EFFECT OF DIETARY SELENIUM AND VITAMIN E ON THE PRIMARY AND SECONDARY IMMUNE RESPONSE IN LAMBS CHALLENGED WITH PARAINFLUENZA3 VIRUS

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ABSTRACT

This study was conducted to determine the effect of dietary selenium (Se) and vitamin E (Vit E) on various blood characteristics and the primary and secondary humoral immune response of lambs challenged with parainfluenza 3 virus (PI3V). Treatments included: 1) +Se/+Vit E, 2) -Se/+Vit E, 3) +Se/-Vit E and 4) -Se/-Vit E. The basal diet (-Se/-Vit E) was deficient in Se and Vit E. Sodium selenite (.2 mg Se/kg diet) and alpha-tocopherol acetate (20 mg Vit E/kg diet) were added to +Se and +Vit E diets, respectively, to provide adequate levels of each according to NRC recommendations. Following a 10-wk dietary adaptation and depletion period, lambs in all treatment groups were intratracheally inoculated with PI3V on d 0 and 35 of the 70-d study. Prior to inoculation, whole blood and plasma glutathione peroxidase (GSH-Px) activities were higher (P < .01) for +Se lambs. Whole blood and plasma GSH-Px increased (P < .01) after primary viral inoculation in +Se lambs but not in -Se lambs. Serum immunoglobulin M (IgM) concentrations were enhanced (P < .05) by Se supplementation on d 14, 35 and 49 of the study. Selenium and (or) Vit E did not affect serum immunoglobulin G (IgG) levels. Serum PI3V antibody titers increased after inoculation on d 0 and 35 in all treatment groups. Titer levels appeared to increase more substantially for +Se lambs after primary inoculation, but increases were greater (P < .01) for +Vit E lambs after secondary challenge. Our results indicate that Se and Vit E independently enhance the immune response of lambs challenged with a viral pathogen.

(Key Words: Lambs, Selenium, Vitamin E, Immune Response, Viral Challenge.)

Introduction

Selenium (Se) and vitamin E (Vit E) both are involved in the mammalian antioxidant defense system. Selenium as an essential component of glutathione peroxidase reduces potentially harmful oxygen radicals such as hydrogen peroxides and lipid hydroperoxides (Rotruck et al., 1973). Vitamin E is a powerful antioxidant that prevents the formation of lipid hydroperoxides from unsaturated phospholipids present in subcellular membranes (Tappel, 1970).

Selenium and Vit E have been found to alter immunocompetence in various species. The etiology of their stimulatory roles in the immune response is unknown; however, it may be related directly to their antioxidant properties (Baumgartner, 1979). Spallholz et al. (1973a) demonstrated that high dietary Se enhanced serum immunoglobulin G (IgG) and immunoglobulin M (IgM) antibody titers in mice challenged with sheep red blood cells. Vitamin E has been implicated in stimulation of serum antibody synthesis, particularly IgG antibodies (Tengerdy et al., 1973). Vitamin E improved disease resistance in chicks infected with Escherichia coli (Heinzerling et al., 1974) and in lambs challenged with Chlamydia (Stephens et al., 1979). Similarly, supplemental Se decreased mortality rates in mice challenged with Staphylococcus aureus (Boyne et al., 1986) and increased survival time of mice infected with Candida albicans (Boyne and Arthur, 1986). However, little effort has been directed toward an understanding of the unified role Se and Vit E may play in resistance to infectious organisms. The present study was designed to determine the effect of Se and Vit E on the primary and secondary immune response of lambs challenged with parainfluenza 3 virus (PI3V).

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## TABLE 1. COMPOSITION OF BASAL DIET FED TO LAMBS

<table>
<thead>
<tr>
<th>Item</th>
<th>Starter diet</th>
<th>Diet A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Torula yeast&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>Cottonseed hulls&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Corn starch&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Dextrose&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Corn oil&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>3</td>
</tr>
<tr>
<td>Mineral mix&lt;sup&gt;e&lt;/sup&gt;, f</td>
<td>.73</td>
<td>.73</td>
</tr>
<tr>
<td>Vitamin E premix&lt;sup&gt;g&lt;/sup&gt;</td>
<td>.27</td>
<td>.27</td>
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### Calculated composition

<table>
<thead>
<tr>
<th></th>
<th>Starter diet</th>
<th>Diet A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, %</td>
<td>92.0</td>
<td>92.0</td>
</tr>
<tr>
<td>Crude protein, %&lt;sup&gt;h&lt;/sup&gt;</td>
<td>16.6</td>
<td>14.4</td>
</tr>
<tr>
<td>Crude fiber, %&lt;sup&gt;h&lt;/sup&gt;</td>
<td>10.2</td>
<td>14.8</td>
</tr>
<tr>
<td>Digestible energy, kcal/g&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3.57</td>
<td>3.40</td>
</tr>
<tr>
<td>Calcium, %&lt;sup&gt;h&lt;/sup&gt;</td>
<td>.40</td>
<td>.38</td>
</tr>
<tr>
<td>Phosphorus, %&lt;sup&gt;h&lt;/sup&gt;</td>
<td>.56</td>
<td>.47</td>
</tr>
</tbody>
</table>

<sup>a</sup>Lake States, Rhinelander Paper Co., Rhinelander, WI.
<sup>b</sup>Southern Oil Co., Rocky Mount, NC.
<sup>c</sup>Clinton Corn Processing Co., Clinton, IA.
<sup>d</sup>C. F. Sauer, Richmond, VA.
<sup>e</sup>Contained in diet (%): CaCO<sub>3</sub>, .46; NaCl, .25; CuSO<sub>4</sub>·5H<sub>2</sub>O, .0004; FeCl<sub>3</sub>·6H<sub>2</sub>O, .0108; MnSO<sub>4</sub>·H<sub>2</sub>O, .0108; ZnSO<sub>4</sub>·H<sub>2</sub>O, .0022; CrCl<sub>3</sub>·6H<sub>2</sub>O, .0002; KI, .00013; CoCl<sub>2</sub>·6H<sub>2</sub>O, .0004.
<sup>f</sup>Contained Se added as sodium selenite to provide .2 mg/kg feed for lambs in +Se/+Vit E and +Se/-Vit E treatment groups.
<sup>g</sup>Contained vitamin E added as alpha-tocopherol acetate in a corn starch carrier to provide 20 mg/kg feed for lambs in +Se/+Vit E and -Se/+Vit E treatment groups.
<sup>h</sup>Dry matter basis.

### Materials and Methods

**Animals and Diets.** Twenty-four Dorset × Suffolk weanling lambs averaging 2 mo of age and 15 kg in body weight were obtained from a university research station where their Se nutrition prior to this study was controlled. Twelve of the lambs were born to ewes fed diets marginal in Se (.03 to .06 mg/kg), whereas the other 12 lambs were produced from ewes that had free choice access to a trace mineral salt containing 20 mg Se/kg. Lambs in the latter group also received a s. c. injection of sodium selenite (2.5 mg Se) approximately 3 wk prior to the initiation of the study. Lambs were not creep fed, but were allowed access to feed and mineral mixes provided to their dams prior to weaning. There was no evidence of white muscle disease (WMD) in lambs born to marginally Se-deficient ewes; however, WMD had been observed in previous years in this flock.

Lambs were assigned according to sex and previous Se status to one of four treatment groups in a 2 × 2 factorial arrangement of treatments. Equal numbers of ewe and wether lambs were distributed within each treatment. Treatments consisted of 1) +Se/+Vit E, 2) -Se/+Vit, 3) + Se/-Vit E and 4) -Se/-Vit E with six animals per treatment group. The basal diet (-Se/-Vit E) was formulated to be low in Se and Vit E (Table 1). Lambs born to marginally Se-deficient ewes were assigned to the -Se treatments. Lambs in the +Se treatments (1 and 3) were born to Se-supplemented ewes and received the basal diet plus .2 mg Se/kg diet, added as sodium selenite. Vitamin E, provided as alpha-tocopherol acetate was added to the diet of lambs assigned to +Vit E treatments (1 and 2) to supply 20 mg Vit E/kg feed. The levels of Se and Vit E supplemented to the basal diet provided adequate amounts of each nutrient according to NRC recommendations (NRC, 1985).

Lambs were housed individually in 1.52-m<sup>2</sup> plastic pens with automatic plastic bowl waterers in a temperature-controlled room. Lambs were dewormed and vaccinated for enterotox-
emia prior to the study. A starter diet was provided to the lambs ad libitum during a 10-wk depletion and dietary adjustment period. During the subsequent challenge period, lambs were fed Diet A ad libitum. The ingredient composition of the basal diets is outlined in Table 1. Dietary Se concentrations averaged .025 and .18 mg Se/kg feed for −Se and +Se diets, respectively.

**Challenge Period.** Lambs were weighed on d 0, 35 and 70 of the challenge phase, and feed intakes were monitored throughout the study. Lambs were intratracheally inoculated with PI3V on d 0 and 35 to measure the primary and secondary immune response to viral infection. The DH-1 strain of PI3V in the inoculum was propagated in a continuous line of ovine fetal corneal cells grown in Eagle minimum essential medium containing 5% bovine fetal calf serum and .15 mg gentamycin/ml. Infectivity titer of the inoculum, expressed as median cell culture infective doses (CCID50/ml) was 1.7 x 10^7.

Lambs were inoculated by the intratracheal instillation of 3 ml of PI3V inoculum on d 0 and 35. Prior to inoculation on both days, lambs were weighed and bled and rectal temperatures were recorded. Rectal temperatures were monitored daily for 7 d after each inoculation. Blood samples were obtained in heparinized vacutainer tubes via jugular puncture on d 0, 3, 7, 10, 14, 28 and 35 after each PI3V inoculation, and whole blood and plasma were retained for subsequent analyses. Jugular blood samples also were collected on the same day in tubes without any additive, and the serum was harvested for the determination of immunoglobulin concentrations and PI3V antibody titers.

**Analyses.** Whole blood and plasma glutathione peroxidase (GSH-Px) were measured by the coupled assay of Paglia and Valentine (1967) using hydrogen peroxide as the substrate. Plasma creatine phosphokinase (CPK) activity was measured as described by Sigma Chemical Co. (1985). Serum albumin, IgM and IgG concentrations were estimated by acrylamide discontinuous gel electrophoresis according to the procedure of Morgan and Glick (1972). Serum antibody titers PI3V were measured by a microtiter method using a constant dose of virus and varying dilutions of serum as described by Fulton et al. (1982). Plasma and serum total protein concentrations were determined by the biuret method as described by Gornall et al. (1949). Selenium in diets was measured by the method of Olson et al. (1975). Data were analyzed statistically by least squares analysis of variance, using the General Linear Model (GLM) procedures of SAS (1982). The model included the main effects of Se, Vit E and Se × Vit E interaction. Variables also were tested for sex effects, but none were observed. Blood data from the viral challenge phase also were analyzed across sampling day, with the model including treatment, day and treatment × day interaction. Differences between sampling days within a treatment were determined using Duncan's multiple range test (Steel and Torrie, 1980).

**Results and Discussion**

Selenium and(or) Vit E had little effect on the intake and growth of lambs challenged with PI3V (Table 2). Selenium depletion tended to decrease feed intake during the first 35 d in lambs receiving no supplemental Vit E but not in lambs supplemented with 20 mg Vit E/kg diet. This resulted in a Se × Vit E interaction (P < .10) for feed intake during this period. Similarly, previous studies indicated reduced feed intake in swine (Peplowski et al., 1981) and rats (Eskew et al., 1985) fed diets deficient in both Se and Vit E. Average daily gains (ADG) were lower (P < .01) for lambs receiving supplemental Vit E during the second 35-d period compared with lambs fed diets low in Vit E. Total gains for the 70-d period also tended to be lower (P < .10) for +Vit E lambs. Barber et al. (1977) observed depressed body weight gains for guinea pigs injected with Vit E and challenged with intraperitoneal doses of Venezuelan equine encephalomyelitis virus. In contrast, Colnago et al. (1984) reported higher gains for Vit E-supplemented chicks challenged in vivo with *Eimeria tenella*, and lambs infected with chlamydia and receiving supplemental Vit E had higher weight gains than control animals during a 10-d postinfection period (Stephens et al., 1979).

Rectal temperatures were monitored after primary and secondary PI3V inoculation to assess the presence and severity of infectious illness. Preinoculation temperatures were simi-

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*Supplied by Dr. H. D. Lehmkuhl, National Animal Disease Center, Ames, IA.

*Becton Dickinson, Rutherford, NJ.*
TABLE 2. FEED INTAKE AND AVERAGE DAILY GAIN (ADG) FOR LAMBS

| Treatment | Item | +Se/+Vit E | -Se/+Vit E | +Se/-Vit E | -Se/-Vit E | SE
|-----------|------|------------|------------|------------|------------|------
| Feed intake, kg/d | D 0–35 | 1.36 | 1.36 | 1.36 | .91 | .13 |
| | D 36–70 | 1.35 | 1.38 | 1.41 | 1.28 | .09 |
| | D 0–70 | 1.35 | 1.37 | 1.39 | 1.10 | .09 |
| ADG, kg | D 0–35 | .27 | .26 | .27 | .26 | .03 |
| | D 36–70 | .12 | .12 | .14 | .22 | .02 |
| | D 0–70 | .19 | .19 | .21 | .24 | .02 |

*Values shown are least squares means, n = 6.

Pooled standard error of the mean.

Dry matter basis.

Selenium X Vit E interaction (P < .10).

Vitamin E effect (P < .01).

lar for all four treatment groups (39.9°C). Neither Se nor Vit E consistently affected body temperatures. Inoculation of lambs with PI₃V caused an elevation in body temperature (data not shown); however, the effect was slight and was not typical of a febrile response. Marshall (1981) observed similar results in calves intranasally inoculated with either SF-4 or PC strains of live PI₃V. After primary oculation, transient signs of fever were noted in calves between 4 and 6 d after inoculation with the SF-4 strain of PI₃V. No clinical signs of infection were noted in either group of calves after reinoculation with PI₃V on d 42.

Whole blood GSH-Px activities were consistently lower (P < .001) for -Se lambs on each sampling date throughout the study (Figure 1). Whole blood GSH-Px for lambs in the -Se treatment groups averaged 64 U/g hemoglobin (Hb) on d 0 and continued to decline, reaching a low mean value of 11 U/g Hb on d 70. Despite their original marginally deficient status and the 10-wk depletion period preceding this study, -Se lambs still had a sizeable pool of whole blood GSH-Px on d 0. However, substantial differences (P < .001) in Se status as measured by GSH-Px were apparent initially between -Se and +Se lambs.

Vitamin E had no influence on whole blood GSH-Px activity regardless of Se status. Previous studies also have reported that Se deficiency reduced whole blood or erythrocyte GSH-Px activity independent of Vit E (Whanger et al., 1977; Siddons and Mills, 1981). Whole blood GSH-Px activity increased (P < .01) greatly in +Se lambs after primary PI₃V inoculation and was consistently high throughout the remainder of the study. This increase in GSH-Px activity may have been induced by an increased production of oxygen radicals caused by viral stress. In order to prevent oxidative tissue damage, an increase in GSH-Px activity may be necessary to rid the system of these oxygen metabolites. In turn, increased enzymatic activity may be dependent on the Se status of the animals and the availability of Se for incorporation into GSH-Px. This can explain why GSH-Px did not increase in Se-deficient lambs following inoculation.

Results for plasma GSH-Px also are presented in Figure 1. Selenium supplementation resulted in higher (P < .01) plasma GSH-Px activity in lambs on all sampling dates. Plasma GSH-Px increased (P < .01) after primary PI₃V inoculation (d 0) in +Se lambs and did not decline until 28 postinoculation, but no such change was apparent in Se-deficient lambs. Previous studies with Se have indicated relationships between infectious disease and plasma GSH-Px. Calves that were either marginally deficient or receiving adequate Se had substantially increased plasma GSH-Px after exposure to Pasteurella hemolytica (Reffett et al., 1986). In a more recent study (Reffett et al., 1988), plasma GSH-Px activity increased in calves supplemented with Se (.2 mg Se/kg diet) but not in Se-deficient calves after primary and secondary challenge with infectious bovine rhinotracheitis virus. Our observation of an increase in plasma GSH-Px is consistent with the increased whole
blood activity observed in +Se lambs after viral inoculation. Glutathione peroxidase may play an important role in disease resistance by increasing the efficiency of the antioxidant defense system. Macrophages and neutrophils produce oxygen radicals such as hydrogen peroxide and superoxide anions during phagocytosis of foreign particles (Boyne and Arthur, 1979). If these radicals are not promptly removed from the system, they may ultimately
damage the membranes of phagocytic cells. In general, scavenger cells reduce the spread of disease through their protective microbicidal activity. Plasma GSH-Px activity declined to preinoculation levels by d 28 followed by a subsequent increase \((P < .01)\) in activity by d 35. Plasma GSH-Px remained elevated in +Se lambs following the secondary PI3V challenge on d 35.

Plasma creatine phosphokinase (CPK) activity was monitored throughout the study as an indicator of oxidative tissue damage (Figure 2). Elevated plasma CPK activities are highly correlated with the incidence of WMD, a condition often precipitated by Se and(or) Vit E deficiencies (Ewan et al., 1968). The combined effect of low dietary Se and Vit E tended to elevate plasma CPK activities on d 14 and 21 of the study. This resulted in a Se \(\times\) Vit E interaction \((P < .10)\) on d 21. Results on d 14 were not significant due to the high degree of variation among animals in this group. After the secondary PI3V inoculation, enzyme activities were relatively similar for all treatment groups until d 49, when -Se/+Vit E lambs had higher \((P < .05)\) CPK activities than lambs in other treatments. A further increase \((P < .01)\) in CPK activity for this group was observed on d 56 and 63 due to an extremely elevated enzyme activity for one animal. Although clinical signs of WMD were not evident, these transient increases in CPK activity may reflect oxidative stress induced by viral exposure.

Serum albumin concentrations were relatively unaffected by viral stress in this study (data not shown). Serum albumin was lower \((P < .01)\) on d 14 and tended to be lower \((P < .10)\) on d 45 for -Vit E lambs. Cipriano et al. (1982) observed lower serum albumin in calves fed diets either deficient in or supplemented with high levels (1,000 mg/d) of Vit E compared with calves with normal vitamin E intakes.

Vitamin E also appeared to have some influence on serum IgM concentrations during the 70-d study (Figure 3). Levels of IgM in the serum were higher \((P < .05)\) in -Vit E lambs on d 7 and 35 but lower \((P < .10)\) on d 42 and 49 after the secondary PI3V challenge. Selenium supplementation enhanced \((P < .05)\) serum IgM on d 14, 35 and 49 of the study, and mean immunoglobulin concentrations tended to be higher for +Se lambs on other sampling dates. In previous studies, Se and Vit E stimulated the humoral immune response in mice challenged

![Figure 2. Plasma creatine phosphokinase activity in lambs. Values shown are least squares means, \(n = 6\). Pooled standard errors of the mean for d 0, 7, 14, 21, 28, 35, 42, 56, 63 and 70 were 3.0, 5.6, 31.6, 24.4, 3.6, 4.7, 4.3, 4.2, 14.4, 194.4 and 17.5.](file)

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with sheep red blood cells (Spallholz et al., 1973b). It has been suggested that Se stimulates synthesis of IgM antibody by increasing the number of IgM-producing cells. In a similar fashion, supplementation with Vit E has been found to enhance IgG antibody production. However, neither Vit E nor Se affected serum IgG concentrations in stressed lambs in our study (Figure 3). Inoculation with PI₃V tended to increase serum IgG in Vit E-supplemented lambs but not in -Vit E lambs.

Selenium and Vit E have been implicated in the immune response mechanisms of animals challenged with exogenous antigens. Deficiencies of these nutrients have depressed serum neutralizing antibody titers in canines following
vaccination with canine distemper-infectious hepatitis virus (Sheffy and Schultz, 1978). Peplowski et al. (1981) noted higher hemagglutinin titers to sheep red blood cells in swine supplemented with Se or Vit E. An additive response in titer level was observed when both nutrients were provided simultaneously either by injection or in the diet.

The presence of serum anti-Pl3V antibodies was determined and expressed as the average reciprocal titer level on d 0, 14, 35 and 49 of the study (Figure 4). On d 0, lambs tested seronegative for the strain of Pl3V used in this study. By d 14, lambs in all four treatments exhibited positive Pl3V seroconversion. Serum titer levels continued to increase (P < .07) between d 14 and 35 for +Se/-Vit E lambs but tended to decrease in other treatment groups. Lambs in the -Se/-Vit E treatment had lower (P < .01) titers on d 35 than on d 14. After secondary Pl3V inoculation (d 35), an anamnestic antibody response resulted in increased (P < .01) titer levels in all treatments except +Se/-Vit E. Lack of a significant increase in serum titers between d 35 and 49 in these +Se/-Vit E lambs may be explained by their previously high titer levels on d 35. A significant Se x Vit E interaction was noted on d 49. Lambs supplemented with vitamin E had higher titer levels in the absence of, but not in the presence of, supplemental Se. Supplemental Vit E appeared to stimulate the secondary immune response, which is generally associated with IgG antibody production. Tengerdy et al. (1973) observed increased serum IgG antibody titers in mice supplemented with 60 to 180 mg Vit E/kg diet and antigenically challenged with sheep red blood cells or tetanus toxoid.

In summary, dietary Se and Vit E supplementation enhanced the immune response of lambs challenged with Pl3V. Serum IgM concentrations tended to be higher for Se-supplemented lambs during the latter 35 d of the study. Anti-Pl3V antibody titers were higher in +Se/-Vit E lambs after primary inoculation; however, secondary Pl3V challenge evoked a more substantial antibody response in -Se/+Vit E lambs. Although Se and Vit E independently stimulated the immune response of stressed lambs, an additive or synergistic effect was not observed. This may be due to the levels of these nutrients present in the +Se/+Vit E diet. Selenium and Vit E were supplied in amounts similar to NRC recommendations for growing lambs (NRC, 1985). Other researchers have found a greater stimulatory effect when Se or Vit E was present in levels considerably above the stated requirement (Spallholz et al., 1973a;
Bendich et al., 1986). It may be necessary to re-evaluate established recommended intakes for nutrients that may be classified as "immuno-stimulatory." Increased intakes of nutrients such as Se and Vit E may prove beneficial during periods of stress such as weaning, marketing and parturition.

**Literature Cited**


