth, NJ.

^bMU-SE, 5 mg of Se and 50 mg of D- α tocopheryl acetate/ml, Schering-Plough Animal Health, Kenilworth, NJ.

^cVacutainer, Beckton-Dickinson, Franklin Lakes, NJ.

^dVarian Model 9000 HPLC, Varian Associates, Sugar Land, Tex.

^eVarian SpectrAA, 20 Atomic absorption spectrophotometer, Varian Assoc, Sugar Land, Tex.

^f4.6 \times 150 mm, 5 μ m, Supelcosil LC-18 column, Supelco, Bellefonte, Pa.

^gSAS, version 6.10, SAS Institute Inc, Cary, NC.

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