Effects of Vitamin E and Selenium on Immune Responses of Peripheral Blood, Colostrum, and Milk Leukocytes of Sows

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ABSTRACT: This study was designed to assess how dietary vitamin E (E) and(or) selenium (Se) concentrations affect immune responses of gestating and peripartum sows. Multiparous sows (24), assigned to one of four groups at breeding, were fed ensiled, shelled corn-soybean meal-based diets without supplemental E or Se (-E–Se), with .3 mg of Se/kg (-E+Se), with 60 IU of E/kg (+E–Se), or with both supplemental E and Se (+E+Se) during gestation and to d 4 of lactation. Blood was obtained on 0, 30, 60, and 90 d of gestation and at parturition for serum E and Se assays. Lymphocytes and polymorphonuclear cells (PMN) were isolated from the blood, colostrum, and 4-d milk samples for immune studies. Compared with the control (+E+Se) diet, the -E–Se diet reduced (P < .05) the serum tocopherol and Se concentrations, the mitogenic responses of lymphocytes of peripheral blood (PBL) and colostrum (CL), the phagocytic activity of blood and colostral PMN, and the microbicidal activity of blood, colostral, and milk PMN. The -E+Se diet reduced (P < .05) the serum tocopherol concentrations, the mitogenic responses of PBL and CL, and the phagocytic activity of PMN. The +E–Se diet reduced (P < .05) serum Se concentrations and the phagocytic activity of PBL. The +E+Se diet reduced (P < .05) serum tocopherol concentrations and the phagocytic activity of PMN. The data indicated that E restriction depressed PBL and PMN immune functions, whereas Se restriction depressed mainly PMN function.

Key Words: Pigs, Selenium, Vitamin E, Immunity, Gestation Period

Introduction

During the last decade, it has been demonstrated in several species that vitamin E (E) and Se influence immune responses (Nockels, 1986). Early research on the effects of nutrients on immunity involved measuring antibody production (Marsh et al., 1981) and host resistance to disease challenges (Teige et al., 1982). More contemporary immune-response measures involve quantifying the ability of lymphocytes to proliferate when stimulated by mitogens (Bendich et al., 1983, 1986) and the abilities of polymorphonuclear cells (PMN) to phagocytize (engulf) and(or) kill invading organisms (Boyne and Arthur, 1986).

The metritis-mastitis-agalactia (MMA) syndrome of sows has been associated with inadequate dietary vitamin E and Se (Whitehair et al., 1983). If the infectious aspects (metritis and mastitis) of this complex were manifestations of impaired immunity, the sow could serve as an animal model for investigations of immunocompetence.

Although Larsen and Tollersrud (1981) and Jensen et al. (1988) have studied the influence of vitamin E and Se on swine lymphocytes, reports of research efforts to quantify the immune actions of PMN of sow blood, colostrum, and milk of swine were not found. We, therefore, elected to study the influence of vitamin E and(or) Se on the immunoresponsiveness of cellular components of peripheral blood of gestating sows and in their colostrum and milk.

Materials and Methods

Animals and Diets. Twenty-four multiparous sows, from the Michigan State University herd of purebred Yorkshires, Duroc x Yorkshire crossbreds, and Landrace x Yorkshire crossbreds, served as experimental animals. The sows averaged approximately 225 kg in BW and were part of an all-in-all-out farrowing system. Because the immunologic analyses required in...
Vitamin-trace mineral premix

Vitamin E,
Mono-dicalcium phosphate
ber. The sows were bred at the first postweaning
basal diet, without supplemental vitamin E or Se, was
conducted on stored samples, four sows (two control
kg constituted the vitamin E-deficient
this study were labor-intensive and could not be
the monthly weaning cycles between June and Novem-

diet. This diet with .3 mg of Sekg (+E-Se), whereas the supplemented diets contained
In a 5-mL plastic vial, and frozen at -20°C for

Table 1. Composition of diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>-E-Se</th>
<th>+E-Se</th>
<th>-E+Se</th>
<th>+E+Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried, high-moisture corn</td>
<td>860.0</td>
<td>858.88</td>
<td>858.5</td>
<td>858.38</td>
</tr>
<tr>
<td>Soybean meal (44% CP)</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Mono-dicalcium phosphate</td>
<td>17.5</td>
<td>17.5</td>
<td>17.5</td>
<td>17.5</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Vitamin-trace mineral premixb</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Vitamin E, 500,000 IU/kg</td>
<td>—</td>
<td>.12</td>
<td>—</td>
<td>.12</td>
</tr>
<tr>
<td>Selenium, 200 mg/kg</td>
<td>—</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*Treatments consisted of an ensiled shelled corn-soybean meal-based diet without supplemental vitamin E or Se (-E-Se), with .3 mg of Se/kg (-E+Se), with 60 IU of E/kg (+E-Se), or with both supplemental E and Se (+E+Se).

Supplied the following per kilogram of diet: 4,000 IU of vitamin A, 800 IU of vitamin D3, 4 mg of riboflavin, 16 mg of d-pantothenic acid, 21 mg of niacin, 23 µg of vitamin B12, 2.6 mg of menadione, 152 mg of choline chloride, 90 mg of Zn, 71 mg of Fe, 4.5 mg of Mn, 12 mg of Cu, and .5 mg of I.

this study were labor-intensive and could not be
conducted on stored samples, four sows (two control and two treated) were assigned to experiment after
feeding of the experimental diets started at the time
of breeding and continued until 4 d postpartum.

The experimental diets (Table 1) were corn and
soybean meal-based. The corn had been ensiled as
high-moisture corn (for destruction of vitamin E) and
dried to approximately 13% moisture before use. This
basal diet, without supplemental vitamin E or Se, was
designated the vitamin E- and Se-deficient
diet. This diet with .3 mg of Se (as sodium selenite)/kg constituted the vitamin E-deficient (-E-SE) diet, with 60 mg of vitamin E (as all-rac-α-tocopheryl acetate)/kg constituted the Se-deficient (+E-SE) diet, and with both .3 mg of Se and 60 mg of vitamin E/kg constituted the control (+E+Se) diet. By analyses, the basal diet contained .29 mg of vitamin E and .089 mg of Se/kg, whereas the supplemented diets contained 55.3 mg of vitamin E and/or .35 mg of Se/kg.

The bred sows were tethered by neck collars in the
gestation facility, fed 2.5 kg/d of the assigned diet, and
allowed ad libitum access to tap water. At approximately
7 d before farrowing, the sows were moved to a
farrowing barn equipped with metal farrowing stalls.

Sample Collections. Blood samples from the jugular vein were collected into plain and heparinized Vacu-
tainer tubes (Beckton Dickinson, Rutherford, NJ) at
breeding, at 30, 60, and 90 d of gestation, and at parturition. The heparinized samples were immediately
placed in an ice bath for subsequent cell isolation
and immunoassays. The nonheparinized samples were
allowed to clot at room temperature for ≥3 h and were
centrifuged at 400 × g for 15 min. Serum from each
sample was withdrawn with a Pasteur pipette, placed
in a 5-mL plastic vial, and frozen at −20°C for
subsequent vitamin E, Se, and glutathione peroxidase
(GSH-px) analyses.

Colostrum samples were obtained during parturition
and milk samples were obtained 4 d postpartum.
Before hand-milking the samples, the udder was
washed with warm water, disinfected with 70% ethanol, and dried with paper toweling. Letdown of
the milk samples was induced by administering .1 µmol of oxytocin intramuscularly. Approximately 50
mL of colostrum and milk was collected into sterile
polypropylene centrifuge tubes, which were then immediately placed in an ice bath.

Isolation of Cells. Peripheral blood lymphocytes (PBL) were isolated from the heparinized blood by
Ficoll-hypaque (specific gravity, 1.357) density gradient
techniques (Barta et al., 1984b). Washed lymphocytes were suspended in 2 mL of Rosewell Park
Memorial Institute (RPMI) 1640 culture media
(Gibco Laboratories, Grand Island, NY) sup-
plemented with fungizone (6 mL/L), HEPES (12 mL/
L), NaHCO3 (1.5 g/L), gentamycin sulfate (16 mg/L),
and heat-inactivated porcine serum (100 mL/L).
Lymphocytes were counted in a Neubauer hemocytom-
eter, and their viability was assessed by trypan blue
exclusion. Dilution with RPMI media was made to
obtain a final concentration of 1 to 2 × 10⁶ viable cells/mL.

Polymeronuclear cells were also isolated from the plasma and Ficoll layers by differential centrifugation
(Boyum, 1968). After lysis of the red blood cells in the
granulocyte pellet, the clean granulocyte preparation
was counted in a hemocytometer and adjusted to 1 ×
10⁶ cells/mL.

For cell isolation from colostrum and milk, the
50-mL samples were diluted 1:5 and 1:2, respectively,
with cold, sterile PBS. The diluted colostrum or milk
was then defatted by centrifugation at 400 × g for 20
min. The supernate and fat layers were discarded. The
cell pellet was then washed twice in cold PBS and
centrifuged at 215 × g for 15 min. The washed cells were suspended in 1 mL of PBS containing 50% inactivated bovine serum and smeared on 2.5-cm × 7.5-cm glass slides for staining and differential cell counting.

For lymphocyte and granulocyte isolation, the washed cells were reconstituted in PBS with .009 M EDTA. Ten milliliters of the cell suspension was then layered onto 3 mL (specific gravity, 1.357) of Ficoll-hypaque and centrifuged at 400 × g for 40 min. After centrifugation, the lymphocyte layer was isolated from the buffy interface and the granulocytes were isolated from the cell pellet lying at the bottom of the tubes. The numbers of lymphocytes and granulocytes were adjusted to 1 × 10^6 cells per milliliter of media.

**Lymphocyte Blastogenesis.** The immunoresponsiveness of the lymphocytes was assessed by measuring their responses to phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed (PW) mitogens (Sigma Chemical, St. Louis, MO) according to procedures of Barta et al. (1984a). These involved incubating the cells at 37°C with [3H]thymidine in an atmosphere of 5% CO₂, harvesting the cells with a Titerette Cell Harvester 550 (Flow Laboratories, Irvine, Scotland, U.K.), and measuring the radioactivity in a beta counter (TM Analytic, Elk Grove Village, IL). The phagocytic and microbicidal activities of the PMN cells were quantified using the method of Simpson et al. (1979). The phagocytic aspects required determining the uptake of yeast cells (Fleischmann's Yeast, Oakland, CA) by the PMN and the microbicidal aspects required determining the ratios of nonviable to viable engulfed yeast particles within PMN.

Vitamin E analyses of the serum and feeds were conducted without saponification and using HPLC equipment (Waters Model 712 sample processor, 501 pump, 490E programmable detector, and M740 data module; Millipore, Milford, MA). The normal phase procedure, adapted from Dennison and Kirk (1979), used an 85:15 mixture of hexane and chloroform as the mobile phase pumped at 1.1 mL/min through a Microporasil column (Waters, Millipore) and detection was by UV light at 280 nm.

Serum Se was assayed using phosphoric-nitric acid digestion (Reamer and Veillon, 1983) followed by fluorometric detection in a Perkin Elmer Model LS-3B fluorometer (Norwalk, CT) according to the procedure of Whetter and Ullrey (1978). The GSHpx assays were conducted on serum by the coupled assay of Whetter and Ullrey (1978). The GSHpx assays were as shown in Table 2. Significant differences between treatments (within period) were determined using Student's t-test. In this study, a difference was considered significant at the level of P < .05 (Gill, 1978). All statistical analyses were performed using SAS (1988).

**Results**

The effects of vitamin E deficiency on the measured variables are summarized in Table 3. Serum tocopherol increased from 1.45 to 2.2 μg/mL in the control sows, whereas in the vitamin E-deficient sows it declined from 1.16 to .36 μg/mL and became (P < .05) lower than the control values within 30 d after the start of the experiment. Serum Se values ranged from 154 to 207 ng/mL and were not affected by vitamin E supplementation. Serum GSHpx values ranged from 1.60 to 2.38 enzyme units (EU)/mL, were correlated (P < .05) with serum Se (r = .62), and were not affected by vitamin E supplementation.

The ability of PBL from sows fed the vitamin E-deficient diet to respond to PHA and PW mitogen was (P < .05) less than that of PBL from the sows fed the control diet by 90 d of gestation. Similarly, the phagocytic and microbicidal abilities of blood PMN from the vitamin E-deficient sows were (P < .05) reduced, compared with these abilities in cells from the control sows by d 90 of gestation.

The effects of Se deficiency are summarized in Table 4. Serum tocopherol increased with time in both the control and Se-deficient sows (range 1.03 to 2.08 μg/mL) and was unaffected (P > .05) by Se deficiency. Serum Se ranged from 156 to 185 ng/mL for the
Table 3. Effects of vitamin E deficiency in gestating sows on their serum tocopherol, serum selenium, serum glutathione peroxidase (GSHpx), blood lymphocyte blastogenesis, blood polymorphonuclear cell (PMN) phagocytic activity, and PMN microbicidal activity

<table>
<thead>
<tr>
<th>Days on experiment</th>
<th>Measures</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>Parturition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>-E</td>
<td>C</td>
<td>-E</td>
<td>C</td>
</tr>
<tr>
<td>Tocopherol, μg/mL</td>
<td></td>
<td>1.45</td>
<td>1.16</td>
<td>1.72</td>
<td>1.10</td>
<td>1.84</td>
</tr>
<tr>
<td>Selenium, μg/mL</td>
<td></td>
<td>161</td>
<td>154</td>
<td>183</td>
<td>166</td>
<td>193</td>
</tr>
<tr>
<td>GSHpx, EU/mL</td>
<td></td>
<td>1.60</td>
<td>1.99</td>
<td>2.05</td>
<td>2.12</td>
<td>2.00</td>
</tr>
<tr>
<td>Lymphocyte blastogenesis</td>
<td>Mitogen</td>
<td>PHA, Bq</td>
<td>4.27</td>
<td>4.89</td>
<td>4.57</td>
<td>5.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PW, Bq</td>
<td>4.42</td>
<td>4.49</td>
<td>4.64</td>
<td>5.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Con A, Bq</td>
<td>3.66</td>
<td>3.26</td>
<td>4.22</td>
<td>4.31</td>
</tr>
<tr>
<td>Blood polymorphonuclear immune response</td>
<td>Phagocytic, %</td>
<td>86.8</td>
<td>85.5</td>
<td>87.2</td>
<td>89.7</td>
<td>88.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbicidal, %</td>
<td>39.5</td>
<td>39.7</td>
<td>41.9</td>
<td>35.7</td>
</tr>
</tbody>
</table>

*Control diet.
**Vitamin E-deficient diet.
^All-rac-α-tocopherol.
$Significantly different (P < .05) from the control at this sampling period.
^Phytohemagglutinin.
^Pokeweed mitogen.
^Concanavalin A.
|Control sows but declined from 165 to 123 ng/mL in the Se-deficient sows and became (P < .05) lower than that in the control sows by 30 d after the onset of gestation. Serum GSHpx values ranged from 1.84 to 2.09 EU/mL in the control sows and became (P < .05) lower in the Se-deficient sows by d 60 of gestation. Serum GSHpx correlated (P < .05) with serum Se with r = .68.

There was no treatment effect on the abilities of PBL to respond to PHA, PW, or Con A mitogens.

Table 4. Effects of selenium deficiency in gestating sows on their serum tocopherol, serum selenium, serum glutathione peroxidase (GSHpx), blood lymphocyte blastogenesis, blood polymorphonuclear cell (PMN) phagocytic activity, and PMN microbicidal activity

<table>
<thead>
<tr>
<th>Days on experiment</th>
<th>Measures</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>Parturition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>-Se</td>
<td>C</td>
<td>-Se</td>
<td>C</td>
</tr>
<tr>
<td>Tocopherol, μg/mL</td>
<td></td>
<td>1.03</td>
<td>1.19</td>
<td>1.18</td>
<td>1.47</td>
<td>1.40</td>
</tr>
<tr>
<td>Selenium, μg/mL</td>
<td></td>
<td>156</td>
<td>165</td>
<td>170</td>
<td>144</td>
<td>176</td>
</tr>
<tr>
<td>GSHpx, EU/mL</td>
<td></td>
<td>1.84</td>
<td>1.93</td>
<td>1.64</td>
<td>1.59</td>
<td>1.71</td>
</tr>
<tr>
<td>Lymphocyte blastogenesis</td>
<td>Mitogen</td>
<td>PHA, Bq</td>
<td>4.85</td>
<td>4.91</td>
<td>4.67</td>
<td>4.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PW, Bq</td>
<td>4.77</td>
<td>4.67</td>
<td>4.62</td>
<td>5.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Con A, Bq</td>
<td>4.32</td>
<td>4.30</td>
<td>4.10</td>
<td>4.28</td>
</tr>
<tr>
<td>Blood polymorphonuclear immune response</td>
<td>Phagocytic, %</td>
<td>85.5</td>
<td>89.9</td>
<td>92.9</td>
<td>90.9</td>
<td>89.9</td>
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<tr>
<td></td>
<td></td>
<td>Microbicidal, %</td>
<td>48.5</td>
<td>47.6</td>
<td>49.6</td>
<td>42.4</td>
</tr>
</tbody>
</table>

*Control diet.
**Selenium-deficient diet.
^All-rac-α-tocopherol.
$Significantly different (P < .05) from the control at this sampling period.
^Phytohemagglutinin.
^Pokeweed mitogen.
^Concanavalin A.
|Percentage of PMN cells containing two or more yeast cells.
|Percentage of PMN cells containing two or more dead yeast cells.
Table 5. Effects of vitamin E and selenium deficiency in gestating sows on their serum tocopherol, serum selenium, serum glutathione peroxidase (GSHpx), blood lymphocyte blastogenesis, blood polymorphonuclear cell (PMN) phagocytic activity, and PMN microbicidal activity

<table>
<thead>
<tr>
<th>Measures</th>
<th>Days on experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Tocopherol, µg/mL</td>
<td>C -E-Se</td>
</tr>
<tr>
<td>Selenium, ng/mL</td>
<td>216</td>
</tr>
<tr>
<td>GSHpx, EU/mL</td>
<td>2.88</td>
</tr>
<tr>
<td>Lymphocyte blastogenesis</td>
<td></td>
</tr>
<tr>
<td>Mitogen</td>
<td></td>
</tr>
<tr>
<td>PHA, Bq</td>
<td>4.60</td>
</tr>
<tr>
<td>PW, Bq</td>
<td>4.83</td>
</tr>
<tr>
<td>Con A, Bq</td>
<td>4.32</td>
</tr>
<tr>
<td>Blood polymorphonuclear immune response</td>
<td></td>
</tr>
<tr>
<td>Phagocytic, %h</td>
<td>82.4</td>
</tr>
<tr>
<td>Microbicidal, %i</td>
<td>40.9</td>
</tr>
</tbody>
</table>

| Measures                               | C -E-Se            | C -E-Se            | C -E-Se            | C -E-Se            | C -E-Se     | SEM        |
| Tocopherol, µg/mL                      | .99                | 1.03               | 1.18               | 1.35               | 1.93        | .45d       |
| Selenium, ng/mL                        | 216                | 186                | 190                | 205                | 228          | 213        |
| GSHpx, EU/mL                           | 2.88               | 2.11               | 2.16               | 2.54               | 2.68         | 2.16d      |

Table 6. Differential cell counts of sow colostrum and milk

<table>
<thead>
<tr>
<th>Sample</th>
<th>Neutrophilsa</th>
<th>Macrophages</th>
<th>Lymphocytes</th>
<th>Eosinophils</th>
<th>Epithelial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum</td>
<td>64.0 ± 4.3b</td>
<td>5.6 ± 1.1</td>
<td>26.5 ± 3.7</td>
<td>.7 ± .3</td>
<td>1.4 ± .4</td>
</tr>
<tr>
<td>Milk</td>
<td>40.7 ± 4.7</td>
<td>15.5 ± 2.3</td>
<td>19.2 ± 3.6</td>
<td>.4 ± .2</td>
<td>23.6 ± 2.0</td>
</tr>
</tbody>
</table>

aPolymorphonuclear cells.
bMean ± SEM; n = 24.

However, the phagocytic and microbicidal abilities of blood PMN from the Se-deficient sows were (P < .05) reduced, compared with these abilities in cells from the control sows at gestation d 90 and at parturition. The effects of the combined vitamin E and Se deficiency during gestation are presented in Table 5. Serum tocopherol increased with time in the control sows (range .99 to 1.93 µg/mL) but declined with time and became (P < .05) lower in the deficient sows by d 60 of gestation. Serum Se ranged from 186 to 228 ng/mL for the control sows, declined in the deficient sows, and became (P < .05) lower than serum Se in the control sows by 30 d after the onset of gestation. Serum GSHpx values ranged from 2.11 to 2.88 EU/mL in the control sows and became (P < .05) lower in the deficient sows by 30 d of gestation. The serum GSHpx was correlated r = .83; (P < .05) with serum Se.

The abilities of PBL from the deficient sows to respond to PHA and PW mitogens were reduced (P < .05) compared with the abilities of PBL from control sows by 60 d gestation. Also, compared with cells from the control sows, the PMN from the deficient sows showed a lesser (P < .05) phagocytic and microbicidal response by d 60 and 90 of gestation, respectively.

Serum cholesterol values for sows in this study were inconsistent and mean values for control sows ranged from 1.9 to 2.7 mmol/L. No differences (P > .05) were observed among the mean serum cholesterol concentrations of any groups of sows at any sampling periods during the trial.

The differential counts of sow colostrum and milk were unaffected by treatment; thus, the data have been combined (Table 6). The PMN (neutrophils) represented 64% of the cells in colostrum and 40% in milk. Epithelial cell concentrations, in contrast, represented 1.5% of the cells in colostrum and 23% in milk.

The responses of colostral and milk lymphocytes to mitogen stimulation are presented in Table 7. In the
VITAMIN E, SELENIUM, AND SWINE IMMUNITY

Table 7. Effects of vitamin E- and(or) selenium-depleted diets on mitogenic stimulation of colostrum and milk lymphocytes of peripartum sows

<table>
<thead>
<tr>
<th>Mammary secretion and group</th>
<th>Mitogen</th>
<th>None</th>
<th>PHA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PW&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Con A&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bq</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colostrum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.4</td>
<td>3.8</td>
<td>3.9</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>+E-Se&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.4</td>
<td>3.8</td>
<td>3.8</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>-E-Se&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.4</td>
<td>3.1&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td>-E-Se&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>3.2&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td>SED&lt;sup&gt;g&lt;/sup&gt;</td>
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<td>.21</td>
<td>.26</td>
<td>.31</td>
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<td>Milk</td>
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<td>2.8</td>
<td>2.7</td>
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<tr>
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<tr>
<td>SED</td>
<td>.14</td>
<td>.29</td>
<td>.28</td>
<td>.21</td>
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</table>

<sup>a</sup>Phytohemagglutinin.  
<sup>b</sup>Pokeweed.  
<sup>c</sup>Concanavalin A.  
<sup>d</sup>Pooled control means, n = 12.  
<sup>e</sup>n = 4.  
<sup>f</sup>Different (P < .05) from actual control means.  
<sup>g</sup>Standard error of the difference.

The case of colostral lymphocytes, stimulation was decreased (P < .05) by the -E+Se and -E-Se diets when PHA was the mitogen but only by the -E-Se diet when PW was the mitogen. Colostral lymphocytes were not affected by Con A, and milk lymphocytes were not affected by any mitogen tested.

The phagocytic and microbicidal activities of colostral and milk PMN are presented in Table 8. The ability of colostral PMN cells to engulf (phagocytize) yeast particles was unaffected by the vitamin E- or Se-deficient diets but was decreased (P < .05) by the combined vitamin E and Se deficiency. There was no influence of diet on the phagocytic activity of milk PMN cells.

The microbicidal activity of colostral PMN cells was decreased (P < .05) by each of the treatments in comparison to the PMN cells from colostrum of the respective controls. The microbicidal activity of milk PMN cells, however, was decreased (P < .05) only by the diet deficient in both vitamin E and Se.

Discussion

This experiment indicates that acceptable serum tocopherol concentrations, based on measures of immunocompetence in gestating sows, range from 1 to 2 µg/mL (2.31 to 4.62 µmol/L). These values were achieved by dietary vitamin E concentrations approximately 60 IU/kg of diet. This serum tocopherol range compares favorably to the range of .9 to 1.2 µg/mL (2.1 to 2.9 µmol/L) believed, at Michigan State University's Animal Health Diagnostic Laboratory, to be indicative of normal vitamin E status of mature swine (H. D. Stowe, unpublished data).

The dietary vitamin E concentration used in this experiment is consistent with vitamin E concentrations used in contemporary feeding programs but is considerably higher than the 11 IU of vitamin E/kg of diet indicated as the requirement of pigs at their mature weight (NRC, 1988).

Previous investigators have determined that diminished immune competence occurs earlier in the course of a vitamin E deficiency than do some of the more classical manifestations of vitamin E deficiency (Bendich et al., 1986). For these reasons, when the National Research Council next reevaluates its interpretations of the vitamin E requirements for swine, it seems appropriate that the criterion of immunoresponsiveness be given serious consideration in determining nutrient adequacy.

The serum tocopherol data demonstrated that a practical, vitamin E-deficient diet can be produced by using dried, ensiled, high-moisture corn, which, experience has demonstrated, becomes severely depleted of its natural vitamin E content by the ensiling process (Young et al., 1975). There was remarkable similarity in the gradual declines in serum tocopherol with duration of consumption of the vitamin E-deficient diets with or without supplemental Se.

The Se data indicate that serum Se concentrations in the range of 170 to 228 ng/mL (2.0 to 2.7 µmol/L) maintained immune competence, based on the end points used. This range compares favorably with the 180 to 220 ng/mL (2.28 to 2.78 µmol/L) range considered normal (Animal Health Diagnostic Laboratory) and was achieved by the dietary Se concentration of .3 mg/kg. This is the Se concentration currently permitted in commercial swine diets (Food and Drug Administration, 1987).
The correlation between GSHpx and serum Se has been previously demonstrated, and the GSHpx concentrations found in the sow serum are comparable to those previously reported (Hakkarainen et al., 1978; Chavez, 1979; Stowe and Miller, 1985).

Serum cholesterol assays were conducted because of previously demonstrated correlations, in other species, between serum tocopherol and cholesterol, a major lipid fraction of serum (Foley et al., 1990). Although the range of serum cholesterol in the sows (1.9 to 2.73 mmol/L) was consistent with the range of 2.20 to 2.82 mmol/L reported for swine by Foley et al. (1990), no association between serum tocopherol and cholesterol concentrations was observed. This may indicate that vitamin E is unevenly distributed between the serum lipoprotein components such as the low- and high-density lipoprotein fractions.

The reduced ability of peripheral blood and colostral lymphocytes from vitamin E-deficient sows to respond to stimulation by mitogens provides additional evidence for a role of vitamin E in immunocompetence. When an impaired response of lymphocytes to PHA or Con A stimulation is observed, this is interpreted as an altered function of the thymic-derived T lymphocytes (e.g., those associated with cell-mediated immunity; Schimizu and Schimizu, 1979). When the impaired response of lymphocytes to PW mitogen stimulation is noted, this is believed to represent an effect on the bone marrow-derived or B lymphocytes (e.g., those associated with antibody production and, thus, humoral immunity; Tizard, 1987). Because the responses of sow PBL to both PHA and PW mitogens declined as the degree of vitamin E deficiency progressed, one can conclude that vitamin E deficiency reduced the blastogenic activity of both thymic and bone marrow-derived lymphocytes. A similar conclusion was reached by Bendich et al. (1983, 1986) in rats when blastogenesis of splenic lymphocytes was measured. The differences between porcine PBL responses to PHA and Con A mitogens, both of which should stimulate T cells, could indicate the presence of different subsets of T cells in the PBL population (Stobo and Paul, 1973).

The fact that impaired mitogenic stimulation of PBL occurred earlier in sows deficient in both vitamin E and Se than in sows deficient only in vitamin E suggests that immunosuppression may also be affected by Se deficiency, even though the impaired responses to mitogens were not significant (\( P > .05 \)) in sows deficient only in Se.

The ability of peripheral blood PMN to engulf yeast cells, in vitro, was impaired by both vitamin E and Se deficiencies and was impaired sooner by the combined vitamin E and Se deficiency than by individual deficiencies of vitamin E or Se. This effect of vitamin E on phagocytosis is consistent with observations of Harris et al. (1980), who reported increased malonaldehyde production, decreased chemotaxis, and decreased ingestion of oil droplets by PMN from vitamin E-deficient rats. Chemotaxis and phagocytosis were restored to normal within 18 h after parenteral administration of vitamin E. These investigators postulated that vitamin E deficiency affected areas of membranes involved in triggering chemotaxis, phagocytosis, and oxidative responses. The apparent effect of Se on phagocytic ability of blood PMN is in contrast to the reports of Boyne and Arthur (1979, 1981), who were unable to demonstrate an effect of Se deficiency on the phagocytic abilities of PMN from cattle blood.

Although the phagocytic ability of colostral PMN was diminished by all treatments, the reductions were only significant in the cells from sows deficient in both vitamin E and Se. The absence of treatment effects on the phagocytic abilities of PMN taken from milk collected at 4 d postpartum suggests that this variable is relatively insensitive to vitamin E or Se intake.

The in vitro ability of peripheral blood PMN to kill engulfed yeast cells was significantly reduced, within

### Table 8. Phagocytic and microbicidal activities of polymorphonuclear (PMN) cells of colostrum and milk from control sows and sows fed vitamin E- and(or) selenium-depleted diets between conception and 4 days postpartum

<table>
<thead>
<tr>
<th>Diet</th>
<th>Phagocytic activity(^a)</th>
<th>Microbicidal activity(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colostrum</td>
<td>Milk</td>
</tr>
<tr>
<td>+E+Se(^c)</td>
<td>50.2 ± 5.4(^d)</td>
<td>27.5 ± 3.0</td>
</tr>
<tr>
<td>-E+Se</td>
<td>43.6 ± 3.2</td>
<td>23.7 ± 1.5</td>
</tr>
<tr>
<td>+E+Se(^f)</td>
<td>44.6 ± 8.3</td>
<td>30.2 ± 5.0</td>
</tr>
<tr>
<td>+E+Se(^g)</td>
<td>33.4 ± 5.1</td>
<td>19.9 ± 3.4</td>
</tr>
<tr>
<td>+E-Se</td>
<td>53.5 ± 2.5</td>
<td>23.2 ± 2.2</td>
</tr>
<tr>
<td>-E-Se</td>
<td>35.2 ± .9(^e)</td>
<td>21.2 ± 2.5</td>
</tr>
</tbody>
</table>

\(^a\)Percentage of PMN cells containing two or more yeast particles.
\(^b\)Percentage of PMN cells containing two or more dead yeast particles.
\(^c\)Control for vitamin E-deficient sows.
\(^d\)Mean ± SEM, n = 12.
\(^e\)Significantly (\( P < .05 \)) different from respective control.
\(^f\)Control for selenium-deficient sows.
\(^g\)Control for vitamin E- and selenium-deficient sows.
similar lengths of time (90 d) after onset of the experiment, by individual and combined deficiencies of vitamin E and Se. A Se effect on the killing ability of blood PMN was observed by Boyne and Arthur (1979, 1986). They found that the microbicidal (C. albicans) activity fell dramatically by 9 wk after the start of Se deficiency in cattle and found the killing ability of PMN from Se-supplemented cattle to be three times that of PMN from Se-depleted cattle.

The killing of engulfed yeast cells was also impaired in colostral PMN by either vitamin E and (or) Se deficiencies but only by the combined deficiency in milk PMN. Thus, the microbicidal activity of colostral PMN seems to be relatively sensitive to vitamin E and (or) Se depletion in comparison to the microbicidal activity of PMN from milk. This difference in sensitivity of these two populations of cells is difficult to explain given their common origin in the bone marrow. The microbicidal process is believed to be associated with an intracellular “respiratory burst,” which involves the production and metabolism of oxidizing radicals (Babior, 1984).

These observations indicate that both vitamin E and Se play roles in the phagocytic functions of granulocytic cells, particularly demonstrable in peripheral blood PMN. It is this relationship of vitamin E and selenium to both phagocytic and microbicidal abilities of PMN that is believed to contribute to the reduction in somatic cell counts and incidence of mastitis among dairy cattle appropriately supplemented with vitamin E and selenium (Smith and Conrad, 1987).

Implications

This research helps document the importance of dietary vitamin E and selenium in maintaining immune function of livestock. Specifically, the data imply that if gestating sows do not obtain adequate vitamin E and (or) selenium, they and their pigs will be more susceptible to disease processes in the peripartum period. Although selenium restriction primarily impaired neutrophil function, vitamin E restriction affected both neutrophils and lymphocytes. This research provides justification for maintaining the allowed selenium supplementation rate for swine at .3 mg/kg diet and may stimulate the National Research Council to consider the criterion of immune responsiveness when the vitamin E requirements of swine are next reexamined.

Literature Cited


Reamer, D. C., and C. Veillon. 1983. Elimination of perchloric acid