EFFECT OF VITAMIN E AND SELENIUM SUPPLEMENTATION ON SOME IMMUNE PARAMETERS FOLLOWING VACCINATION AGAINST BRUCELLOSIS IN CATTLE¹

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ABSTRACT

Twenty-four 7-mo-old beef heifers (Charolais Simmental cross), weighing 213 kg, were used to determine the effect of vitamin E (VitE) and(or) selenium (Se) supplementation on the humoral response to a standard dose of Brucella abortus strain 19 vaccine and on the levels of naturally occurring immunoglobulins (Ig) to several antigens. The treatments were as follows: Group 1, no supplement; Group 2, supplementation with 6 g of elemental Se; Group 3, supplementation with 1,400 IU/d of VitE; and Group 4, Se and VitE supplements combined. There were no significant differences in anti-B. abortus IgG1, IgG2, or IgM antibody levels due to Se, VitE or Se/VitE treatments; the concentrations of IgA antibody were too low to be measured with the ELISA test used. Statistical analysis revealed that the levels of total and IgM natural antibody to Salmonella typhimurium were higher in Group 3. Perhaps VitE supplementation given in conjunction with B. abortus vaccine enhanced the production of antibody to S. typhimurium in several animals whose humoral system had been activated by previous exposure to this organism.

(Key Words: Vitamin E, Selenium, Feed Supplements, Brucella abortus, Salmonella typhimurium, Immune Response.)

Introduction

Vitamin E (VitE) is an essential antioxidative component of cell membranes that prevents peroxidative damage to the cell membrane and membranes of subcellular organelles by free radicals. Selenium (Se) has a biological function related to VitE in that Se is an essential component of glutathione peroxidase (GSHPx), an enzyme involved in detoxification of hydrogen peroxide and lipid hydroperoxides (Rotruck et al., 1973). The antioxidative role becomes very important during the immune response when neutrophils produce large quantities of superoxide and hydrogen peroxide from molecular oxygen to destroy ingested foreign organisms (Ross, 1977). The lymphocytes seem to be especially susceptible to peroxidative damage because their membranes have a relatively high free fatty acid content (Kigoshi and Ito, 1973). These cell membranes are involved in transport of soluble substances; they are of a great importance in the binding of mitogens and antigens. Several studies with small animals suggest that both humoral and cellular immune response are enhanced by VitE and Se supplementation (Nockels, 1979; Sheffy and Schulz, 1979). Inoculation with Pasteurella haemolytica increased immunoglobulin M (IgM) antibody levels in Se-supplemented calves (Stabel et al., 1989), whereas steers given a combined VitE and Se (VitE/Se) treatment showed enhanced immunoglobulin G

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(IgG) antibody titers to the same organism (Droke and Loerch, 1989).

Our study was conducted in Northern Ontario, an area where the soil is known to be deficient in Se (Jenkins and Hidiroglou, 1972). It was designed to determine the effect of Se and VitE supplementation in cattle on the humoral immune response to immunization with Brucella abortus and also on the natural antibody levels to selected antigens.

Materials and Methods

Animals and Diets. Twenty-four crossbred heifers (Charolais x Simmental), 7 mo old and weighing 213 kg, kept at the Kapuskasing Experimental Farm were divided randomly into four groups of six animals each and were given one of the following treatments: Group 1, no supplement (control); Group 2, Se supplement; Group 3, VitE supplement; and Group 4, combined VitE and Se supplement. The cattle had ad libitum access to direct-cut orchardgrass silage. The diet supplement, containing 300 g/(hd.d) of soybean meal and 200 g/(hd.d) of rolled barley, was spread daily on top of the silage. The heifers were offered 1 \text{kg}(\text{hd.d}) of mineral mix\textsuperscript{5} and were treated with 1 ml Ivomec\textsuperscript{6}/50 kg body weight. The Se supplement was administered orally on d -84\textsuperscript{7} in two 30-g iron boluses\textsuperscript{8}, each containing 3 g of elemental Se (Hidiroglou et al. 1985).

The VitE supplement (DL-alpha-tocopherol) was administered at an average dietary intake of 1,400 IU/d (orally in gelatin capsules).

Blood Collection and Immunization. Blood was collected into heparinized vacuum tubes by jugular venipuncture. Plasma was separated by centrifugation and frozen immediately in three fractions for antibody, Se and VitE determination. All animals were vaccinated subcutaneously with 3 \times 10^{10} colony-forming units (cfu) in 5 ml of Brucella abortus strain 19 vaccine on d 0. The bleeding schedule was as follows: -84, -56, -28, 0, +14, +16, +18, +21, +23, +26, +30, +51, +79, +107 and +135.

Enzyme-Linked Immunosorbent Assay (ELISA). Indirect ELISA (Henning and Nielsen, 1987) was performed using the following antigens: smooth lipopolysaccharide (SLPS) from B. abortus (strain 413), Salmonella typhimurium SLPS, Keyhole limpet hemocyanin (KLH), Escherichia coli K235, E. coli 055:B5, Serratia marcescens, Pseudomonas aeruginosa and Klebsiella pneumoniae\textsuperscript{9}.

Mean optical densities (OD) were calculated and corrected, so that OD of positive control serum (OD<sub>pos</sub>), included in duplicate on each plate, was 1.0 (all OD values divided by OD<sub>pos</sub>). The background conjugate activity was subtracted. The corrected mean OD for each group and each bleeding date were statistically analyzed.

Hemagglutination Test. Bovine plasma samples were doubly diluted in .15 M NaCl, starting at dilution 1:20. Volumes of 100 \mu l of each dilution were added to wells of round-bottomed, 96-well polystyrene plates and mixed with 100 \mu l of 2.5% guinea pig erythrocytes (washed three times and resuspended in .15 M NaCl). After a 2-h incubation the results were read. A mantle of erythrocytes was considered a positive reaction; a “button” pellet was considered negative (Herbert, 1978).

Analytical Methods. Selenium in the blood plasma of cows was measured by the method of Hoffman et al. (1968). The Se content of roughage and diet supplement, after the wet digestion, was determined by fluorometry (Hoffman et al., 1968). On d -7, +26, and +173 of the experiment, GSHPx activity in the EDTA-treated whole blood was measured according to the coupled assay of Paglia and Valentine (1976). Determination of alphatocopherol was performed by high-pressure liquid chromatography equipped with spectrofluorimetric detection (McMurray and Blanchflower, 1979).

Statistical Analysis. The effect of different treatments on blood antibody levels at each bleeding date was analyzed by comparing mean OD values obtained from each group. The statistical analysis was done by conventional one-way ANOVA. The following linear model of SAS (1982) was used: \[ Y_{ijk} = u + V_i \]
Figure 1. Mean concentrations of Se (ng/ml) in plasma of heifers from four experimental groups, treated as follows: no supplement (--); Se supplement (⋯); VitE supplement (--; and Se/VitE combined supplement (--). The standard B. abortus strain 19 vaccine (3 × 10^10 cfu) was administered on d 0. The statistical significance refers to the effect of VitE supplement and is indicated for bleeding days by vertical bars as follows: three vertical lines, $P < .0009-.001$; two vertical lines, $P < .009-.001$; one vertical line, $P < .05-.01$; no indication if not significant. The effects of Se or its interaction with VitE were not significant or as stated in the text.

Results

The mean concentrations of Se in the roughage and diet supplement, sampled at the same time as blood, ranged from .02 mg/kg to .05 mg/kg. Plasma Se concentrations in animals given Se supplement were higher ($P < .0001$) than those of animals not supplemented with Se. This difference was observed 4 wk after the introduction of the Se supplement and was maintained until the end of experiment (Figure 1). In blood plasma, the GSHPx activity was higher ($P < .002$ to $P < .0001$) in the Se-supplemented groups (results not shown). The plasma VitE levels were elevated ($P < .02$ to $P < .0001$) in the VitE-supplemented animals; this difference lasted throughout the experiment (Figure 2). The parallel trends of VitE concentrations among all experimental groups may be related to changes in feed intake. An apparent interaction of Se with VitE was observed from d 23 ($P < .04$).

Figure 2. Mean concentrations of VitE (μg/ml) in plasma of heifers from four experimental groups. See Figure 1 for key.

The IgG1, IgM and total antibody levels increased following the vaccination, but no statistical differences associated with VitE, Se or VitE/Se supplementation were detected (Figure 3, 4, 5). Although an increase in the levels of IgG2 was evident (Figure 6), the mean OD obtained in the tests did not exceed 3. This is the minimum value considered significant (93.6% level of confidence) in the diagnostic ELISA test used (Nielsen et al., 1987). The IgA antibody could not be detected.

The agglutination of guinea pig erythrocytes due to the specific IgG and IgM antibody was observed at sample dilutions of 1/4 and 1/8. However, no consistent increase in the extent of agglutination was observed.

The antibody levels to S. typhimurium (Figure 7), in the presence of VitE supplement (Groups 3 and 4), were higher ($P < .03$ to $P < .0005$) from d +14 to +26. Similarly, the levels of IgM antibody (Figure 8) were elevated ($P < .02$) from d +14 to +23. However, all but the VitE-supplemented group showed levels below those considered significant in the ELISA test used.

The antibody levels to the remaining bacterial antigens and KLH were relatively low, remaining virtually unchanged for the duration of the experiment (results not shown).

Discussion

The diet fed to the cattle was deficient in Se, as shown by Se concentrations below .1 mg/kg, a value indicating that Se is deficient in animal feed (Underwood, 1980). The effect of Se dosing was reflected in blood GSHPx of the
animals. In unsupplemented cattle, GSHPx was less than 40 IU/g Hb, which is considered critical and indicative of Se deficiency (McMurray, 1980). Plasma Se concentrations in Se-supplemented animals remained between 60 and 80 ng/ml, whereas the groups lacking Se supplement stabilized at about 20 ng/ml. Any value below 20 ng/ml is indicative of a Se deficiency in cattle (Perry et al., 1976; Fenimore et al., 1983). Buckley et al. (1987) defined the marginally deficient serum Se concentration in cattle to be 20 to 40 ng/ml. Thus, the groups not supplemented with Se could be considered deficient in this element. This reflects the fact that nutritional muscular dystrophy, induced by such a deficiency, is endemic to the area of Northern Ontario (Nelson et al., 1964). The VitE concentrations in the unsupplemented and Se-supplemented group remained between 2 and 3 µg/ml; in the VitE-supplemented group between 3 and 6 µg/ml, for most of the experiment. A VitE deficiency in cattle is characterized by plasma concentrations below 1.5 µg/ml (Hidiroglou et al., 1988); thus, none of our groups was deficient in this vitamin.

The IgG1 subclass of antibody to B. abortus LPS was found to reach peak concentrations about 3 wk after vaccination. The profiles were nearly identical for all the experimental groups. The lack of measurable differences may be due to an excessive stimulation of humoral response by the full dose of B. abortus strain 19 vaccine. Thus, small differences between the groups may have been masked. Also, differences in the nutritional status may have been insufficient to affect the humoral response. However, neither VitE nor Se supplementation, at the doses used, improved the long-term humoral response in cattle, based on vaccination with a standard
dose of *B. abortus* strain 19.

The IgM antibody profile indicates that the VitE/Se- supplemented group tended to respond less than the remaining experimental groups. One possibility, that the IgM antibody levels in the VitE/Se-supplemented group increased more rapidly than those of the other groups and reached a peak prior to the first bleeding 2 wk after vaccination, was examined. In such a case, the first bleeding should reflect the decline in IgM antibody levels. However, the IgM antibody levels in cattle, followed every 2 d after vaccination in a similar experiment, peaked 2 wk after vaccination and then declined steadily to nearly background activity at three months. This correlated quite well with what is considered to be a “normal” IgM antibody response, which results in a peak at about 1 mo and a return to the background activity at 4 mo after vaccination (Nielsen and Duncan, 1988).

Therefore, the probable cause lies in extensive variation in IgM antibody response, even within a single experimental group, and in unsuccessful randomization, which resulted in assignment of several “low-responders” to one group. In any case, the IgM antibody profiles were not affected by the dietary supplementation.

The IgG2 subclass of antibody was found in the samples at relatively low concentrations, with a high degree of variability among animals. Subsequently, no statistically significant effect of the various treatments could be detected. The lack of positive effect of VitE and(or) Se supplementation on IgM and IgG antibody responses contradicts the results of several studies. VitE and Se supplementation was shown to improve the hemagglutinin titers to sheep red blood cells and IgG and IgM concentrations to various antigens in chickens and mice (Tengerdy et al., 1972, 1973; Spallholz et al., 1973; Franchini et al., 1986). In calves challenged with viral antigens, VitE (Reddy et al., 1986, 1987) and Se (Reffett et al., 1988a) supplementation has improved IgM antibody titers but left the IgG antibody levels unaffected. The humoral immune response to various antigens in horses was the highest in the VitE/Se-supplemented group (Raalsrud and Ovemes, 1986).

The lack of detectable levels of IgA antibody in plasma was not surprising because this class of antibody is predominant in seromucous secretions such as saliva, colostrum and milk.

The levels of total antibody to several different bacterial antigens, KLH and guinea pig erythrocytes were measured. We hoped that such measurement would reveal small differences in antibody response due to a nonspecific increase in antibody production either following the supplementation or after the vaccination. A significant difference between the experimental groups was detected only with *S. typhimurium* LPS, 14 d after the immunization with *B. abortus*. Closer examination of total and IgM antibody levels in four animals from the VitE-supplemented group showed a tendency for higher titers (not significantly as shown by analysis of variance) even prior to the introduction of the supplements or the vaccine. Present *S. typhimurium* exposure or sustained high levels of antibody following some previous exposure could produce such a result. An effect of VitE on
antibody production by an activated humoral immune system was observed in lambs challenged with parainfluenza virus (Reffett et al., 1988b). They reported that, although the IgM antibody levels increased more substantially in Se-supplemented animals, the increase was greater with VitE supplement after a secondary challenge. Although, in our experiment, VitE supplementation may have been involved, additional work may be necessary to investigate the interaction of immune system activation and dietary supplementation on the humoral response to the vaccine in cattle.

Our data indicate that Se and VitE, alone or combined, at the levels indicated did not affect the anti-B. abortus IgG subclass or IgM antibody levels following a standard B. abortus strain 19 vaccination. Some studies have reported that VitE has a positive effect when used as an adjuvant in sheep (Tengerdy et al., 1983; Afzal et al. 1984). Effects of such a mode of VitE administration on humoral response in cattle need further study.

Implications

For beef cattle, dietary supplementation with vitamin E at levels above those recommended as nutritional requirements and given in conjugation with B. abortus strain 19 vaccine may have enhanced the levels of total and IgM natural antibody to Salmonella typhimurium. However, neither Se nor vitamin E supplementation altered the immune response to B. abortus.

Literature Cited


