Effect of vitamin E on the immune system of ewes during late pregnancy and lactation

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ABSTRACT

The present experiment was designed to determine the effects of a regimen of repeated, intramuscular (i.m.) injections of vitamin E (VE) on innate and humoral components of the immune response of pregnant and lactating ewes. Pregnant ewes were randomly assigned to two treatments consisting of i.m. injections of either VE (30 IU α-tocopherol/kg BW, n = 10) or equivalent volume of a placebo (emulsified base, n = 8). Injections were administered at 2-week (wk) intervals to all ewes as a group, for a total of 24 wk. Lambing occurred over a 5-wk period, starting wk 7 of the study. All ewes were administered a booster vaccination for Clostridium tetani and Clostridium perfringens (C + D) at wk 3. Blood and colostrum were analyzed for α-tocopherol and selenium content, and extracellular glutathione peroxidase (GPX3) activity as measures of antioxidant status; lysozyme activity as a measure of innate immunity and immunoglobulin G (IgG), and anti-tetanus toxoid IgG (IgG-T) concentration as a measure of humoral immune status. Three ewes in the placebo group did not lamb and were therefore excluded from all analyses. Results showed a significant treatment × week effect of VE supplementation on IgG concentration. VE supplementation increased IgG concentrations in ewes after their yearly booster vaccination against C. tetani and C. perfringens; placebo-treated ewes showed no such response. The extent, magnitude and persistence of the decreased IgG concentration during the transition period were less in VE-supplemented ewes than controls. There was no effect of VE supplementation on the concentration of IgG-T after vaccination. VE-supplementation of pregnant ewes had no effect on serum lysozyme activity. GPX3 increased in both treatment groups, peaking during the transition period. The α-tocopherol content of the colostrum of VE-supplemented ewes was greater than that of controls. In conclusion, VE supplementation of pregnant and lactating ewes had an equivocal effect on variables used to assess immune function. The benefit of VE supplementation to pregnant and lactating ewes requires further research however it may have utility in blunting the immune suppression that occurs during the transition period.

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1. Introduction

The maternal immune system during pregnancy and lactation is under increased physiological stress. During the transition period, defined in dairy cows and ewes as the three-week period before and several weeks after
parturition (Caroprese et al., 2006; Drackley, 1999; Spears and Weiss, 2008; Theodorou et al., 2007), animals are more susceptible to disease (Mallard et al., 1998). The stress of pregnancy and increased metabolic demands during parturition and lactation result in increased production of reactive oxygen species (ROS), leading to reductions in neutrophil function, antibody responses and cytokine production by immune cells (Spears and Weiss, 2008). The unprepared dam may be compromised in the ability to repair tissues damaged during parturition while maintaining resistance to infection (Andrieu, 2008).

The immune status of the ewe impacts the maternal transfer of immune cells in the colostrum. Efforts to enhance immune function with vitamin E (VE) supplementation have produced inconsistent results in adult sheep (Hatfield et al., 2002), pregnant ewes (Daniels et al., 2000; Gentry et al., 1992; Giadinis et al., 2000; Larsen et al., 1988) and lambs (Daniels et al., 2000; Gentry et al., 1992).

In 2007, the National Research Council (NRC, 2007) increased the recommended intake of VE for pregnant and lactating ewes from 1.2 IU/kg BW to 5.6 IU/kg BW to ensure provision of sufficient quantities of VE to the neonate from the colostrum. This recommendation, however, lacks empirical support.

The present experiment was designed to determine the effects a regimen of repeated, i.m. injections of VE on innate and humoral components of the immune response of pregnant and lactating ewes.

2. Materials and methods

2.1. Animals

Eighteen Dorset ewes, from the University of Rhode Island’s Peckham farm were used in this study. The ewes ranged in age from 1 to 9 year and were confirmed pregnant via ultrasound. The majority of ewes (15) were born and raised at Peckham Farm. There were 2 sets of twins and the rest of the ewes were a half-sibling of at least one other ewe on the study. There was only one ewe that did not have any shared ancestry. Two ewes were primiparous and the rest multiparous. Ewes were fed to meet or exceed the nutritional requirements of the ‘average’ ewe and the ‘average’ was adjusted over time to reflect changes in the ewes. Ewes were provided with constant access to clean drinking water and loose sheep mineral. General health, body condition and weight were monitored throughout the study. This study was conducted under the approval of the Institutional Animal Care and Use Committee of the University of Rhode Island according to the USDA and the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Study design

Ewes were randomly assigned to two treatments consisting of either i.m. injections of VE (30 IU n-α-tocopherol (Natural E-300, Neogen Corp., Lexington, KY)/kg BW (n = 10) or equivalent volume of a placebo (emulsified base, n = 8). Injections were administered in the neck, cranial to the scapula, at 2-week (wk) intervals to all ewes, as a group, for a total of 24. Lambing occurred over a 5-wk period, starting wk 7 of the study. There was an average of 2.2 and 2.3 lambs born per ewe in the VE and placebo group, respectively. Ewes, with an average weight of 102 ± 2 kg at the beginning of the study, were weighed prior to each injection to account for losses in weight at parturition and BW changes during lactation. All ewes were given a yearly booster vaccination for Clostridium tetani and Clostridium perfringens (C + D) (Colorado Serum Company, Denver, CO) at week 3 of the study. Blood and colostrum were analyzed for α-tocopherol concentration, selenium (Se) concentration, and glutathione peroxidase (GPX3) activity as a measure of antioxidant status; lysozyme activity as a measure of innate immunity and immunoglobulin G (IgG), and anti-tetanus toxoid IgG (IgG-T) concentration as a measure of humoral immune status.

2.3. Sample collection

2.3.1. Blood and colostrum collection

Blood samples were collected via jugular puncture every two weeks throughout the study period. Blood was collected into sterile serum separator and EDTA vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) for the analysis of serum and plasma, respectively. Tubes were centrifuged (1750 x g) at 4 °C (for plasma) or 25 °C (for serum). Plasma and serum were aliquoted and stored at –80 °C until analysis. All blood samples for analyses were obtained prior to bi-weekly VE and placebo injections. Colostrum from the ewes was collected immediately after parturition and stored at –80 °C until analysis. Immediately prior to analysis, colostrum was thawed and centrifuged (24,400 x g for 20 min at 4 °C) and the fat layer was removed.

2.3.2. Feed sampling and analysis

Weekly samples of hay (mixed grass hay) and a commercial grain pelleted (16% sheep pellet, Central Connecticut Co-op, Manchester, CT) were obtained for nutrient and α-tocopherol analysis and stored at –20 °C. At the conclusion of the study composite samples of the hay and grain were made and stored at –20 °C until nutrient analysis at Dairy One Inc. (Ithaca, NY) and vitamin E analysis at the Diagnostic Center for Population and Animal Health (DCPAH, Lansing, MI). The loose mineral mix (Sheep Mineral 20 S, Blue Seal Inc., Muscatine, IA) was analyzed for selenium content (DCPAH, Lansing, MI).

2.4. Sample analysis

2.4.1. α-Tocopherol analysis

Serum, tissue, and feedstuffs were analyzed for α-tocopherol content at DCPAH (Lansing, MI). For the analysis of serum α-tocopherol, serum was extracted into hexane; extracts were analyzed by reverse-phase high-performance liquid chromatography (HPLC) using a C18 column (Waters Corp., Milford, MA) and acetone:methylene chloride:methanol (70:20:10) mobile phase, with detection at 292 nm using a photo diode array detector. Results are expressed as μg α-tocopherol/mL.

2.4.2. Immunoglobulin analysis

A sheep IgG ELISA Quantitation Kit (custom kit, discontinued catalog #E130-101, Bethyl Laboratories, Montgomery, TX) was used to determine total serum IgG. Unless otherwise indicated, all steps were performed for 1 h at room temperature in Nunc 96-well C-bottom immunoplates (Thermo Fisher Scientific, Rochester, NY). Wells were coated with affinity purified rabbit anti-sheep IgG (10 μg/mL), diluted in 0.05 M carbonate–bicarbonate buffer (pH 9.6, Sigma–Aldrich, St. Louis, MO) and then blocked with wash buffer (PBS with 0.05% Tween 20). Reference serum was serially diluted to generate a standard curve and experimental samples were diluted (1:100,000) in wash buffer. A standard sample serum was run on each plate to control for intra- and inter-assay variability; a CV ≤ 5% for duplicate determinations was considered acceptable. Rabbit anti-sheep-IgG-HRP conjugate (0.01 μg/mL) was diluted in wash buffer for the detection step and then enzyme substrate solution (TMB, H2O2, Kirkegaard and Perry, Gaithersburg, MD) was added, allowed to develop for 15 min, and the reaction stopped with 2 M hydrogen peroxide. Absorbance at 450 nm was determined using a colorimetric plate reader (BioTek Instruments Inc., Winooski, VT). Results are expressed as total serum IgG concentration (mg/mL).

Total IgG produced against tetanus toxoid (IgGT) was measured as previously described (Roskopf et al., 2005). Costar 96-well microplates (Thermo Fisher Scientific, Rochester, NY) were coated with 0.2 μL of tetanus toxoid (Colorado Serum Co., Denver, CO) diluted in 0.05 M carbonate–bicarbonate buffer, incubated for 18 h at 4 °C. All subsequent steps were performed for 1 h at 37 °C with shaking, unless otherwise indicated. Plates were blocked and lamb serum was diluted (1:1000) in wash
buffer. A standard serum sample was run on each plate to control for inter- and intra-assay variability; a CV < 5% for triplicate determinations was accepted. Donkey anti-sheep-HRP conjugate (Bethel Laboratories, Montgomery, TX) was diluted (0.05 µg/ml) in wash buffer for detection, and substrate solution was added to each well, incubated for 10 min at room temperature, in the dark, before being stopped with the addition of 1.25 M H₂SO₄. Absorbance at 450 nm was determined as previously described (Adams et al., 1997).

2.4.3. Lysozyme activity

Lysozyme activity was determined by the radial diffusion of samples through a suspension of live culture of *Micrococcus luteus* (ATCC 27141, Manassas, VA) in M/15 phosphate buffered agarose, pH 6.3, following the procedure of (Sotirov et al., 2005). The diameters of the lytic zones were compared with those of the standard, hen egg white lysozyme (Sigma–Aldrich, St. Louis, MO). Results are expressed as µg of hen egg white lysozyme equivalent.

2.4.4. Selenium and GPX3

Plasma samples were analyzed at the USDA Grand Forks Human Nutrition Research Center (Grand Forks, North Dakota) for Se content and the activity of the extracellular Se-dependent glutathione peroxidase (GPX3). Selenium was determined by automated electrothermal atomic absorption spectrophotometry using a reduced palladium matrix modifier graphite tubes fitted with L’Vov platforms, and automated Zeeman-effect background correction. Results are expressed as ng/ml. GPX3 was assayed using the glutathione reductase-coupled assay (Paglia and Valentine, 1967), modified by using 0.25 mM H₂O₂ as substrate (Lawrence and Burk, 1976). Results are expressed as nanomoles of NADPH/min/mg of protein.

2.4.5. Statistical analysis

The mixed procedure in SAS (SAS Inst. Inc., Cary, NC) was used to analyze the effect of vitamin E supplementation of dams on serum α-tocopherol, lysozyme activity, antibody concentrations, selenium, and GPX3. The random effect of ewe was accounted for and a repeated measures approach was used. The appropriate error structure was assessed and applied for each outcome variable. Treatment, week and treatment × week remained in all models. The ewes in this study ranged from 1 to 9 years of age; therefore, ewes were classified as younger (<7 years of age, n = 11) or older (≥7 years of age, n = 4), and the binary age variable was offered into each model and subsequently removed from the model if not significant. Tukey’s adjusted P-values were calculated for each 2-way comparison to adjust for multiple 2-way comparisons within each model. Once the final model was chosen, residuals were checked for homoscedasticity, outliers, leverage cases, and normal distribution. Student’s t-test was used to statistically analyze colostral content of α-tocopherol, selenium, GPX3 activity, lysozyme activity, IgG and IgG-T content. Significance was defined for all tests as P ≤ 0.05.

### 3. Results

The data from these animals were excluded from all analyses.

3.1. Feed analyses

The nutritional content of the composite grain and hay sampled fed over the study period is presented in Table 1. VE content of the grain and hay was 46 and 50 IU/kg DM, respectively.

3.2. Alpha-tocopherol analyses

There was a treatment x week effect for serum α-tocopherol (P ≤ 0.0001, Fig. 1). Serum α-tocopherol content of VE-supplemented ewes increased from the baseline level, showing a maximum at the last time point sampled (week 16) and exceeding the serum α-tocopherol levels of controls at weeks 8, 12 and 16 (Fig. 1). The α-tocopherol

<table>
<thead>
<tr>
<th>Components</th>
<th>Grain mix</th>
<th>Hay</th>
<th>Mineral mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (%)</td>
<td>88.6</td>
<td>91.2</td>
<td></td>
</tr>
<tr>
<td>CP (%)</td>
<td>18.8</td>
<td>12.1</td>
<td></td>
</tr>
<tr>
<td>NDF (%)</td>
<td>32.6</td>
<td>59.2</td>
<td></td>
</tr>
<tr>
<td>ADF (%)</td>
<td>11.5</td>
<td>35.5</td>
<td></td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>1.44</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.91</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Magnesium (%)</td>
<td>0.40</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>0.91</td>
<td>2.01</td>
<td></td>
</tr>
<tr>
<td>NEm (Mcal/kg)</td>
<td>1.86</td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>NE (Mcal/kg)</td>
<td>1.23</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>Vitamin E (IU/kg)²</td>
<td>46.3</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol (µg/g)³</td>
<td>42.14</td>
<td>45.47</td>
<td></td>
</tr>
<tr>
<td>Selenium (µg/g)³</td>
<td>1.13</td>
<td>0.03</td>
<td>44.0</td>
</tr>
</tbody>
</table>

³ Proximate analysis conducted by Dairy One Cooperative, Inc. (Ithaca, New York) except as noted, results expressed on a dry matter basis.  
² Analyzed at the Diagnostic Center for Population and Animal Health (Michigan State University, Lansing, MI).

### 3.3. Immunoglobulin analyses

There was a treatment x week effect of VE supplementation on IgG concentration (P = 0.03, Fig. 2). There was an increase in IgG concentrations observed in vitamin E supplemented ewes after their yearly booster vaccination against *Clostridium tetani* and *C. perfringens*. This vaccination response did not occur in placebo ewes. The extent, magnitude and persistence of the decreased IgG concentration during the transition period were less in VE-supplemented ewes than controls (Fig. 2). Vitamin E supplementation did not affect the IgG content of colostrum (P = 1.00).

![Fig. 1. Serum α-tocopherol concentrations of ewes supplemented with d-α-tocopherol (●, 30 IU d-α-tocopherol/kg BW/2 wks, n = 10) or placebo (■, emulsified base, n = 5) during late pregnancy and lactation. Ewes were vaccinated against *Clostridium tetani* and *Clostridium perfringens* week three, approximately four weeks prior to the start of lambing (•—•). Mean ± SEM. P < 0.0001 versus placebo within week. *P < 0.0001 versus baseline (week 0) within treatment, †P < 0.0001 versus week 16 within treatment.](image-url)
Effect of treatment on study variables in colostrum from ewes supplemented with δ-α-tocopherol (30 IU δ-α-tocopherol/kg BW/2 weeks, n = 10) or placebo (emulsified base, n = 5) during late pregnancy and lactation.a

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol (µg/mL)</td>
<td>Placebo</td>
<td>Vitamin E</td>
</tr>
<tr>
<td>IgG (mg/mL)</td>
<td>2.63 ± 0.60</td>
<td>13.37 ± 2.90</td>
</tr>
<tr>
<td>IgGT (OD450)</td>
<td>0.61 ± 0.13</td>
<td>0.56 ± 0.13</td>
</tr>
<tr>
<td>GPX3 (µg/mL)</td>
<td>0.24 ± 0.16</td>
<td>0.22 ± 0.06</td>
</tr>
</tbody>
</table>

Mean ± SEM; a IgG against tetanus toxoid measured in absorbance at 450 nm; b Selenium dependent glutathione peroxidase, nmole NADPH/min/mg protein.

**Fig. 2.** Serum IgG concentrations of ewes supplemented with δ-α-tocopherol (■), 30 IU δ-α-tocopherol/kg BW/2 wks, n = 10) or placebo (□, emulsified base, n = 5) during late pregnancy and lactation. Ewes were vaccinated against Clostridium tetani and Clostridium perfringens week three of the study. Means within columns with differing superscripts differ (P ≤ 0.05).

<table>
<thead>
<tr>
<th>Week of study</th>
<th>IgGT (OD450)</th>
<th>Lysozyme (µg/mL)</th>
<th>Selenium (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.59 ± 0.09ab</td>
<td>0.19 ± 0.01a</td>
<td>192 ± 5a</td>
</tr>
<tr>
<td>2</td>
<td>0.45 ± 0.06a</td>
<td>0.21 ± 0.01ab</td>
<td>205 ± 7ab</td>
</tr>
<tr>
<td>4</td>
<td>1.06 ± 0.15cd</td>
<td>0.20 ± 0.02ab</td>
<td>199 ± 8ac</td>
</tr>
<tr>
<td>6</td>
<td>1.17 ± 0.11ce</td>
<td>0.22 ± 0.02ac</td>
<td>197 ± 7ed</td>
</tr>
<tr>
<td>8</td>
<td>1.42 ± 0.11e</td>
<td>0.22 ± 0.02ad</td>
<td>205 ± 7 ze</td>
</tr>
<tr>
<td>10</td>
<td>1.10 ± 0.12ef</td>
<td>0.22 ± 0.02ae</td>
<td>200 ± 7f</td>
</tr>
<tr>
<td>12</td>
<td>0.92 ± 0.10gh</td>
<td>0.25 ± 0.02g</td>
<td>192 ± 7i</td>
</tr>
<tr>
<td>14</td>
<td>0.83 ± 0.09hi</td>
<td>0.26 ± 0.02dehi</td>
<td>198 ± 8j</td>
</tr>
<tr>
<td>16</td>
<td>0.99 ± 0.11hi</td>
<td>0.22 ± 0.02dh</td>
<td>203 ± 8k</td>
</tr>
<tr>
<td>18</td>
<td>1.04 ± 0.11bc</td>
<td>0.23 ± 0.02db</td>
<td>209 ± 8l</td>
</tr>
<tr>
<td>20</td>
<td>0.76 ± 0.08bc</td>
<td>0.30 ± 0.02b</td>
<td>216 ± 7b</td>
</tr>
<tr>
<td>24</td>
<td>0.71 ± 0.09bc</td>
<td>0.29 ± 0.02b</td>
<td>224 ± 7b</td>
</tr>
</tbody>
</table>

* Lambing occurred over a 5-week period starting week 7 of the study. Ewes were vaccinated against Clostridium tetani and Clostridium perfringens week three of the study. Means within columns with differing superscripts differ (P ≤ 0.05).

Effect of treatment on study variables in ewes supplemented with δ-α-tocopherol (30 IU δ-α-tocopherol/kg BW/2 weeks, n = 10) or placebo (emulsified base, n = 5) during late pregnancy and lactation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol (µg/mL)</td>
<td>Placebo</td>
<td>Vitamin E</td>
</tr>
<tr>
<td>IgG (mg/mL)</td>
<td>2.14 ± 0.09</td>
<td>3.45 ± 0.11</td>
</tr>
<tr>
<td>IgGT (OD450)</td>
<td>0.95 ± 0.13</td>
<td>0.90 ± 0.12</td>
</tr>
<tr>
<td>Lysozyme (µg/mL)</td>
<td>0.28 ± 0.01</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>Selenium (ng/mL)</td>
<td>215 ± 4a</td>
<td>197 ± 2</td>
</tr>
</tbody>
</table>

* There was a treatment x week interaction, see Fig. 1.

Lysozyme activity tended to be greater in control ewes (P = 0.07, Table 3) and increased over time (across treatments, Table 4). VE supplementation did not affect the IgG-T content of colostrum (P = 0.8, Table 2). Older ewes had greater serum IgG-T than younger ewes (P = 0.05, Table 5).

3.4. Lysozyme

Lysozyme activity increased over time (across treatments, Table 4). VE supplementation did not affect the lysozyme activity of colostrum (P = 0.44, Table 2).

3.5. Selenium

Plasma Se was slightly less in VE-supplemented ewes compared to controls (P = 0.04, Table 3) and in older ewes versus younger ewes (P = 0.03, Table 5). Plasma Se increased in both treatments during the study (P ≤ 0.0001, Table 4). VE supplementation did not affect the Se content of colostrum (P = 0.15, Table 2).

3.6. Selenium-dependent glutathione peroxidase

There was a treatment x week effect on GPX3 activity (P ≤ 0.05, Fig. 3), which showed a slight increase during the middle of the lambing period (week 10). VE supplementation did not affect the GPX3 activity of colostrum (P = 0.88, Table 2).

4. Discussion

The objective of this study was to determine the effect of a regimen of VE supplementation (30 IU δ-α-tocopherol/kg BW/everly 2 weeks), delivered as a.i.m. bolus dose, on parameters of innate and humoral immunity in ewes during late pregnancy and lactation. Results demonstrated that VE increased total serum IgG concentration following vaccination, and reduced the extent, magnitude and persistence of decreased serum IgG concentration...
Table 5
Effect of age of ewe on study variables in ewes supplemented with α-α-tocopherol (30 IU α-α-tocopherol/kg BW/2 wks, n = 10) or placebo (emulsified base, n = 5) during late pregnancy and lactation.a

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age of ewes</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥7 years old (n = 4)</td>
<td>&lt;7 years old (n = 11)</td>
</tr>
<tr>
<td>α-Tocopherol (µg/mL)</td>
<td>2.82 ± 0.17</td>
<td>3.06 ± 0.12</td>
</tr>
<tr>
<td>IgG (mg/mL)</td>
<td>18.68 ± 0.64</td>
<td>17.94 ± 0.38</td>
</tr>
<tr>
<td>IgGT (OD60) b</td>
<td>1.2 ± 0.05</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>Lysozyme (µg/mL)</td>
<td>0.25 ± 0.01</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>GPX3 c</td>
<td>0.95 ± 0.10</td>
<td>0.88 ± 0.03</td>
</tr>
<tr>
<td>Selenium (ng/mL)</td>
<td>188 ± 4</td>
<td>209 ± 2</td>
</tr>
</tbody>
</table>

* Mean (±SEM).

b IgG against tetanus toxoid measured in absorbance at 450 nm.

c Selenium dependent glutathione peroxidase, nmol NADPH/min/mg protein.

during the transition period. However, there was no effect of VE-supplementation on IgG-T response to vaccination, lysozyme activity or GPX3 activity.

As expected, VE-supplementation increased serum and colostral α-tocopherol levels. Serum α-tocopherol concentrations in control ewes (2.14 µg/mL) were similar to those observed by Hatfield et al. (2002) of 1.8–2.3 µg/mL, and were greater than those reported by Daniels et al. (2000) of 1.2µg/mL. The serum α-tocopherol concentration for the VE-supplemented ewes in the present study (3.5 µg/mL) were greater than those reported by Daniels et al. (2000), 1.9 µg/mL, in which ewes were orally supplemented with 400 IU/day. The present finding that VE increased colostrum content of α-tocopherol are consistent with previous findings (Bass et al., 2001; Pinelli-Saavedra et al., 2008; Weiss et al., 1990).

Although serum α-tocopherol concentrations of <2.0 µg/mL have been considered deficient (Maas et al., 1984; McMurray, 1982), the current range considered adequate for adult sheep is 1.5–3.0 µg/mL (DCPAH, Lansing, MI). The increase in serum α-tocopherol concentrations over the study is reflective of a successful supplementation strategy.

There was a 21% versus 37% decrease in pre-partum serum IgG in the VE-supplemented versus placebo-supplemented ewes. The biologic significance of the difference between treatment groups in the magnitude and persistence of the drop in serum IgG concentration is not clear. As the total IgG content of the colostrum for both groups (57 mg/mL) was at the high end of the normal range for sheep (40–60 mg/mL, Tizzard, 2013), it is possible that VE-supplementation dampened the immunosuppression that occurs in the ewe during the transition period without compromising the quantity of immunoglobulins transferred to the offspring.

As expected, all ewes produced a greater amount of anti-tetanus antibodies in response to vaccination against tetanus toxoid however; there was no additive effect of VE supplementation on IgG-T concentration. While the present results are consistent with those of Daniels et al. (2000) and Hatfield et al. (2002), they contrast with the findings of others who found supplemental VE to increase antibody titers to specific antigens, such as anti-tetanus (Larsen et al., 1988), anti-C. perfringens (Tengerdy et al., 1983) and anti-parainfluenza type 3 (Reffett et al., 1988). These studies, however, were focused on vaccination responses in lambs, which do not have the memory response of the aged ewes in this study.

To date, there has been little research on the effects of VE on innate immunity in ruminants. Lysozyme activity can reflect the ability of the serum to lyse potential pathogens (Firth et al., 2005). Few studies have examined the effect of VE-supplementation on lysozyme activity. Lysozyme activity was shown to be responsive to VE supplementation in piglets and rats (Babinszky et al., 1991; Jakubowski et al., 2004). In the present study, VE-supplementation of pregnant ewes had no effect on serum lysozyme activity. Similar findings were observed in ewes supplemented with both VE and Se before lambing (Morgante et al., 1999). The increase in lysozyme activity over time across treatments may be a reflection of the response of the ewes to the changing seasons as has been observed in goats (Semerdjiev et al., 2009) but contrary to what has been previously observed in sheep (Bivolarski and Sotirov, 2001).

Due to the well-documented interactions between VE and Se, Se status was assessed on the basis of plasma Se concentration and GPX3 activity. Unexpectedly,
VE-supplementation was associated with a small (8%) but significant reduction in plasma Se within the range of nutritional adequacy (70–400 ng/mL, Raisbeck, 2000). This effect was not associated with a change in GPX3 activity. Studies in humans would suggest that plasma Se levels, such as observed in this study, are well in excess of those associated with optimal expression of the two selenoenzymes in plasma, GPX3 and selenoprotein P (SePP1). Thus, it is likely that this small effect reflects a difference in the dominant component of plasma Se, non-specific Se present as selenomethionine in plasma proteins (Combs et al., 2011), which is not known to have any immediate nutritional significance.

Ewe age was a significant a factor of two variables in this study, IgG-T concentration and plasma Se content. That older ewes produced more IgG-T than younger ewes can be attributed to an increased memory response to vaccination in the older animals. The small (10%) increase in plasma Se in younger ewes likely reflects a higher non-specific incorporation of selenomethionine into albumin and other plasma proteins that can contain that dominant form of Se in foods (Combs et al., 2011). Furthermore, the small number of animals used in this study limits the scope and conclusions that can be drawn from the results. It is possible that some differences between treatment groups (e.g. lysozyme in colostrum) that were not significant could reach significance given a higher number of animals.

5. Conclusion

The results of this study demonstrate that supplemental VE increased serum IgG concentration following vaccination of ewes in late pregnancy and reduced the magnitude of the pre-partum drop in serum IgG in the ewes without changing the amount of IgG transferred by the ewes to the colostrum. This response would indicate the potential for use of this treatment to counter the immune suppression that occurs in ewes during the transition period.

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References


Tizzard, I.R., 2013. Veterinary Immunology, 9th ed. Elsevier, St. Louis, MO.