PORCINE IMMUNOGLOBULIN TRANSFER AFTER PREPARTUM TREATMENT WITH SELENIUM OR VITAMIN E 1, 2, 3

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ABSTRACT

Responses to prepartum injection of sows with Se and vitamin E (E) were evaluated by determining immunoglobulin (IgA, IgM, IgG) levels in the colostrum and serum of the sows and the serum of their offspring. Fifty-four sows (40 multiparous, 14 primiparous) receiving diets adequate in E and Se according to current NRC (1988) standards were randomly allotted to four treatment groups in which a single i.m. injection of saline (controls), 5 mg of Se, 1,000 IU of E, or both Se and E were given on d 100 of gestation. Sows were bled prior to and 7 d after injection, at farrowing and on d 14 and 28 of lactation. Colostral samples were collected at the initiation of farrowing. Pigs were bled 20 h postpartum and at 14 and 28 d of age. Major immunoglobulin changes in the serum of the sows due to treatment were not seen prior to parturition. Injections of Se and(or) E resulted in higher colostral IgM levels (8.4, 10.7, 9.8 and 9.6 mg/ml, respectively), but only the response from Se was significant (P < .05). Concentrations of colostral IgA or IgG were not affected by treatment (P > .30). Compared with controls, all three treatments increased (P < .10) IgM concentrations in serum from pigs at birth (28.3, 33.3, 36.0 and 33.5 mg/ml, respectively), whereas IgA and IgG concentrations were not affected (P > .30). On d 14, IgM concentrations in pig serum from the sows treated with Se and Se + E remained elevated (10.2, 13.4, 12.3 and 12.9 mg/ml, P < .05), whereas all three treatments increased IgG concentrations (2.1, 3.0, 3.1 and 3.6 mg/ml, respectively). These results indicate that prepartum injection of sows with E and Se influences immunoglobulin transfer to their progeny.

(Key Words: Pigs, Vitamin E, Selenium, Immunoglobulins.)

Introduction

Vitamin E (E) and Se supplementation was demonstrated to benefit the immune system of mice (Tengerdy et al., 1972, 1973; Spallholz et al., 1973a,b). Since these studies, the enhancement of the immune system by E and Se supplementation has been demonstrated with lambs (Nockels, 1979; Turner et al., 1985), chickens (Heinzerling et al., 1974; Marsh et al., 1981) and cattle (Cipriano et al., 1982; Reddy et al., 1986). In young pigs, E and Se supplementation have been beneficial in increasing whole cell agglutination against Escherichia coli (Ellis and Vorhies, 1976), increasing the response against sheep red blood cells (Peplowski et al., 1981) and enhancing the response of pig lymphocytes to phytohemagglutin (Larson and Tollersud, 1981). Yet other studies have shown no change in the immune response due to supplementation with either E or Se (Blodgett et al., 1986; Komegay et al., 1986). As a result of placental structure and function, the newborn pig is devoid of circulating antibodies (Bourne et al., 1978) and must attain maternal immunoglobulins by receiving colostrum during the first few
TABLE 1. PERCENTAGE COMPOSITION OF DIETS

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Gestation</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground corn</td>
<td>77.90</td>
<td>68.35</td>
</tr>
<tr>
<td>Dehulled soybean meal</td>
<td>13.50</td>
<td>13.50</td>
</tr>
<tr>
<td>Alfalfa meal</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Beet Pulp</td>
<td></td>
<td>10.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.20</td>
<td>2.25</td>
</tr>
<tr>
<td>Ground limestone</td>
<td>.55</td>
<td></td>
</tr>
<tr>
<td>Iodized salt</td>
<td>.50</td>
<td>.50</td>
</tr>
<tr>
<td>Trace mineral premix a</td>
<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>Selenium premix a</td>
<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>Vitamin premix c</td>
<td>.20</td>
<td>.20</td>
</tr>
<tr>
<td>Antibiotic d</td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>Antibiotic e</td>
<td></td>
<td>.10</td>
</tr>
</tbody>
</table>

*Supplied the following (mg/kg of diet) Fe, 88; Zn, 75; Mn, 30; Cu, 9; I, 1.

bSupplied .1 mg Se/kg of diet.

cSupplied the following (per kg of diet): 6,600 IU vitamin A, 880 IU vitamin D, 22 IU vitamin E, 4.4 mg vitamin K, 8.8 mg riboflavin, 22 mg pantothenic acid, 44 mg niacin, 434 mg choline and 22 µg vitamin B₁₂.

dAureomycin-50 supplied 55 mg of chlortetracycline/kg of diet.

eNeo-terramycin supplied 55 mg of chlortetracycline/kg of diet.

hours after birth (Butler, 1974). Acquisition of these colostral immunoglobulins is crucial to the pig's immunity and survival (Leece, 1971; Blecha and Kelly, 1981).

The objective of this study was to determine whether single prepartum injections of E and/or Se would increase colostral transfer of immunoglobulins to the pig as indicated by serum immunoglobulin concentrations at birth and at 2 and 4 wk of age.

Materials and Methods

Fifty-four crossbred sows (40 multiparous, 14 primiparous) from three farrowing groups (December 1985; February 1986; August 1986) were allotted randomly from parity outcome groups to four treatment groups consisting of single i.m. injections of either saline (control), 5 mg Se as sodium selenite (Se), 1,000 IU of E as dl-alpha-tocopherol or 5 mg Se plus 1,000 IU E (Se + E) administered on d 100 of gestation. During gestation, the sows were fed 1.8 kg/d of a corn-based gestation diet; upon farrowing they were switched to 5.7 kg/d of a lactation diet (Table 1). These diets were supplemented with .1 mg Se and 22 IU vitamin E as dl-alpha tocopherol acetate/kg. The total diet was calculated to contain .15 mg of Se and 45 IU of E/kg, which is at current NRC (1988) standards for Se and above NRC standards for E for gestating and lactating sows.

Jugular blood (20 ml) was taken from the sows prior to and 1 wk after treatment, at farrowing, and on d 14 and 28 of lactation. This sample was divided into aliquots of whole blood (4 ml), plasma (heparin, 143 USP units/8 ml) and serum (8 ml).

Beginning 1 d before expected farrowing, sows were checked every 2 h for signs of parturition. Only 28 sows that farrowed 13 to 15 d after treatment were used in the experiment (control, n = 11; Se, n = 9; E, n = 9; Se + E, n = 9). At parturition, colostrum was composited from glands from the right side of each sow. Blood samples (5 ml) were collected approximately 18 to 22 h after parturition on d 14 and on d 28 via jugular puncture from the first two healthy pigs born. Plasma or serum was obtained by centrifugation at 1,290 × g for 15 min; colostral samples were centrifuged under refrigeration at 1,290 × g for 15 min, and the fat-free colostrum was then centrifuged at 45,000 × g for 30 min and the whey (supernatant fluid) was retained. All samples were stored at −20°C until analysis. Selenium in whole blood was analyzed using a fluorometric procedure developed by Olson et al. (1975). Plasma vitamin E was measured by a modification (excitation wavelength 205 nm) of an HPLC procedure developed by Collins and Chow (1984).
Concentrations of IgA, IgM and IgG were measured in serum and colostrum from pigs and sows using the single radial immunodiffusion technique (SRID) developed by Fahey and McKelvey (1965) and Mancini et al. (1965). Porcine IgA, IgM and IgG antisera were diluted in a barbital buffer solution (pH 8.6). The dilutions were 1:5 for IgA, 1:10 for IgM and 1:40 for IgG. This diluted antiserum was mixed with 2% agarose in barbital buffer, and approximately 8 ml of this agar-antibody mixture was poured onto 98- x 45- x 7-mm polystyrene SRID plates. After solidification of the agar-antibody mixture, 17, 2-mm wells were cut into each plate. Duplicate 5-μl samples of serum or colostrum were added to the wells. Purified IgM, IgG and IgA standards also were prepared for each plate. The purity of these reagents was checked by radial immunodiffusion. No cross-reactivity was detected. The IgA and IgM plates were incubated (25°C in a humid chamber overnight and IgG was incubated for 8 h; diameters of immunoprecipitate rings were measured. If samples did not fit within the standard curves, they were diluted with saline and reassayed. All samples were assayed in duplicate. For colostral immunoglobulins, the intraassay CV was determined to be 8%, and the interassay CV was 13%. For serum immunoglobulins, the intraassay CV was 8%, and the interassay CV was 10%.

The data were analyzed with a repeated measures analysis (Winer, 1971) using the GLM procedure (SAS, 1985). Variables measured were not affected (P > .10) by farrowing period and there was no evidence of a treatment × trial interaction for any of the measurements; consequently, data were pooled across farrowing periods. The overall treatment changes were analyzed using a between-animal error term; the time and time × treatment interaction were analyzed using the within-animal error term. Because baseline values for the various parameters of the sows tended to be different for each treatment group, a profile analysis was performed on the variables comparing the change in the slopes of the treatment groups from one time point to the next time point. Assumptions of normality and homogeneity of variance were tested using the Discr ib procedure (SAS, 1985) as well as the Fmax test (Winer, 1971). If both of these assumptions could not be rejected, then a third assumption, that of compound symmetry, was tested using Mauchly’s test. If any of these assumptions were violated, a conservative one degree of freedom, Greenhouse-Geisser test (Winer, 1971) was performed on time and time × treatment effects. Individual comparisons between treatment groups were performed with nonorthogonal contrasts. Contrasts were designed to compare: C vs E; C vs E + Se; C vs Se; E vs Se; E vs E + Se; and Se vs E + Se. Analysis also was run with parity as a covariate for colostral immunoglobulins and with parity, litter number, size and birth weights as covariates for pig immunoglobulin levels.

Results and Discussion

Immune responses could be expected in this experiment only if the requirement for E and(or) Se for maximal immune response is greater than that required for normal growth and reproduction. Data from the sows were taken to provide information concerning their immune status and responsiveness of concentrations of E and Se in their blood to the injected nutrients. Colostrum was collected and analyzed because it is the primary vehicle for transfer of immunity from sows to pigs. Concentrations of IgA, IgG and IgM in blood from the pigs were selected for measurement as indicators of immune competency.

Selenium concentrations in whole blood of sows were not affected (P > .10) by treatment (Figure 1). However, there was a sharp decrease in blood Se concentrations at parturition that returned to baseline values at the 2-wk bleeding period. This could indicate an increased Se requirement as suggested by Smith and Picciano (1986) who made similar observations in rats, but this also could reflect a short-term Se loss during parturition.

Vitamin E levels are not reported in the present study because December and February samples were lost due to a freezer malfunction. However, E concentrations in sow plasma collected in the August farrowing paralleled blood Se levels. A similar reduction of E levels at parturition has been observed by Loudenslager et al. (1986).
The IgA, IgG and IgM concentrations in serum from sows are presented in Figures 2, 3 and 4, respectively. The sows presumably had well-developed immunoglobulin levels, and their blood concentrations were not consistently altered by treatment. Serum IgG concentrations were relatively constant before farrowing then increased modestly during lactation (Figure 2). Vitamin E, Se and E + Se administration resulted in less rapid increases \((P < .05; P < .01 \text{ and } P < .10, \text{ respectively})\) in serum IgA from parturition to wk 2 of lactation than in controls. Watson et al. (1981) found that high dietary intakes of E suppressed serum IgA levels while elevating intestinal IgA secretions in mice. Perhaps the slower increases in serum IgA levels in the sows also may be due to increased intestinal or mammary IgA secretions.

The most variable of the immunoglobulins in sow serum was IgG, which dropped sharply at parturition and rebounded precipitously during early lactation (Figure 3). Serum IgG concentrations declined more sharply \((P < .10)\) in sows injected with E + Se during the 1st wk after treatment than controls. The slight increase in IgG levels in the Se group compared with controls during the same time period was not significant \((P > .10)\). The IgM concentrations in serum from sows from 2 wk prepartum to 4 wk postpartum declined in all groups, but the decline was more rapid \((P < .10)\) in the E + Se group than in controls (Figure 4). In addition, Se injection reduced the downward
VITAMIN E AND Se UPON IMMUNOGLOBULIN TRANSFER

TABLE 2. IMMUNOGLOBULIN LEVELS IN SOW COLOSTRUM

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
<th>Control</th>
<th>Vit E</th>
<th>Se</th>
<th>Vit E + Se</th>
<th>SEM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>13.8</td>
<td>12.7</td>
<td>14.5</td>
<td>12.4</td>
<td>1.9</td>
</tr>
<tr>
<td>IgM</td>
<td>8.4</td>
<td>9.8</td>
<td>10.0</td>
<td>9.6</td>
<td>.8</td>
</tr>
<tr>
<td>IgG</td>
<td>54.0</td>
<td>63.9</td>
<td>64.3</td>
<td>64.1</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*Controls = 1 ml saline i.m.; Vit E = 1,000 IU of alpha-tocopherol i.m.; Se = 5 mg i.m.; Vit E + Se = 1,000 IU alpha-tocopherol + 5 mg Se i.m. All injections given d 100 of gestation.

Se vs control (P < .05).

**Pooled standard error of the mean.

Colostrum is the key to transfer of immunocompetency from the sows to their pigs. The increase (P < .05) in colostral IgM concentrations for sows injected with Se (Table 2) provides a basis for enhanced immune transfer. Responses to E or E + Se were not significant (P > .10), but the observed concentrations were intermediate between those for sows treated with Se and controls. Direct supplementation with E and Se had been reported to increase antibody production in both mice (Spallholz et al., 1975) and pigs (Peplowski et al., 1981). Colostral IgA and IgG concentrations were not affected (P > .10) by treatment, but their patterns followed the same trend as that observed for IgM. Although concentrations of immunoglobulins have been reported to increase with age of the sow (Klobasa and Butler, 1987), parity was not a significant (P > .10) covariate in the present experiment.

The final test for immune transfer is based on evaluation of immunoglobulin concentrations in pig serum. Serum IgA concentrations in 20-h-old pigs were not affected (P > .10) by treatment and were not detectable in our system on d 14 and 28. This is consistent with the lack of an IgA response in colostrum. Although the IgM concentrations in pig serum declined rapidly from 20 h after birth until the end of the experiment (Figure 5), pigs from treated sows consistently had higher concentrations than those from controls sows. Injection of E, Se and E + Se increased (P < .01, P < .10 and P < .10, respectively) serum IgM concentrations over controls at 20 h postpartum. Serum IgM concentrations were higher (P < .05) on d 14 in pigs from sows receiving Se and higher on d 14 (P < .10) and 28 (P < .05) in pigs from sows given E + Se (P < .10) than in control sows. Serum IgM concentrations tended (P > .10) to be higher on both d 14 and 28 in pigs from sows given E injections compared with controls. Vitamin E treatment resulted in a steeper decline (P < .10) in serum IgM concentrations from birth to d 14 compared with controls.

Whereas serum IgG concentrations in pigs at 20 h postpartum were not affected (P > .10) by treatment, serum IgG concentrations at d 14 were elevated by E (P < .05), Se (P < .01) and E + Se (P < .05) (Figure 6). However,
treatment differences were no longer detectable ($P > .10$) on d 28. Declines in serum IgG concentrations from birth to d 14 were lower for the Se ($P < .10$) and E + Se ($P < .01$) treatments than for controls. In contrast, from d 14 to 28, serum IgG concentrations declined more sharply in pigs from sows given E ($P < .05$), Se ($P < .05$) and E + Se ($P < .01$) than in those from control sows. As for the IgM responses, the IgG responses in pigs were more dramatic than those observed in colostrum. Pig IgG also was the only parameter in which there was a time × treatment interaction ($P < .05$).

Because parity, litter size and birth weights have been suggested as factors that influence the immune response of pigs (Klobasa and Butler, 1987; Warner et al., 1987), serum immunoglobulins were analyzed using these parameters as covariates. Litter size was a significant covariate for IgM ($P < .05$) but did not alter the probability of detecting treatment differences when added to the model. Immunoglobulin concentrations in serum from sows did not appear to correspond meaningfully with colostral immunoglobulin changes. However, because significant amounts of IgM are produced locally by the mammary gland (Bourne and Curtis, 1973), serum IgM levels may not be indicative of total IgM synthesis. Furthermore, this may indicate effects of Se and E on IgM synthesis in the mammary gland. Increased colostral IgM levels translated into higher IgM levels in serum of the 20-h pig. Additionally, elevated serum IgM levels at birth appeared to correspond with higher serum IgM and IgG levels in the pigs during the suckling period (birth to d 28). The reason for the higher IgG levels on d 14 may be similar to observations noted by Klobasa et al. (1981). In their study, oral administration of 3.5 g of IgG at birth resulted in increased synthesis not only of IgG but also of IgM and IgA. Perhaps the slightly higher absorption of IgM increased IgG synthesis in these pigs by d 14, but these IgM levels were not high enough to sustain these influences throughout the suckling period.

Elevation of immunoglobulin levels in pig serum could be very beneficial during the suckling period. Factors that influence the ability of the pig to obtain or absorb colostrum or alter blood immunoglobulin levels may markedly affect the survivability of the pig (Leece, 1971; McCallum et al., 1977; Hendrix et al., 1978). As suggested by Hendrix et al.
(1978), increased mortality rates observed for pigs of low birth weight may reflect, in part, reduced colostrum intake resulting from competition with larger littermates at parturition. Hence, by increasing the immunoglobulin content of colostrum and the ultimate quantity of immunoglobulins consumed by the pig, factors increasing the requirement for or reducing the absorption of immunoglobulins may be compensated partially.

These data indicate that supplementation of E and/or Se as single injections can augment the transfer of immunity to pigs from sows fed levels of E and Se that approximate the sow’s requirement (NRC, 1988). Supplements of E or Se in excess of the requirements also have been reported to improve immune status of mice (Tengerdy et al., 1973; Heinzerling et al., 1974), lambs (Stephens et al., 1979), chickens (Tengerdy et al., 1972), cattle (Smith et al., 1984) and pigs (Ellis and Vorhies, 1976). This suggests that current estimates of the requirements for these nutrients for normal growth and development may be too low for optimum immune function. Further studies are needed to ascertain the relationship of E and Se to the immune system and to establish the levels of E and Se supplementation that optimize immune function.

**Literature Cited**


