HUMORAL AND CELL-MEDIATED IMMUNE RESPONSE AND PERFORMANCE OF WEANED PIGS FED FOUR SUPPLEMENTAL VITAMIN E LEVELS AND HOUSED AT TWO NURSERY TEMPERATURES

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ABSTRACT

Three trials using 80 Yorkshire × Hampshire × Duroc crossbred pigs (avg initial wt, 6.9 kg) were conducted to determine the effects of four dietary vitamin E levels (11, 110, 220 and 550 IU/kg of feed) on the humoral and cell-mediated immune response and performance of 4-wk-old weanling pigs housed at two nursery temperatures (19 or 30°C). Interactive effects of temperature and vitamin E were not observed for any measurements. Serum and liver vitamin E levels increased linearly with increasing level of dietary vitamin E, but performance, cortisol, antibody levels and mitogen-induced stimulation indices were not affected by supplemental levels of vitamin E. Average daily gain and feed intake were higher (P < .01) for pigs housed at 19°C than for pigs housed at 30°C, but feed:gain ratios, mitogen stimulation index of white blood cells, plasma cortisol levels and antibody titers were not altered. Although supplemental vitamin E above the NRC estimated requirement increased serum and liver vitamin E concentrations, no differences were observed in humoral and cell-mediated immune response, cortisol levels or performance for weanling pigs housed at either 19 or 30°C temperatures.

(Key Words: Vitamin E, Environmental Temperature, Piglets, Immune Response.)


Introduction

The immune response of young pigs is believed to be compromised when they are subjected to a stress, such as weaning and(or) exposure to suboptimal environmental temperature (Kelley, 1980). Supporting this idea was the experiment by Furuuchi and Shimizu (1976), who suggested that cold temperature may increase the incidence of disease in weaned pigs.

Recent evidence (Ellis and Vorhies, 1976; Lim et al., 1981; Reddy et al., 1986) suggests that vitamin E also may play an important role in maintenance of optimal humoral and cell-mediated immune responses. Although the National Research Council (NRC, 1979) estimated requirement for vitamin E will prevent the onset of overt deficiency signs, Heinzerling et al. (1974) and Peplowski et al. (1981) have suggested that levels of vitamin E that are 6 to 20 times higher will optimize the immune response in chicks and pigs, respectively. Therefore, the objectives of this experiment were to determine whether changes in housing temperature would be detrimental to humoral and cell-mediated immune responses or performance and to determine whether supplemental dietary vitamin E would enhance the immune response and thus alleviate any problems caused by this potential stress.
Materials and Methods

In three trials, 80 Yorkshire × Hampshire × Duroc crossbred pigs (16, 32, and 32 for Trials 1, 2, and 3, respectively) were used in a split-plot arrangement of treatments to evaluate the effects of four supplemental vitamin E levels and two environmental temperatures on performance and immune response of weanling pigs. This was accomplished by housing pigs in rooms maintained at either 19 or 30°C and designed to provide 13.5 air changes per hour. All pigs (6.9 kg average initial wt) had ad libitum access to a 20% CP basal diet (meeting or exceeding all NRC estimated nutrient requirements and analyzed to contain an average of .2 ppm Se) with one of the following four supplemental vitamin E (α-tocopherol acetate; 551,876 IU/kg) levels (Table 1): 1) 11 IU of vitamin E added/kg of diet (NRC, 1979, basal diet); 2) 110 IU/kg (10× NRC); 3) 220 IU/kg (20× NRC) and 4) 550 IU/kg (50× NRC). At 28 d of age, eight pigs within each litter were paired by weight and sex; each pair was assigned randomly to a dietary vitamin E treatment. Temperature treatments were assigned randomly within each pair of pigs.

Pigs were weaned, mixed and moved to nursery pens on the same day. Pigs were housed (two per pen) in 1.6×1.3-m raised deck nursery pens. Lights were on continuously for 24 h per day. Pigs were weighed and feed consumption was recorded each week. Humoral immune response was measured by determining the antibody response to sheep red blood cells5 injected i.m. at the time of weaning and 17 d postweaning. Blood samples were collected initially and weekly for 5 wk after weaning. Serum was separated from whole blood by centrifugation (500×g) and frozen (−20°C) in polypropylene tubes for later determination of serum vitamin E, cortisol and antibody levels.

At the end of each trial (5 wk), pigs were killed and livers were removed for determination of vitamin E and Se concentrations (16 pigs per trial, two pigs selected randomly from each diet × temperature treatment). Diet, serum and liver vitamin E concentrations were determined in an independent laboratory by high performance liquid chromatography (HPLC) procedure (Bendich et al., 1984). Liver and dietary Se levels also were determined in an independent laboratory by hydride generation and atomic absorption spectroscopy (Brodie, 1979). Cortisol levels were analyzed using a commercial RIA kit6. Cross-reactivity of cortisol antisera as reported by the supplier were cortisone 1.6%, corticosterone .6%, dexamethasone .1%, prednisolone 35.0%, prednisone .2%, progesterone .05%, cholesterol less than .05% and testosterone less than .05%. Also, when known quantities of pure crystalline cortisol were added to pooled serum samples, the mean recoveries were between 96% and 102%.

Antibody titers to sheep red blood cells were measured using a hemagglutination assay (Schurig et al., 1978). Cell-mediated immune response was measured using a modified cell blastogenic assay reported by Bendich et al. (1984). White blood cells were separated using Histopaque 1077, then diluted in RPMI 1640 media (modified with HEPES buffer and 10% porcine serum) to 2×10° cells/ml. The

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5A 1-ml mixture of 10 μl (approximately 10° cells) packed sheep red blood cells, 10 μg lysozyme (L6876, Sigma Chemical Co., St. Louis, MO), .1 ml Freund’s complete adjuvant, .8 ml physiological saline, .1 ml AIOH and MgOH mixture.
6Amersham Corp., Arlington Heights, IL.
7Sigma Chemical Co., St. Louis, MO.
8LM 263-5, K. C. Biological, Lenexa, KS.
93007-5, K. C. Biological, Lenexa, KS.
diluted cells were added to a microtiter plate already prepared in triplicate wells to contain each of the following mitogens: concanavalin A (diluted either 100 or 10 μg/ml of media, respectively), phytohemagglutinin (M form, diluted either 1:10 or 1:500 in the media) and lysozyme (diluted either 1.0 or .1 mg/ml of media). After 48 h in a 37°C and 5.0% CO₂ environment, cells were pulsed with tritiated thymidine (1 μCi per well). Eighteen hours after pulsing, cells were frozen, harvested on filter paper and counted in a scintillation counter using Ecoscint scintillation cocktail. Counts per minute of stimulated cells (cpm) were divided by cpm of unstimulated cells and reported as a stimulation index.

All data were analyzed using the GLM procedure of SAS (1986) with the model including the effects of diet, temperature, litter, trial, time (week) and individual pig. Pen was the experimental unit for performance parameters. For the other measured parameters, pigs were the experimental units because each litter contained each of the treatment combinations (a replicate). All main effect and time interactions (two- and three-way) were included. No diet x temperature interactions were noted for any of the measurements; therefore, only main effect means are presented.

Results and Discussion

Analyzed dietary vitamin E levels were as follows: diet 1 (NRC = x), 12 IU/kg; diet 2 (lox), 101 IU/kg; diet 3 (O OX), 235 IU/kg; and diet 4 (O OX), 512 IU/kg. All diets were analyzed and found to contain an average of .2 ppm Se. Livers from pigs fed each of the diets were found to contain an average of .25 ppm Se (wet weight basis), which is comparable to levels in liver from pigs fed .12 ppm Se (Meyer et al., 1981).

Daily room temperatures were recorded, and the means averaged across mal are shown in Figure 1. Temperatures generally were constant except for a few mechanical problems, primarily in Trial 3. No apparent detrimental effects on immune function or performance were noted for the exposure to high temperature, probably due to the short duration of exposure (Minton et al., 1988).

Supplementing vitamin E in the diet (Table 2) did not affect ADG, avg daily feed intake or efficiency of feed utilization. Peplowski et al. (1981) and Bonnette et al. (1990) also found little effect of dietary supplemental vitamin E on performance. On the other hand, temperature (19 vs 30°C) did influence (P < .01) performance of the pigs (Table 2). Avg daily feed intake generally was increased (P < .05) for pigs housed at 19°C compared with 30°C with no effect on feed:gain. Average daily gain was generally also increased (P < .01), reflecting increased feed intake. Crenshaw et al. (1986) noted an opposite effect when pigs weighing approximately 6.0 kg were exposed to 18 vs 25°C rooms for 24 d; pigs in the 25°C room gained more and utilized feed more efficiently.

Differences between our results and those reported by Crenshaw et al. (1986) may be due to a lower starting weight in our trials (6.0 vs 6.9 kg) coupled with lower temperatures (25 vs 30°C and 18 vs 19°C). Pigs used by Crenshaw et al. (1986) were slightly smaller and the...
TABLE 2. LEAST SQUARES MEANS OF PEN PERFORMANCE VALUES FOR WEANLING PIGS FED VARYING LEVELS OF SUPPLEMENTAL DIETARY VITAMIN E AND HOUSED AT TWO NURSERY TEMPERATURES

<table>
<thead>
<tr>
<th>Item</th>
<th>110</th>
<th>110</th>
<th>220</th>
<th>220</th>
<th>550</th>
<th>550</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final wt, kg</td>
<td>22.3</td>
<td>20.6</td>
<td>20.5</td>
<td>20.5</td>
<td>21.0</td>
<td>21.0</td>
<td></td>
</tr>
<tr>
<td>ADG, kg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.42</td>
<td>.39</td>
<td>.39</td>
<td>.39</td>
<td>.40</td>
<td>.40</td>
<td></td>
</tr>
<tr>
<td>Daily feed, kg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.71</td>
<td>.78</td>
<td>.76</td>
<td>.76</td>
<td>.70</td>
<td>.70</td>
<td></td>
</tr>
<tr>
<td>Feed:gain</td>
<td>1.72</td>
<td>1.76</td>
<td>1.95</td>
<td>1.95</td>
<td>1.78</td>
<td>1.78</td>
<td></td>
</tr>
<tr>
<td>Final wt, kg</td>
<td>22.5</td>
<td>24.5</td>
<td>23.6</td>
<td>23.6</td>
<td>22.9</td>
<td>22.9</td>
<td>1.1</td>
</tr>
<tr>
<td>ADG, kg</td>
<td>.46</td>
<td>.50</td>
<td>.47</td>
<td>.47</td>
<td>.45</td>
<td>.45</td>
<td>.02</td>
</tr>
<tr>
<td>Daily feed, kg</td>
<td>.77</td>
<td>.87</td>
<td>.82</td>
<td>.82</td>
<td>.84</td>
<td>.84</td>
<td>.04</td>
</tr>
<tr>
<td>Feed:gain</td>
<td>1.70</td>
<td>1.76</td>
<td>1.76</td>
<td>1.76</td>
<td>1.86</td>
<td>1.86</td>
<td>.09</td>
</tr>
</tbody>
</table>

<sup>a</sup>Estimated NRC (1979) requirement for vitamin E is 11 IU/kg. These levels represent 1, 10, 20 and 50 times the estimated requirement.

<sup>b</sup>Five pens per treatment mean with two pigs per pen. Initial weight was 6.9 kg.

<sup>c</sup>Main effect of temperature (P < .05).

temperature of the low treatment group may have been below the thermoneutral zone; thus, the resulting reduced performance may have been due to stress. In our study, the upper temperature treatment may have been above the thermoneutral zone during the latter part of the trials, which may have depressed feed intake and increased feed:gain; the lower temperature treatment may have been close enough to the critical temperature to stimulate feed intake without stressing the pigs. Morrison et al. (1975) found that a decrease in temperature increased the rate of gain and feed consumption in pigs, but it did not affect the feed:gain ratio. Also, Henken et al. (1982), using young chickens, suggested that a temperature stress that did not cause a decrease in the animal's weight gain may indicate that the animals were never outside their thermoneutral zone, and therefore they were not stressed.

Increasing dietary vitamin E levels increased (quadratic, P < .01) the serum concentration of vitamin E (Table 3), which is in agreement with other reports (Peplowski et al., 1981; Blodgett et al., 1988). Also, liver vitamin E concentration increased linearly (P < .01) as the level of vitamin E in the diet increased (Table 3), with the magnitude of the increase in vitamin E concentration being much greater in liver than in serum. Rousseau et al. (1957) and Bonnette et al. (1990) reported similar results.

The dietary vitamin E levels × week interaction was highly significant (Figure 2). Serum vitamin E values increased (P < .01) over time for pigs fed dietary vitamin E levels above NRC (1979), with the increase proportional to the levels of vitamin E fed. Mahan and Moxon (1980) also reported that vitamin E serum concentrations of nursing pigs (similar to the values obtained in this experiment) were greater than concentrations obtained from weaned pigs fed a diet deficient in vitamin E. Initially, serum vitamin E levels were high, possibly due to the high availability of vitamin E in the milk compared with the feed (Meyer et al., 1981). Serum vitamin E levels dropped in the week following weaning as the animals were switched from milk to a dry feed, which was associated with low feed consumption and a decrease in absorption and/or retention of dietary vitamin E (Meyer et al., 1981).

Dietary vitamin E levels did not affect the level of cortisol in the blood (Table 3). Across dietary vitamin E levels, cortisol levels were high initially (14.5 ng/dl) but decreased to an average of 6.2 ng/dl after the 1st wk. Afterward, cortisol levels increased linearly (P < .01) to 12.2 ng/dl by the end of the experiment. Similar results were noted by Bonnette et al. (1990). The high initial values may have been due to a combination of several factors, including weaning, moving, bleeding, mixing with non-littermates and fighting to establish social dominance, all of which occurred prior to blood sampling and many of which have been suggested to cause stress (Kelley, 1980).

Antibody titer to sheep red blood cells were not influenced by supplementing vitamin E in the feed (Table 3). Antibody titers to sheep red blood cells followed a typical
antibody response (Figure 3): a primary response to the antigen was noted at wk 1 with a slight decrease at wk 2 as the antigen was being processed by the immune system. The next exposure to the antigen at d 17 resulted in a secondary response as seen by the increase in antibody titers after wk 3. Bonnette et al. (1990) noted a similar response for sheep red blood cells.

The antibody titers in this experiment were lower ($24.3$ vs $26$) than those reported by other researchers (Blecha and Kelley, 1981; Peplowski et al., 1981). This lower antibody titer may be due to suboptimal visual detection of the hemagglutination assay. The lower titer also may be due to the antigen being injected in combination with two different adjuvants, AlOH and Freund's. There has been increasing concern about using adjuvants to elicit an immune response. Adjuvants may work by enhancing phagocytosis and providing continuous release of the antigen (e.g., alum or AlOH) or adjuvants may present the antigen continuously in slowly disappearing mineral oil (Freund's) as well as providing activation of macrophages and some T-cell stimulating properties (Benjamini and Leskowitz, 1988). If the adjuvants caused an accumulation of macrophages in the inflammation site, antigen processing may be hindered. And, if a nutrient affects a function, such as antibody production, the addition of an adjuvant may overshadow any benefit due to the nutrient (Kelley, personal communication).

In contrast to our results, Peplowski et al. (1981) reported an increase in antibody titers when $20 \times NRC$ dietary levels of vitamin E were fed. This difference between experiments may have been due to the initial high serum vitamin E level ($0.20$ mg/dl) of pigs in our experiment compared with low initial serum vitamin E levels ($0.04$ mg/dl) reported by Peplowski et al. (1981). Sows in the study by Peplowski et al. (1981) were not given any vitamin E for several generations; sows in our studies were fed adequate levels of vitamin E (NRC, 1979). Also, when drawing a conclusion about experiments involving vitamin E, the concentration of Se must be noted because of the interrelationship between these two nutrients. Several of the vitamin E reference papers may have used animals or diets that were deficient in Se.

The low initial concentration of serum vitamin E in experiments by Peplowski et al.
TABLE 3. EFFECT OF ENVIRONMENTAL TEMPERATURES AND SUPPLEMENTAL VITAMIN E ON LEAST SQUARES MEANS OF THE OVERALL SERUM AND LIVER VITAMIN E CONCENTRATIONS, CORTISOL LEVEL, ANTIBODY TITERS AND STIMULATION INDICES

<table>
<thead>
<tr>
<th>Nursery temperature and supplemental vitamin E</th>
<th>30°C</th>
<th>19°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11¢</td>
<td>110</td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum, mg/dl</td>
<td>.10</td>
<td>.23</td>
</tr>
<tr>
<td>Liver, mg/100 g(^{cd})</td>
<td>.25</td>
<td>1.26</td>
</tr>
<tr>
<td>Cortisol, ng/dl</td>
<td>10.4</td>
<td>9.4</td>
</tr>
<tr>
<td>Antibody titer to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sheep red blood cells(^e)</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Stimulation indices(^f)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysozyme</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 mg/ml</td>
<td>2.49</td>
<td>3.20</td>
</tr>
<tr>
<td>.1 mg/ml</td>
<td>2.41</td>
<td>3.48</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>118.9</td>
<td>126.5</td>
</tr>
<tr>
<td>10 µg/ml</td>
<td>12.9</td>
<td>22.3</td>
</tr>
<tr>
<td>Phytohemagglutinin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:10</td>
<td>161.5</td>
<td>255.9</td>
</tr>
<tr>
<td>1:500</td>
<td>8.3</td>
<td>10.8</td>
</tr>
</tbody>
</table>

\(^{ab}\)Estimated NRC (1979) requirement for vitamin E is 11 IU/kg. These levels represent 1, 10, 20 and 50 times the estimated requirement.

\(^{cd}\)Five pens per treatment mean with two pigs per pen except liver vitamin values, which had six pigs per treatment mean.

\(^{cd}\)Cubic effect (P < .01) for serum vitamin E and linear effect (P < .01) for liver vitamin E.

\(^{ef}\)Main effect of vitamin E level (P < .01).

\(^{g}\)Pigs were injected with sheep red blood cells (10 µl) and lysozyme (1 µg) on d 0 and d 17. Titers were measured using a hemagglutination assay. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.

\(^{f}\)Mitogen concentration/ml of media. Background cpm = 380.

(1981) may suggest an explanation for the positive effects noted when supplementing higher levels of dietary vitamin E. Marsh et al. (1986) suggested that the primary lymphoid tissues were the main target for a vitamin E deficiency, and Baustad and Naftstad (1972) suggested that vitamin E is a hematopoietic (pertaining to the production and development of blood cells) factor in newborn piglets. Thus, the lack of an effect on antibody titer, mitogen stimulation or cortisol in our experiment may be due to the initial antigens being injected while the tissue stores of vitamin E were sufficient (while the pigs were nursing), thereby leaving lymphoid tissues unaffected.

Increasing serum vitamin E levels did not affect stimulation indices in either of the two levels of lysozyme and concanavalin A (ConA) or in the two dilutions of phytohemagglutinin (PHA), as shown in Table 3. No vitamin E × week or temperature × week interactions (P > .10) were noted for the stimulation indices. The two polyclonal T-cell antigens, ConA and PHA, which should give an indication of the general state of cellular immunity, did not show a consistent response over time for the two levels or dilutions used (Figure 4). Concanavalin A, however, at higher levels (100 µg/ml) produced a response curve similar to that of lysozyme and sheep red blood cells.

The lysozyme-induced stimulation indices shown in Figure 4 were used as an indicator of an antigen-specific T-cell response. Clonal expansion of a specific T-cell population was similar to the specific humoral response to sheep red blood cells (i.e., showing both a primary and secondary response) (Figure 3). Cells in both concentrations of lysozyme (1.0 mg/ml and .1 mg/ml; Figure 4) responded in the same fashion. An increase in stimulation index would suggest that the cell is more capable of responding to the specific antigen (lysozyme). Also, there were no significant differences of diet or time on the cpm of unstimulated cells (average 380 cpm).

In contrast to our results of no effects of supplemental levels of vitamin E, other re-
searchers have shown a positive effect of supplemental vitamin E on the cell-mediated immune response. Tanaka et al. (1979) reported that supplemental vitamin E (200 IU/kg of diet) in diets for mice stimulates helper T-cells. Corwin et al. (1981) suggested that vitamin E (5 IU/kg of diet) enhanced the stimulation of rat lymphocytes by mitogens. Reddy et al. (1986) found that a single injection of 2,000 IU of vitamin E to calves increased PHA-induced lymphocyte stimulation indices compared with preinjected values of the same animal; however, when vitamin E was added to the cell cultures, no increases in PHA-induced lymphocyte stimulation indices were noted. This finding suggests that the increase may not be due to vitamin E directly, but to some factor influenced by vitamin E.
Decreasing the environmental temperature (19 vs 30°C) of the weanling animals did not influence the cortisol concentration, serum or liver vitamin E content, antibody titer to sheep red blood cells or the mitogen stimulation indices (Table 3). In agreement, Crenshaw et al. (1986) reported that an 18°C environmental temperature compared with 25°C did not detrimentally influence antibody titer levels to human red blood cells (humoral immunity) or skin-fold thickness due to an intradermal injection of PHA (cell-mediated immunity) in pigs 4 and 8 d postweaning.

Elevated cortisol values in mice can suppress the cellular immune response (Blecha et al., 1983). Westly and Kelley (1984) reported that adding various levels of cortisol to the media decreased the mitogen stimulation index that adding various levels of cortisol to the media decreased the mitogen stimulation index of lymphocytes of adult sows. The average cortisol value was determined to be around 25 ng/ml, but levels as low as 13 ng/ml and as high as 135 ng/ml caused a significant reduction in blastogenesis. We did not observe the same dramatic effects on the immune response in this experiment. This may be due to the constant levels of cortisol or lack of glucocorticoid binding protein in an in vitro experiment that would not be seen in vivo.

Colder temperatures (8 to 12°C), however, have been reported to increase susceptibility of weaned pigs to disease (Furuuchi and Shimizu, 1976). Further support for the detrimental effect of cold temperature was reported by Blatchford et al. (1978). They indicated that temperatures between 5 and 30°C rarely caused a significant increase in cortisol, whereas temperatures above 40°C or below -5°C increased plasma adrenocorticotropic hormone and cortisol values. Blecha and Kelley (1981) reported an increase in antibody titer when 21- and 35-d-old pigs were exposed to a cold temperature (0 vs 25°C) for 4 d. Therefore, the lack of an immune response to an injected antigen (sheep red blood cells) or blastogenic stimulation of white blood cells to PHA, ConA and lysozyme in our experiment may suggest the environmental temperature differences were not overly stressful, and they still were within a functionally acceptable physiological range for the weanling pig.

Implications

The results of this experiment would suggest that pigs, nursed by sows fed the NRC estimated nutrient requirements for vitamin E and selenium, may have adequate stores of vitamin E at weaning to circumvent benefits from feeding levels of vitamin E (110, 220, and 550 IU/kg) higher than the estimated requirement (11 IU/kg) on performance, cortisol concentration, antibody production or mitogen-induced white blood cell proliferation. Also, housing weanling pigs at temperatures of 19 or 30°C did not create a sufficiently stressful situation to alter cortisol concentration, antibody production or mitogen-induced cell proliferation. However, pigs in the 19°C temperature ate more feed and gained faster than pigs housed at 30°C.

Literature Cited


