ABSTRACT

Thirty-two Holstein heifer calves, eight per group, were fed 0, 125, 250, or 500 IU/d of supplemental vitamin E/calf, from birth to 24 wk of age, in order to determine the effect on their immune responses. Overall mean lymphocyte blastogenic responses to various T-cell and B-cell mitogens were higher in supplemented calves than in control calves. Mean concentrations of cortisol in serum were lower in all supplemented calves than in control calves. Antibovine herpesvirus type 1 antibody titer (IgG) at 8 and 9 wk, in response to a commercial modified-live intranasal vaccine at 7 wk, was similar in all treatment groups. At 24 wk, in response to a booster at 21 wk, titer was higher in calves given 125 IU of vitamin E/d than in control calves. Based on the concentrations used, it is concluded that supplementation of conventional rations with 125 IU of vitamin E/d may maximize immune responses in calves and may be cost effective.

INTRODUCTION

Vitamin E enhances humoral immune (HI) response to various living and nonliving antigens in chicks, mice, turkeys, dogs, and pigs (18, 27), and cell-mediated immune response (CMI) in dogs (24), pigs (15), calves (7, 23), and laboratory animals (3, 9, 10, 17, 28). Plasma α-tocopherol concentrations less than 150 to 200 μg/dl are considered diagnostic of vitamin E deficiency in cattle and sheep (6, 12). Recently, Mass et al. (19) reported cases of nutritional myodegeneration in suckling lambs and yearling ewes that received adequate selenium. Average plasma α-tocopherol in these affected animals was 65 μg/dl. The vitamin E requirement of dairy calves has not been determined nor has the effect of supplementation on the immune response been studied adequately. Our earlier studies (22, 23) indicated that calves fed conventional calf starter could become vitamin E-deficient and supplementation could enhance their general performance and immunocompetency.

Although the antioxidant action of vitamin E is probably the important mechanism in enhancing the immune response, vitamin E also reduces the synthesis of certain prostaglandins (PG) (16) and lower serum corticosterone in mice (17, 28). Both cortisol and some PG are potent inhibitors of lymphocyte functions, such as mitogen responsiveness and lymphokine production (5, 20).

The objective of the present research was to study the effect of various doses of vitamin E on the immune response of calves from birth to 24 wk of age. Specifically, we have studied the effect on in vitro lymphocyte blastogenic response to different T-cell and B-cell mitogens, antibovine herpesvirus type 1 (BHV-1) antibody response to vaccine, and concentrations of cortisol in serum.

MATERIALS AND METHODS

Experimental Procedures

Thirty-two Holstein heifer calves were used from birth to 24 wk of age. Calves were blocked into eight groups of four calves each, based on their birth dates. Calves in each group were allotted randomly at birth to four treatments: 0 (control), 125, 250, or 500 IU supplemental vitamin E. DL-α-toco-
pherol\textsuperscript{3} was used up to 8 wk of age, mixed in milk (morning feeding) or in a small amount of water after weaning at 6 wk of age. After 8 wk, vitamin E was supplied by DL-\(
 \alpha\)-tocopheryl acetate in .45 kg of dry feed. Calves were housed in hutches and received milk at 8% of birth weight until weaning at 6 wk of age and a conventional calf starter until 8 wk of age. They were then moved to group pens where they received alfalfa hay ad libitum. They were individually fed a concentrate mixture, as much as they could eat in about 2 h daily, but only after eating .45 kg of the concentrate mixture containing the specified amount of vitamin E. The remaining managerial procedures that were common to all calves, the composition of the dry feeds used, and methods used for statistical analysis of data were presented by Reddy et al.\textsuperscript{(21)}.

All calves were vaccinated with a commercial modified live virus BHV-1 and parainfluenza-3 intranasal vaccine at 7 and 21 wk of age. Calves also received other routine vaccines and were dehorned at about 6 wk of age.

**Analytical Procedures**

**Enzyme-Linked Immunosorbent Assay for Antibovine Herpesvirus Type 1 Antibody Titers.** Blood was collected at 6, 7, 9, 10, 12, 18, 21, 23, and 24 wk of age for determination of anti-BHV-1 antibody titers in serum. The procedure described by Collins et al.\textsuperscript{(8)} was used with some modifications. Briefly, 96-well microtiter plates were coated with .1 ml of partially purified (sucrose gradient) BHV-1 (10\textsuperscript{8} pfu/ml) diluted 1:200 in carbonate buffer (pH 9.6) overnight at 5°C. After washing three times with phosphate-buffered saline (PBS), uncoated sites were blocked with PBS containing 1% goat serum. After 30 min, blocking solution was aspirated and .1 ml of serial doubling dilutions of test serum was added (three wells/dilution) and incubated at 37°C for 1 h. The wells were washed three times and .1 ml of peroxidase-linked goat anti-bovine IgG\textsuperscript{4} (diluted 1:2000) was added. After incubating for 1 h at 37°C, washing was repeated three times and .1 ml of substrate was added [2-2-Azino-Di(3-ethylbenzthiazoline sulfonic acid) diammonium salt]. Optical density was read after 30 min in a Titertec\textsuperscript{5} photometer at 405 nm. Titers were expressed as the reciprocal of the highest dilution that gave an absorbance value at least .1 above background.

**Lymphocyte Blastogenesis Test.** Blood was collected at 4, 6, 7, 8, 9, 12, 18, 21, and 23 wk of age. Lymphocytes were isolated from freshly heparinized blood and used in the test with slight modifications of the procedure described earlier (23). Lymphocyte suspension was adjusted to contain 2.5 \times 10\textsuperscript{5} cells/ml. The cell suspension with or without specified concentrations of mitogens was distributed in 200/al/well in quadruplicate in a flat-bottom, tissue culture plate. After 48 h of incubation at 37°C in a humidified 5% carbon dioxide incubator, [\textsuperscript{3}H]-thymidine\textsuperscript{6} (1 \muCi/culture) was added. Cultures were harvested 24 h later on glass fiber filters in an automated cell harvester.\textsuperscript{7} Filters were counted in a liquid scintillation counter\textsuperscript{8} to determine the incorporation of [\textsuperscript{3}H]-thymidine in lymphocytes.

Lymphocyte stimulation indices (LSI) were calculated as: LSI = disintegrations per minute (dpm) of stimulated lymphocyte cultures/dpm of control cultures. Concentrations of mitogens used were phytohemagglutinin (PHA)\textsuperscript{9} 5 \mug/ml, Concanavalin A (Con A)\textsuperscript{9} 12.5 \mug/ml, pokeweed mitogen (PWM)\textsuperscript{9} 12.5 \mug/ml, and lipo-polsaccharide (LPS)\textsuperscript{10} 25 \mug/ml. Concentrations were predetermined in pilot experiments in our laboratory to give maximum lymphocyte stimulation.

**Serum Cortisol.** Blood was collected at 2, 4, 6, 7, 8, 9, 12, 18, and 24 wk of age and the separated serum was frozen at -20°C until analyzed for cortisol by radioimmunoassay (25).
VITAMIN E IS IMMUNOSTIMULATORY IN CALVES

RESULTS

General Observations. One calf receiving 250 IU vitamin E died at 9 d of age and was replaced. A control calf died at 20 wk of age and was not replaced. Another control calf contracted "pink eye" at about 13 wk of age. Symptoms of mild respiratory infections were observed in most calves of all groups when they were in group pens but no calf was removed for treatment. Symptoms of cough and nasal discharge were more pronounced in two calves given vitamin E at 500 IU/d. Data on weight gains, feed consumption, fecal scores, serum enzymes indicative of cell membrane damage, metabolic profile, and some hematological responses are presented elsewhere (21). Fecal scores were similar among treatments, but one calf given 500 IU/d was treated for diarrhea at 2 wk of age.

Antibovine Herpesvirus Type 1 Antibody Titers. Mean serum anti BHV-1 antibody (IgG) titers at different wk are presented in Figure 1. Although there were no significant differences among treatments, calves given supplemental vitamin E showed a trend toward higher IgG response to vaccination at 7 wk of age. Response at 24 wk, following a booster vaccination at 21 wk of age, was higher for calves given 125 IU/d (P=.05) than control calves; response of calves given 500 and 250 IU/d was intermediate.

In all calves, seroconversion at 9 and 10 wk of age in response to vaccination at 7 wk was minimal. However, vaccination at 21 wk of age elevated (P=.01) anti-BHV-1 antibody titers at wk 23 and 24 only in calves given 125 IU/d compared with respective prevaccination titers at 21 wk.

Lymphocyte Stimulation Indices. Mean LSI averaged across weeks are presented in Table 1 and at different weeks in Figures 2 to 5. With PHA (Figure 2), a T-cell mitogen, overall mean LSI was higher for calves fed 250 IU than for control calves. At wk 4, lymphocytes from calves given 250 IU showed higher (P=.01) blastogenic response than those from control calves.

With Con A (Figure 3), another T-cell mitogen, overall means were higher with calves fed 125 or 500 IU than with control calves. Calves fed 125 IU showed higher (P=.03) values than calves given 0 or 250 IU at wk 6, while those given 250 IU showed higher (P=.03) values than calves given 500 IU at wk 8. Calves given 500 IU showed higher (P=.05) values than calves given 0 or 250 IU at wk 9, and calves given 500 IU showed higher (P=.03) values than calves given 0 or 125 IU at wk 12.

With PWM (Figure 4), a mitogen for both T-cells and B-cells, overall means were higher for calves given 500 IU than for control calves. Compared with control calves, those given 500 IU vitamin E showed higher (P=.01) values at wk 4 and calves given 125 IU showed higher (P=.03) values at 6 wk. With LPS (Figure 5), a B-cell mitogen, overall means were higher with calves given 125 or 500 IU vitamin E than with control calves, but no differences were observed among treatments at any of the individual weeks.

Serum Cortisol. Overall values were lower in all supplemented calves than in control calves (Table 2). At wk 4 calves given 500 IU and at wk 8 calves given 250 IU had lower (P=.03) serum concentrations than control calves.

Figure 1. Effect of supplemental vitamin E on serum antibovine herpesvirus type 1 antibody titers in calves. * Means within a week with different superscripts differ (P<.05). ELISA = Enzyme-linked immunosorbent assay.
TABLE 1. Effect of supplemental vitamin E on lymphocyte stimulation indices with different T and B-cell mitogens. (Least square means averaged across weeks.)

<table>
<thead>
<tr>
<th>Mitogen</th>
<th>Supplemental vitamin E, IU/calf per d</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>125</td>
<td>250</td>
<td>500</td>
<td>SEM</td>
</tr>
<tr>
<td>Phytohemagglutinin</td>
<td>31.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.9</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>29.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>36.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2</td>
</tr>
<tr>
<td>Pokeweed mitogen</td>
<td>20.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>23.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>26.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.6</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within a row with different superscripts differ (P<.05).

DISCUSSION

Our results further confirm the beneficial effects of vitamin E on several immunostimulatory responses of calves. Lymphocyte proliferative responses obtained with both T-cell and B-cell mitogens indicated that vitamin E plays a role in the regulation of both CMI and HI responses. Lymphocyte proliferative response did not increase linearly with increased vitamin E supplementation. During the initial weeks, response appeared to be more favorable with 125 IU/d. Although in this experiment we did not test amounts lower than 125 IU/d, to some extent the data on immune responses presented here and responses presented elsewhere (21) indicated that requirement for supplemental vitamin E might have been slightly less than 125 IU/d during the initial weeks and increased later. This increase in requirements was probably due to increasing body weights, decreased absorption in postweaning period, and destruction of vitamin E in the rumen (11). Variations among calves within treatment groups and variations within calves at different weeks for LSI values necessitate long-term experiments with repeated measures on calves in order to find differences among treatments.
VITAMIN E IS IMMUNOSTIMULATORY IN CALVES

Plasma \( \alpha \)-tocopherol concentrations less than 150 to 200 \( \mu \)g/dl indicate vitamin E deficiency (6, 12, 19). Data on serum \( \alpha \)-tocopherol concentrations (21, 22) of calves fed conventional, unsupplemented diets indicated vitamin E deficiency. Our data on serum enzymes (21, 22) indicated that in unsupplemented calves, cell membranes were prone to damage. Membranes of white blood cells may be more vulnerable to peroxidative damage and free radical attack because they contain more free fatty acids than most cells of the body (13). Based on several response criteria evaluated, we think performance of calves will be maximum if their serum \( \alpha \)-tocopherol concentration is increased gradually to about 200 to 250 \( \mu \)g/dl in

![Figure 4](image1.png)  
Figure 4. Effect of supplemental vitamin E on mean lymphocyte stimulation indices with pokeweed mitogen as the mitogen. \( ^{a,b} \)Means within a week with different superscripts differ \((P<.05)\).

![Figure 5](image2.png)  
Figure 5. Effect of supplemental vitamin E on mean lymphocyte stimulation indices with lipopolysaccharide as the mitogen.

**TABLE 2. Effect of supplemental vitamin E on concentrations (ng/ml) of cortisol in serum of calves at different weeks.**

<table>
<thead>
<tr>
<th>Week</th>
<th>Supplemental vitamin E, 1U/calf per d</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>125</td>
</tr>
<tr>
<td>2</td>
<td>4.5</td>
<td>3.4</td>
</tr>
<tr>
<td>4</td>
<td>5.0(^a)</td>
<td>2.2(^{ab})</td>
</tr>
<tr>
<td>6</td>
<td>4.6</td>
<td>4.0</td>
</tr>
<tr>
<td>7</td>
<td>5.3</td>
<td>3.3</td>
</tr>
<tr>
<td>8</td>
<td>8.4(^a)</td>
<td>5.5(^{ab})</td>
</tr>
<tr>
<td>9</td>
<td>3.2</td>
<td>1.9</td>
</tr>
<tr>
<td>12</td>
<td>3.3</td>
<td>2.6</td>
</tr>
<tr>
<td>18</td>
<td>6.5</td>
<td>4.2</td>
</tr>
<tr>
<td>24</td>
<td>6.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Overall mean</td>
<td>5.2(^a)</td>
<td>3.3(^b)</td>
</tr>
</tbody>
</table>

\( ^{a,b} \)Means within a row with different superscripts differ \((P<.05)\).
the first 4 wk and then is stabilized at least at that concentration. From the amounts used in this experiment and the benefits cited, supplementation with vitamin E at 125 IU/calf/d may be most cost effective.

Overall performance of calves supplemented with 500 IU was not superior to performance of calves supplemented with 125 IU/d. It is not clear if this was due to excess vitamin E during early life. Although there is no evidence of depressed lymphocyte proliferative response to mitogens because of supplementation with high amounts, there may have been compromised functioning of the other immune cells such as polymorphonuclear leukocytes (PMN) during the early weeks of life. Baehner et al. (2) found that administration of 1600 IU of vitamin E to normal humans increased the ability of PMN to ingest particles but slightly decreased their bactericidal potency. Although respiratory burst and superoxide anion release increased slightly, H$_2$O$_2$ release from the PMN was impaired. Research is needed to examine the effect of supplemental vitamin E on the functioning of PMN cells in calves. High vitamin E may impair absorption of vitamins A and K (4), especially during early life when calves are not receiving supplements. Vitamin A also is involved in the regulation of immune response (26).

To our knowledge, this is the first report showing the effect of supplemental vitamin E on concentrations of cortisol in serum of calves. Mice fed diets high in vitamin E had lower corticosterone in serum than controls (17, 28). Whether vitamin E has a direct effect on the adrenal gland or whether vitamin E-supplemented calves are less stressed than control calves is not clear. However, reduced cortisol in serum may partially explain the higher lymphocyte proliferative responses obtained with supplemented calves. Weaning, dehorning, vaccinations, change from calf starter diet to adult diet, shifting from hutches to group pens, inclement weather, flies, and diseases are some of the stress factors encountered by calves. Supplemental vitamin E may offset some of the ill effects of these stress factors on the immune function. Recently, Lawrence et al. (16) demonstrated lower prostaglandin (PG) E$_2$, PGF$_2$-a, and 6-keto-PGF$_1$ in the bursa and lower PGF$_2$-a in the spleen of *Escherichia coli*-infected chicks receiving supplemental vitamin E than in infected, unsupplemented chicks. It is thought that PG acts by stimulating adenylate cyclase in the lymphocyte membrane, which converts ATP to cyclic AMP, thereby increasing intracellular concentrations of cyclic AMP (14). It has been proposed that the ratio of cyclic AMP to cyclic guanosine monophosphate may determine the proliferative response of lymphocytes. When this ratio is high, lymphocyte activities are inhibited and vice versa (1). Whether supplemental vitamin E also exerts immunostimulatory effects in calves by altering concentrations of PG in immune organs should be examined.

Calves supplemented with vitamin E tended to produce more antibodies in response to BHV-1 vaccine. The response was more evident in the latter part of the experiment. Response to vaccine at 7 wk of age was minimal. Prevalence of maternal antibody inhibits antibody production by calves by a negative feedback mechanism. Although most calves exhibited symptoms of respiratory infections while in group pens, especially around 10 to 18 wk of age, the causative organism may not be BHV-1, since no anamnestic response was observed in our enzyme-linked immunosorbent assay results.

REFERENCES

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