Influence of Dietary Vitamin E on Phagocytic Functions of Macrophages in Broilers

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ABSTRACT

Vitamin E (VE) is known for its antioxidant properties and has been shown to modulate immune system functions in various species. This study examined the influence of different levels of dietary VE (α-tocopherol acetate) on phagocytic functions of macrophages (abdominal exudate cells) in broiler chickens at 3, 5, and 7 wk. Birds were fed commercial diets containing 16 (control), 110, or 220 mg of VE/kg of feed. Macrophages were elicited into the abdominal cavity by injecting a 3% Sephadex solution prepared in PBS (G50-50, 1 mL/100 g of BW) 42 h prior to harvest. The percentage of phagocytically active macrophages and the number of SRBC phagocytosed per macrophage for unopsonized and antibody-opsonized SRBC were determined. These aspects of macrophage function were assessed based on 900 macrophages per sample. When unopsonized SRBC were used, dietary VE supplementation above control level did not affect phagocytic function of macrophages at wk 3, 5, or 7. With antibody-opsonized SRBC, the percentage of phagocytically active macrophages and the number of SRBC phagocytosed per macrophage were higher (P = 0.08 and P = 0.01, respectively) in 3-wk-old birds fed 110 and 220 mg of VE/kg of feed compared with age-matched controls. This enhancing effect of VE supplementation on macrophage function was not observed in 5- and 7-wk-old broilers. It appears from this study that supplemental VE enhances Fc-receptor-mediated macrophage phagocytic activity at early stages of broiler growth.

(Key words: broiler, immune function, macrophage, phagocytosis, vitamin E)

INTRODUCTION

Crucial involvement of monocytes and macrophages in humoral and cell-mediated immunity makes them indispensable factors in host defense. Macrophages by way of phagocytosis eliminate necrotic cells, circulating immune complexes, tissue debris, and invading microorganisms (Qureshi, 1998). Additional important functions of macrophages include antigen presentation and secretion of a number of cytokines, prostaglandins, and free radicals. Increased presence of free radicals can cause oxidation of biomolecules, leading to cell death and tissue injury involved in the pathogenesis of several disease states (Kehrer, 1993). Toxic effects of free radicals may be reduced by the presence of antioxidants, such as vitamin E (VE). Evidence gathered until now shows that VE is a highly lipophilic free radical scavenger that is preferentially distributed in cell membranes where it breaks lipid peroxidation chain reactions (Yu, 1994). The antioxidant properties of VE have been credited for the enhancement of humoral and cell-mediated immune functions that are observed in avian and mammalian species (Tengerdy and Brown, 1977; Moriguchi et al., 1990; Meydani and Beharka, 1998). Yet, the mechanisms of VE action on the cells of the immune system and the most optimal route and program of VE administration in broilers have not been completely elucidated.

Early research examining the effect of dietary VE (300 mg of VE/kg) on the immune system of broilers has shown that VE-supplemented broilers exhibit a 3- to 8-fold reduction in mortality caused by Escherichia coli infection (Tengerdy and Nockels, 1975; Tengerdy and Brown, 1977). Although macrophage phagocytic functions were not examined in those studies, the increased clearance of E. coli in VE supplemented broilers points to enhanced macrophage phagocytosis. Administration of 10 mg of VE to broiler and poult embryos in ovo (d 18) improved humoral and cellular effector components of the immune system (Gore and Qureshi, 1997). In ovo VE-treated birds that were challenged with SRBC at 7 d of age exhibited increased antibody response to SRBC at d 14 and 21. Additionally, at 4 wk of age, abdominal exudate macrophages of in ovo VE-treated birds showed increased ability to phagocytose SRBC and to produce nitric oxide in response to LPS stimulation in vitro (Gore and Qureshi, 1997).
Evidence that has been generated over the last few decades highlights the importance of nutrients (including VE) for the optimal functioning of the immune system. Thus, immune functions should be one of the criteria upon which nutrient requirements are based (Bendich, 1995). The recognition of the importance of VE for broiler health and production has resulted in practices of supplementing broiler diets with levels of VE exceeding those recommended by the NRC (BASF, 1994). Established NRC requirements (NRC, 1994) may be optimal for growth but not necessarily for best health and disease resistance. Therefore, it is of particular interest to further examine the role that VE plays on specific functions of the immune system of poultry. The objective of this study was to determine the effect of supplemental dietary VE (above NRC levels) provided throughout a 7 wk growing period on macrophage functions in broilers. Specifically, the effect of supplemental dietary VE on the in vitro adherence ability of abdominal exudate cells and the ability of abdominal exudate macrophages to phagocytose unopsonized or antibody-opsonized SRBC were examined in broilers at 3, 5, and 7 wk of age.

MATERIALS AND METHODS

Birds and Diet

One hundred 1-d-old male broiler chicks (Cobb 500) were randomly assigned to 1 of 3 dietary VE treatments and placed in floor pens in 2 environmentally controlled chambers on wood shavings litter. Average chick weight used for the study was approximately 42 g. Chicks were vaccinated for Marek’s disease in ovo (d 18) and for Newcastle disease and infectious bronchitis at hatch. Broilers were maintained on a 23L:1D lighting schedule and were placed on a 35-mm culture dish that contained 4 glass cover slips. The chicks received either a basal corn-soybean diet, formulated to meet or exceed NRC requirements (NRC, 1994), or the basal diet with additional 110 and 220 mg of VE/kg feed in a form of DL-α-tocopherol acetate. For the first 3 wk, chicks were fed a broiler starter diet (CP, 25%; ME, 3,200 kcal/kg), and for the remaining 4 wk they were fed a grower diet (CP, 18%; ME, 3,200 kcal/kg).

Elicitation of Abdominal Macrophages

The abdominal exudate cells (AEC) were harvested as described by Qureshi et al. (1986). Briefly, at 3, 5, and 7 wk, 15 randomly selected birds from each treatment were weighed and given an intra-abdominal injection of a 3% Sephadex G-50 in sterile PBS solution (1 mL/100 g of BW). After 42 h, Sephadex-injected chicks were euthanized by i.v. injection of 6.5% pentobarbital solution. The AEC were harvested by flushing the abdominal cavity with 20 mL of heparinized (0.5 U/mL) Dulbecco’s PBS solution. The collected AEC suspensions obtained from 10 birds per treatment were placed into siliconized glass tubes and allowed to sit on ice for 10 min. Supernatant fluid that contained the AEC was transferred into a new tube, and AEC were pelleted by centrifuging the sample at 150 × g for 10 min at 4°C. The supernatant fluid was discarded, and the pelleted AEC were resuspended in 4 mL of LM Hahn medium (Qureshi et al., 1986).

Lymphoid Organ Weights

After AEC were harvested, thymus, spleen, and bursa were collected from each chick. Adherent fat was removed from these tissues, and the tissues were weighed. The data were presented as a percentage of tissue weight relative to the BW (relative organ weight).

AEC Adherence Assay

To determine the in vitro phagocytic potential of macrophages, the AEC suspension was adjusted to contain 1 × 10^6 viable macrophages per milliliter. To establish macrophage monolayers, 1 mL of this suspension from each chick was pipetted into a 35-mm tissue culture dish that contained 4 glass cover slips. To allow cells to adhere, culture dishes were incubated at 37°C in a humidified chamber with 5% CO2 for 1 h. After drying, cells were fixed and stained with Leukostat and mounted on microscope slides, 3 coverslips per slide. Proportions of macrophages and other nonerythroid cells in the AEC suspension were determined by morphological classification (Lucas and Jamroz, 1961). The number of adherent cells per microscope viewing field (at 1,000×) was determined by counting all leukocytes until at least 300 abdominal exudate macrophages per cover slip were examined while keeping track of the number of microscope viewing fields and the numbers of macrophages observed. In addition, the total number of adherent leukocytes and macrophages identified was recorded, and the percentage of adherent leukocytes that were macrophages was calculated.

SRBC Phagocytosis Assay

To determine the in vitro phagocytic potential of macrophages, the AEC suspension was adjusted to contain 1 × 10^6 viable macrophages per milliliter. To establish macrophage monolayers, 1 mL of this suspension from each chick was pipetted into a 35-mm tissue culture dish that contained 4 glass cover slips. The culture was then incubated for 1 h at 37°C to allow cells to adhere. Macrophage monolayers were then incubated for 1 h with 1 mL of a 3% SRBC solution in a humidified chamber with 5% CO2 at 37°C. The SRBC used were either unopsonized or opsonized with a nonagglutinating concentration of quail anti-SRBC antibodies. After the incubation, cover slips were washed with PBS, dried, fixed, and stained with Leukostat and mounted on microscope slides, 3 coverslips per slide. A total of 300 macrophages per cover slip (900 macrophages per slide) were examined by bright field microscopy.
microscopy at 1,000× to determine the proportion of phagocytically active macrophages and the number of SRBC phagocytosed per phagocytically active macrophage.

**Statistical Analysis**

Data were analyzed separately for each sampling point (wk 3, 5, and 7) using the GLM procedures of SAS software package (SAS Institute Inc., 1997). Treatment means for all measurements were separated by LS means procedure and declared significantly different at $P \leq 0.05$.

**RESULTS AND DISCUSSION**

There is a small amount of information regarding the importance of dietary VE for the functioning of macrophages in broilers. The environmental conditions under which broilers are raised subject them to a number of stressful agents, including the constant exposure to various microorganisms. Macrophages phagocytose and kill ingested microbes by generating large amounts of highly reactive molecules such as reactive oxygen intermediates (ROI) (Qureshi, 1998; 2003). Therefore, the autooxidative damage of macrophages as well as tissue injury due to overproduction of ROI is an unavoidable consequence of macrophage activation (Splettstoesser and Schuff-Werner, 2002). Thus, it is important that macrophages have a number of antioxidant systems available to cope with oxidative stress. VE has been recognized as a very important hydrophobic antioxidant that can arrest or entirely prevent lipid peroxidation of the cell membrane (Tappel, 1970). This function of VE is particularly crucial for maintaining cell membrane integrity and enabling macrophages to efficiently phagocytose, contain, and kill ingested microbes (Tappel, 1970; Sakamoto et al., 1990).

Based on the above-mentioned information, this study was designed to examine the effect of dietary VE (above NRC requirements) on the in vitro phagocytic potential of abdominal exudate macrophages of broilers when VE was supplemented from day of hatch.

**Body Weights and Relative Organ Weights**

There were no differences in the BW of broilers fed different VE levels at wk 3, 5, or 7 (data not shown). No differences among treatments were observed in relative weights of lymphoid organs (data not shown) with the exception of the relative spleen weight at 7 wk, which was higher in chicks fed 110 and 220 mg of VE/kg compared with chicks fed the control diet (0.14 ± 0.01, 0.18 ± 0.01 and 0.10 ± 0.02, respectively; $P \leq 0.05$). Previously, several authors have reported that VE deficiency depletes leukocytes in the spleen (Scott et al., 1955; Dietert et al., 1983), whereas VE supplementation increases splenocyte proliferation (Sakai and Moriguchi, 1997). Hence, the increase in spleen weight with supplemental VE observed here is likely to represent an increase in the number of lymphocytes.

**Yield of AEC and Incidence of Adherent AEC and Macrophages**

An increase in the yield of Sephadex-elicited AEC has been reported for broilers injected with VE (Gore and Qureshi, 1997). However, we did not observe differences in the numbers of AEC harvested from broilers fed different VE diets (data not shown). At 3 and 5 wk, the number of adherent AEC and macrophages per microscope field were highest in broilers fed 110 mg of VE/kg (P = 0.05, Table 1). At 7 wk, broilers fed 16 and 110 mg of VE/kg had higher numbers of adherent AEC and macrophages per microscope field compared with broilers fed 220 mg of VE/kg ($P \leq 0.05$, Table 1). It appears that higher numbers of adherent AEC were due to increased numbers of adherent macrophages, but this trend was not apparent when the percentage of macrophages within adherent AEC were determined (Table 1). Hence, in this study additional VE supplementation did not influence the yield of adherent AEC and macrophages harvested.

**Phagocytic Potential of Abdominal Exudate Macrophages**

The percentage of phagocytically active macrophages incubated with unopsonized SRBC and the number of unopsonized SRBC phagocytosed per individual macrophage were not affected by dietary VE at any age examined (Figure 1). The percentage of phagocytically active macrophages incubated with antibody-opsonized SRBC for 100 and 200 mg of VE/kg and the number of antibody-opsonized SRBC phagocytosed per individual macrophage were higher in 3-wk-old broilers fed supplemental VE ($P = 0.08$ and $P = 0.05$, for 100 and 200 mg of VE/kg,

### Table 1. Influence of dietary vitamin E (VE) on numbers of adherent abdominal exudate cells (AEC) and macrophages per microscope field and the percentage of adherent macrophages in broilers at wk 3, 5 and 7

<table>
<thead>
<tr>
<th>VE (mg/kg)</th>
<th>Week</th>
<th>Adherent AEC$^2$</th>
<th>Adherent Macrophages$^2$</th>
<th>Macrophage [%]$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>3</td>
<td>60 ± 6$^a$</td>
<td>36 ± 4$^a$</td>
<td>67.6 ± 3.2</td>
</tr>
<tr>
<td>110</td>
<td>3</td>
<td>77 ± 6$^a$</td>
<td>53 ± 4$^a$</td>
<td>69.9 ± 3.9</td>
</tr>
<tr>
<td>220</td>
<td>3</td>
<td>66 ± 5$^b$</td>
<td>48 ± 3$^b$</td>
<td>72.4 ± 1.4</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>50 ± 7$^b$</td>
<td>34 ± 5$^b$</td>
<td>65.8 ± 2.8</td>
</tr>
<tr>
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<td>5</td>
<td>80 ± 4$^b$</td>
<td>49 ± 4$^b$</td>
<td>63.2 ± 4.5</td>
</tr>
<tr>
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<td>51 ± 7$^b$</td>
<td>34 ± 5$^b$</td>
<td>71.4 ± 3.7</td>
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<tr>
<td>16</td>
<td>7</td>
<td>73 ± 13$^b$</td>
<td>44 ± 9$^b$</td>
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</tr>
<tr>
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<td>7</td>
<td>90 ± 8$^b$</td>
<td>57 ± 7$^b$</td>
<td>61.1 ± 2.7</td>
</tr>
<tr>
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<td>7</td>
<td>55 ± 8$^b$</td>
<td>31 ± 4$^b$</td>
<td>58.8 ± 2.6</td>
</tr>
</tbody>
</table>

$^a,b$Means within an age group and column with different superscripts are significantly different ($P \leq 0.05$).

$^1$Cobb 500 broilers were fed diets supplemented with 16, 110, or 220 mg VE (α-tocopherol)/kg of feed from day of hatch up to 7 wk.

$^2$At wk 3, 5 and 7, 1 mL of elicited AEC suspension was incubated on glass coverslips for 1 h. All adherent AEC were scored at 1,000 magnification until a count of 900 macrophages per bird was reached. Data was expressed as the number of adherent cells per microscope field.

$^3$The percentage of macrophages (Mo) was calculated by the formula $Mo \% = (Mo \ number/total AEC \ number) \times 100$. Data were expressed as mean ± SEM based on 10 broilers.
Conclusions

Under the experimental conditions used in this study (i.e., environmental chambers) the standard VE supplementation (16 mg VE/kg) was optimal for most aspects of macrophage functions examined. However, supplemental dietary VE increased the receptor-mediated phagocytic potential of elicited abdominal exudate macrophages in 3-wk-old broilers and increased their ability to make effective use of maternal antibodies. When translated into large-scale broiler production, this seemingly modest enhancement of immune system function on aspects of innate immunity early in the life of broilers together with previous reports on beneficial effects of VE on the development and function of adaptive immunity in 5- to 7-wk-old broilers (Erf and Bottje, 1996; Erf et al., 1998) suggest that additional VE supplementation of broiler diets may have a substantial positive impact with respect to disease resistance and improved broiler health.

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REFERENCES


