Vitamin E and Selenium for Reproduction of the Dairy Cow

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ABSTRACT

Selenium injections and oral vitamin E supplementation prepartum were related to incidence of retained placenta, metritis, and cystic ovaries in a 2 × 2 factorial experiment. Groups were: 1) selenium and vitamin E, 2) vitamin E, 3) selenium, and 4) control. Incidence of retained placenta was 17.5% in cows of groups 2, 3, and 4, whereas it was reduced to 0% in cows receiving both selenium and vitamin E. Incidence of metritis was 60% for cows injected with selenium and 84% for those not receiving selenium. Cystic ovaries were diagnosed in 19% of cows injected with selenium, and incidence was 47% for cows not treated with selenium. Supplementation of vitamin E was required in addition to selenium for prevention of retained placenta of cows fed stored ensiled forage, and prepartum selenium injections were effective for reducing the incidence of metritis and cystic ovaries during the early postpartum period.

INTRODUCTION

The normal incidence of retained placenta (RP) in dairy cattle is about 10% in North America (11, 29, 53). However, in areas where dairy herds are fed selenium (Se) deficient rations the incidence may be as high as 50% (19, 20, 51). The efficacy of Se-vitamin E injections for reducing the incidence of RP was shown by Trinder et al. (51) and since has been confirmed (19, 20, 37, 43). However, Gwazdauskas et al. (13) observed no decrease of incidence when cows were injected with 21.9 mg of Se and 500 mg of vitamin E at 28 to 30 days prior to projected calving. Schingoethe et al. (39) reported no benefit from Se-vitamin E injections, 50 mg vitamin E, and 5 mg Se per 45.4 kg body weight when cows consumed diets adequate in Se. More recently, Segerson et al. (43) concluded that Se-vitamin E injections (50 mg Se and 500 mg vitamin E) were not effective for reducing incidence of RP in herds either adequate or extremely deficient in Se prior to supplementation. In all experiments noted the amount of vitamin E injected was small relative to dietary intake.

Most studies have emphasized prepartum Se-vitamin E treatment on RP, whereas few investigators (13, 25, 40) have evaluated Se and vitamin E status of cows and related it to subsequent reproductive performance. Two of these had an abundance of Se in the diet (25, 40).

Feeding amounts of vitamin E characteristically in fresh forage diets may be required to prevent RP if cows are not responding to selenium treatments. For cows eating fresh forage by grazing, a single injection of 50 mg of Se prevented RP, but that amount was not effective for a similar group of cows fed alfalfa haylage during the prepartum period (37). Freshly cut forages in the vegetative stage contained five to six times as much vitamin E as was in feeds after several months of storage (24, 30). Dry cows grazing pasture plants in the vegetative stage would consume 1.0 to 1.5 g of vitamin E per day. We selected 1.0 g of vitamin E, therefore, as an optimum amount for experimental testing, because daily oral supplementation of vitamin E with Se treatment by injection had not been tested as a treatment for RP.

The specific interaction of prepartum
Se-vitamin E treatment was determined by comparing a single Se injection and daily oral vitamin E (E), alone or in combination, on postpartum uterine health, ovarian function, and incidence of RP. Preliminary findings are in abstract (14).

MATERIALS AND METHODS

Cows and Rations

Seventy-eight multiparous dry cows were in a 2 x 2 factorial experiment. Animals were assigned at random to one of two dietary groups for the entire prepartum period and subdivided for Se treatment at 21 days before projected calving. Treatments were: 1) Se + E, 2) E, 3) Se, and 4) control without supplement. Each group was housed indoors in group pens during the prepartum period. All cows received a legume-grass haylage provided for ad libitum consumption and supplemented with .5 kg of concentrate per cow per day as a total mixed ration. Rations were fed to allow for a 10% refusal over intake, and animals were fed once daily. Haylage had been stored ≥ 5 mo at initiation of the experiment. Ingredients in these diets and their nutrient content are in Table 1. Vitamin E-supplemented groups (1 and 2) received supplemental (d,l)-alpha-tocopheryl acetate to provide an average of .74 g vitamin E ([d] -α-tocopherol equivalents/cow per day). Ad libitum consumption of haylage provided an estimated .32 g of vitamin E/cow per day.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Vitamin E supplemented</th>
<th>Unsupplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haylage b (ad libitum)</td>
<td>Vitamin E-mineral supplement c,h</td>
</tr>
<tr>
<td></td>
<td>%.5 kg</td>
<td>%.5 kg</td>
</tr>
<tr>
<td>% Dry matter (DM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>15.9</td>
<td>9.9</td>
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<tr>
<td>Available crude protein</td>
<td>12.6</td>
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</tr>
<tr>
<td>Acid detergent fiber</td>
<td>44.5</td>
<td>33.7</td>
</tr>
<tr>
<td>Ca</td>
<td>.81</td>
<td>.84</td>
</tr>
<tr>
<td>P</td>
<td>.34</td>
<td>.24</td>
</tr>
<tr>
<td>K</td>
<td>3.26</td>
<td>.78</td>
</tr>
<tr>
<td>Mg</td>
<td>.23</td>
<td>.19</td>
</tr>
<tr>
<td>Mn</td>
<td>76</td>
<td>62</td>
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<td>Fe</td>
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<tr>
<td>Cu</td>
<td>7.8</td>
<td>9.9</td>
</tr>
<tr>
<td>Zn</td>
<td>30</td>
<td>83</td>
</tr>
<tr>
<td>Se</td>
<td>.059</td>
<td>.047</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>32^e</td>
<td>2200^f</td>
</tr>
</tbody>
</table>

a One-half of animals injected 21 days prepartum with selenium (.1 mg/kg/body weight).
b,c,d Means of 11, 12, 13, b samples.
^e Mean α-tocopherol content of 4 monthly composites (range 15 to 48 µg/g DM).
^f Supplemental [d,l]-α-tocopheryl acetate added (1474 µg of d-α-tocopherol equivalents).
^g Calculated using oats as the major contributor of vitamin E and National Research Council composition data (30).
^h Supplement contained 30% oats, 30% soybean flakes, 31% #4 corn cobs, 5% vitamin E (29,480 mg d-alpha-tocopherol equivalents), 2% trace mineral salt, and 2% sodium bentonite.
^i Supplement contained 30% oats, 30% soybean flakes, 36% #4 corn cobs, 2% trace mineralized salt, and 2% sodium bentonite.
Groups 1 and 3 were injected intramuscularly with Se at .1 mg/kg of body weight 21 days before projected calving. Feed intake and refusal were recorded daily. Water and trace mineralized salt blocks were provided for ad libitum access. Body weights were obtained approximately 21 days before calving. Number of animals per group is in Table 2.

Postpartum Cows and Rations

Animals were assigned to several unrelated experiments during the postpartum period. Daily rations contained corn silage (20 to 60%), hay (20 to 40%), and grain concentrate (20 to 50%) dry matter. Selenium intake, estimated from periodic analyses during the postpartum period, was 1 to 3.5 mg/day. Vitamin E content of postpartum diets was not analyzed. Crude protein content was 14 to 16%.

Selenium Treatment, Sampling, and Analysis

Selenium injections were prepared to contain 5.0 mg of Se (sodium selenite, 5-hydrate) and 250 mg of polysorbate (polyoxyethylene sorbitan mono-oleate) per milliliter. Subsamples of haylage (45 to 60% DM) and grain concentrate were obtained weekly and composited by month. Selenium content of the diets was determined by the method of Olson (31) and Olson et al. (32). Analysis for crude protein, available crude protein, dry matter, acid detergent fiber, and minerals was in the Ohio Ration Evaluation Analytical Laboratories (38) as outlined by Pritchard et al. (36).

Thirty-five milliliters of whole blood was collected in heparinized tubes from the jugular vein of Se treated cows on days 21 (prior to Se injection), 20, 19, 14, 7, and day of calving; and days 21, 20, 14, 7, and day of calving in non-Se treated cows (Figure 2). Plasma Se was measured as described (31, 32). Plasma Se-dependent glutathione peroxidase (Se-GSHpx) (glutathione:H2O2 oxido reductase, EC 1.11.1.9) was measured by the method (33) as modified (26) with glutathione increased to 10 mM. This concentration was determined in preliminary studies to give the optimum ratio of enzymatic/nonenzymatic rates. Plasma concentrations of vitamin E (α-tocopherol) and vitamin A (retinol) were measured by high pressure liquid chromatography (HPLC) (2). Alpha-tocopherol content of haylage was measured by HPLC after acetone extraction, saponification (8), and Sep-pak7 clean-up. Recoveries of known amounts of [d]-α-tocopherol were 65 ± 5%, mean ± SE. Total plasma carotenoids were measured spectrophotometrically (22).

Reproductive Criteria

Animals were diagnosed with RP if fetal membranes were retained for 24 h postpartum.

TABLE 2. Incidence of reproductive disorders during the first 12 wk postpartum after prepartum treatment of selenium (Se) and vitamin E (E).a,b

<table>
<thead>
<tr>
<th>Group</th>
<th>Retained placenta</th>
<th>Metritis</th>
<th>Cystic ovaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE x E</td>
<td>0/21 = 0c</td>
<td>12/21 = 57c</td>
<td>4/21 = 19c</td>
</tr>
<tr>
<td>E</td>
<td>4/20 = 20d</td>
<td>16/19 = 84d</td>
<td>8/18 = 44d</td>
</tr>
<tr>
<td>Se</td>
<td>3/18 = 17d</td>
<td>11/17 = 65c</td>
<td>3/16 = 19c</td>
</tr>
<tr>
<td>Control</td>
<td>3/19 = 16d</td>
<td>15/18 = 83d</td>
<td>9/18 = 50d</td>
</tr>
</tbody>
</table>

aSelenium injected 21 days prepartum (.1 mg/kg body weight). Vitamin E supplemented orally as [d,l]-α-tocopheryl acetate to provide an average of .74 g vitamin E ([d]-α-tocopherol equivalents/cow per day).

bOnly a single incidence observation of cystic ovarian condition was recorded per cow during the first 12 wk postpartum.

c,dMeans in a column with different superscripts differ (P<.05).

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Retained placenta were not removed manually. Only animals with RP > 3 days or those showing anorexia and elevated body temperature (39.5°C) were treated (500 ml, .0014% iodine solution). Animals were observed daily for vaginal discharge and twice daily for signs of estrus. Reproductive examinations were on all cows between 14 and 28 days postpartum, at which time they were diagnosed as normal or abnormal (metritis). The diagnosis of metritis (MET) was based on uterine size (28), or when uterine size was intermediate, diagnosis was based on vaginal examination with speculum and light source. Most cows received a vaginal examination. Purulent material found during vaginal examination was considered diagnostic for MET (46, 48). Cystic ovarian disease (CO) was diagnosed when a follicular structure (1.0 to 2.5 cm) persisted for 1 to 2 wk in the absence of a corpus luteum or when a follicular structure greater than 2.5 cm was detected. No attempt was made to differentiate between luteal and follicular ovarian cysts. Treatment for CO was not considered until 45 days postpartum because of the reported high rate of spontaneous recovery of cases diagnosed during the early postpartum period (28).

Uterine infections were treated by intrauterine infusion (60 to 360 ml) of dilute iodine solution (.0014%). Animals diagnosed with cystic ovarian disease were injected intramuscularly with Cystorelin. The option interval for first breeding was 60 days, and all cows were bred artificially 12 h after first detected in estrus. Pregnancy was determined by rectal palpation between 30 and 60 days after the last service.

The identity of experimental treatments was not available to the diagnosing veterinarian, and only one veterinarian performed reproductive examinations.

Statistical Analysis

Data for the proportion of cows with RP, CO, and abnormal uterine health (MET) were analyzed for treatment effects by Chi-square test (45). Plasma data and reproductive performance measures from the 2 x 2 factorial design were analyzed by the least squares method for unequal subclass numbers as outlined by Harvey (16).

RESULTS AND DISCUSSION

Prepartum Diet Composition, Feed Intake, and Body Weights

The nutrient content of prepartum diets is summarized in Table 1. Basal rations were formulated to be deficient in Se and vitamin E. Analysis of monthly composite feed samples indicated that rations were adequate for minerals and crude protein (30). As mentioned, the basal ration was low in vitamin E content which agrees with reported amounts (24, 30). Because of dietary design of the experiment the estimated dry matter intake, Se intake, and ratio of Ca to P are the same for vitamin E supplemented (groups 1 and 2) and unsupplemented groups (3 and 4). Dry matter intake (kg/day) was: vitamin E supplemented, 9.55 ± .34, 10; (X ± SE, n = number of months averaged); and vitamin E unsupplemented, 10.22 ± .61, 12. Intake of Se (μg/day) was: vitamin E supplemented, 552 ± 34, 10; and vitamin E unsupplemented, 582 ± 40, 12. Ca to P ratio of the total ration was: vitamin E supplemented, 2.46 ± .20, 10; and vitamin E unsupplemented, 2.40 ± .16, 12. Average body weights (kg) at 21 days before projected calving were: Se × E, 666 ± 25, 21; (X ± SE, n); E, 714 ± 29, 17; Se, 657 ± 27, 18; and control, 684 ± 20, 17.

Reproductive Disorders

Incidence of RP was zero in cows treated with Se and vitamin E (P<.05), whereas incidence in cows treated with Se or vitamin E (16 to 20%) did not differ from control animals (Table 2). Retained placenta was not affected by sex of calf (male:female = 5:3). Reinhardt et al. (37) first reported that feeding stored haylage during the dry period increased the need for Se to protect against RP. This study supported their observation and indicated that animals consuming stored haylage low in Se and vitamin E (Table 1) required supplemental vitamin E as well as Se treatment for prevention of RP. The vitamin E content of the experimental haylage was ≈ 32 μg/g in agreement with (24, 40).

Data may explain the lack of response to Se-vitamin E injections for prevention of RP as
Before, Se-vitamin E injections contained only 500 mg of vitamin E. Because of the rapid disappearance of injectable vitamin E (54) and the relatively small quantities provided to the prepartum animal in previous studies, it appears 500 mg would have no efficacy for RP when diets are consumed which contain low concentrations of vitamin E. Vitamin E content of the diet, vitamin E status of the animal, and route and form of vitamin E supplemented must be evaluated in future experiments to establish Se and vitamin E required by the dairy animal for prevention of RP.

Uterine contamination with bacteria occurs in most cows during the postpartum period (47). During the early postpartum period 85 to 93% of cows were contaminated (10, 18). Size of the previously gravid uterine horn has been used to determine the rate of uterine involution. Rate of uterine involution is adequate for diagnosis of MET only during the early postpartum period (28, 48). Visual examination of vaginal mucous through a speculum has indicated accurately uterine health in the bovine (46, 48). Using both criteria incidence was lowest \( P < .05 \) in animals receiving Se prepartum (Table 2). Supplementation with vitamin E during the prepartum period did not affect incidence of MET.

We did not attempt to establish the mechanism of Se action that resulted in improved uterine health. We suggest Se, as Se-GSHpx in the polymorphonuclear (PMN) cell, may allow the animal to overcome the onset of a clinical uterine infection. Support for this contention is that Se-GSHpx deficient bovine PMN were less able to kill ingested \( C. \textit{albicans} \) (3). Ullrey et al. (52) reported positive effects of Se, vitamin E, and choline supplementation in swine for prevention of a disease complex, which includes MET. The disease complex, mastitis-metritis-agalactia, was reduced from 39 to 24% \( P < .05 \).

The incidence of CO was lowest \( P < .05 \) in animals injected with Se during the prepartum period (Table 2). As with MET, supplemental vitamin E did not affect incidence of CO. The accumulative percent CO (Figure 1) indicates that by 12 wk postpartum \( \approx 50\% \) of animals not injected with Se were diagnosed as having CO while only 19% of Se treated animals developed CO. Most cases of CO occurred by 12 wk postpartum which agrees with (12, 28).

The high incidence rate of CO (50%) contradicts reports of 7 (7) to 38% (28) but agrees with more recent data (23, 35). The high percentage detected may have been the result of the thorough reproductive examination program. All cows were examined by rectal palpation for ovarian activity during the postpartum period, and this intensive examination program has been recommended to increase the chance of CO observation (44). Only those animals that had a persistent CO past 45 days postpartum were included in Table 2 and Figure 1. Morrow et al. (28) reported that many CO spontaneously will regress during the early postpartum period. In our study, only 27% (4/15) of those cows diagnosed with CO before 45 days postpartum spontaneously regressed. Statistical analysis of plasma Se versus incidence of CO and publication (15) indicated that plasma concentration of 0.06 \( \mu g/\text{ml} \) or greater at the time of calving are adequate to minimize the risk of onset of CO. Diets with inadequate phosphorus, cobalt, copper, manganese, and iodine (1, 27, 30) have been implicated to induce abnormal ovarian function.

The role of Se in ovarian function has not been established; however, Buck et al. (5) reported Se-GSHpx in ovarian tissue from the goat. A subsequent report (4) showed that \( ^{75}\text{Se} \) was accumulated preferentially by the placenta, ovary, pituitary, and adrenal glands, which suggests a need for Se in these body...
tissues. Buck et al. (4) speculate that Se deficiency in these reproductive and endocrine tissues may lead to reproductive dysfunctions such as retained placentas and reduced fertility. The results of our study support their contention. This element should be studied further in relation to ovarian function in view of the antiperoxidative role of Se (17).

Reproductive Performance

Measures of reproductive performance of the experimental animals were: days to first observed estrus, days to first breeding, days to conception, and services per conception. Differences of number of animals per reproductive measure group vary as a result of animals leaving the experiment. The only reproductive reason animals were removed from the experiment was repeat breeding. The occurrence of repeat breeding among groups was not different with one animal leaving each group for this reason.

Days to first observed estrus were: Sex E, 66.5 ± 7.1, 21 (X ± SE, n); E, 66.1 ± 7.7, 18; Se, 96.4 ± 8.7, 14; and control, 70.4 ± 7.9, 17. Days to first breeding were: Sex E, 96.4 ± 6.3, 21; E, 88.2 ± 6.8, 18; Se, 106.5 ± 7.7, 14; and control, 98.3 ± 7.2, 16. Days to conception were: Sex E, 117.9 ± 16.5, 17; E, 161.6 ± 18.8, 13; Se, 175.0 ± 20.5, 11; and control, 135.8 ± 17.5, 15. Services per conception were: Sex E, 2.06 ± .37, 17; E, 2.69 ± .42, 13; Se, 2.64 ± .46, 11; and control, 2.13 ± .39, 15.

Statistical analysis (16) of measures of reproductive performance from the 2 × 2 factorial design indicated that vitamin E supplementation decreased (P<.04) days to first observed estrus. An interaction of Se × vitamin E (P<.03) was observed for days to conception. Days to first breeding and services per conception did not differ between experimental groups. The basis for the Se and Sex vitamin E relationships is not known. We suggest the Se and vitamin E requirement of the dairy cow deserves further study in factorialized experiments that utilize controlled diets during the nonlactating and lactating periods to clarify these observations.

Plasma Data

Plasma concentrations of Se and Se-GSHpx were measured to assess Se treatment. Plasma Se-GSHpx has been considered more sensitive in the short term to Se supplementation than whole blood Se-GSHpx (49). Plasma Se concentration (μg/ml) at 21 days prepartum were low in all experimental groups: Sex E, 0.0375 ± 0.0023 (X ± SE); E, 0.0383 ± 0.0023; Se, 0.0318 ± 0.0024; and control, 0.0352 ± 0.0024. Data (19, 37) showed that prepartum Se concentrations less than .05 μg/ml increase the risk of RP. Statistical analysis of plasma Se concentrations at 21 days prepartum indicated a marginal effect (P<.07) of supplemental vitamin E. Greater plasma concentrations of Se in vitamin E supplemented groups cannot be established firmly as a vitamin E treatment effect because plasma concentrations of Se were not determined prior to vitamin E supplementation. Scott (42) suggested that Se may spare vitamin E requirement. Our observation would indicate that the reverse is also a possibility, and this interaction needs further investigation. The plasma Se response to Se injection is in Figure 2. All animals responded to Se treatment with plasma Se increasing to ≥ .09 μg/ml, a three-times increase over 24 h. After 1 day post-injection, plasma Se concentrations steadily declined to ≥ .057 μg/ml at calving. This agrees with accelerated clearance of Se during the immediate prepartum period (37). Concentrations of Se in plasma in noninjected animals tended to decline over the late prepartum period. At calving the concentration (μg/ml) of plasma Se in injected animals was greater (P<.001) than in un.injected: Se injected, .0570
Concentrations of Se-GSHpx in plasma [μmoles NADPH (nicotinamide adenine dinucleotide phosphate, reduced) oxidized/min/ml plasma] at 21 days prepartum were: Se × E, .163 ± .009 (X ± SE); E, .164 ± .009; Se, .137 ± .009; control, .160 ± .009. Statistical analysis of plasma Se-GSHpx at 21 days prepartum indicated a marginal effect (P<.10) of supplemental vitamin E as for plasma Se concentrations.

Plasma Se-GSHpx responded rapidly to Se treatment (Figure 3). Concentration in plasma increased steadily during the 2 days following injection and plateaued thereafter until 7 days before calving (Figure 3). The reason for the marked rise of Se-GSHpx at calving in Se injected animals is not known. Mean concentrations in plasma of Se-GSHpx in non-injected animals remained constant during the late prepartum period. The concentration of plasma Se-GSHpx was approximately twice as much (P<.001) in injected as un.injected animals at calving: Se injected, .291 ± .019; and un injected, .162 ± .009. The response of Se-GSHpx confirms results of (49). High correlations between blood Se and red blood cell Se-GSHpx (r = .97 to .98) were reported (6, 50). Our data indicate that the relationship between plasma Se and Se-GSHpx is not as close as the relationship in whole blood. We observed a correlation of .57 (P<.0003) between plasma Se-GSHpx and Se at 21 days before calving.

Means and standard errors of plasma concentrations of vitamins A, E, and total carotenoids are in Table 3. Plasma concentrations of vitamins A, E, and total carotenoids were measured to assess the effect of dietary vitamin E supplementation on these fat soluble vitamins.

The mean total caroteneid concentrations in plasma at 21 days before calving were ≈ 16% less (P<.08) in vitamin E supplemented animals (Table 3). This interaction between fat soluble vitamins probably is related to competitive absorption (41). Mean concentration of total carotenoids in each group decreased during the late prepartum period and at calving was approximately 60 to 74% of concentrations 21 days prepartum. Total carotenoids in plasma were less (P<.08) at calving in Se injected groups. This observation may be the result of increased tissue uptake. Selenium supplementation to lambs increased carotene uptake into liver and heart tissue (9).

Vitamins A and E were measured on different cows in each treatment group on different days prepartum. Therefore, regression (day) was included in the analysis of variance to test for treatment effects (16). Supplementing animals in groups 1 and 2 with vitamin E was effective (P<.001) for elevating plasma concentrations of α-tocopherol by ≈ 50% (Table 3). Result was similar by Parish (34), who reported total tocopherol concentrations in plasma of 4.0 to 5.0 μg/ml during the late prepartum period in animals supplemented with .5 to 1.0 g of vitamin E per day. Decline (P<.001) of plasma α-tocopherol was linear (Table 3) in agreement with (34).

Vitamin E supplementation affected (P<.08) concentrations of vitamin A in plasma (Table 3). Vitamin A concentrations (Table 3) increased (P<.01) during the late prepartum period to greatest concentrations around parturition in contrast to (21).

General Conclusions

This study indicates Se status of the dairy animal at calving has an effect on uterine health and ovarian function during the early postpartum period (Table 2, Figure 1). Deficiency
of Se during the prepartum period has a continuing effect and predisposes the dairy animal to increased risk of MET and CO in the subsequent postpartum period. Providing supplemental vitamin E during the prepartum period appears to be an important factor in the efficacy of Se treatment for prevention of RP in dairy cattle. Vitamin E-supplemented animals received ~1.0 g of vitamin E ([d]-α-tocopherol equivalents) per day from the combined intake of added supplement and haylage. We conclude that supplementation of vitamin E is indicated during the prepartum period when diets are fed that supply inadequate quantities of vitamin E. Further experimentation with graded quantities of vitamin E will be required to establish the minimum vitamin E requirement during the prepartum period.

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