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# Effects of Dietary Zinc and Endotoxin Challenge on Immune Function in Weanling Pigs

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## Story in Brief

Twenty-four pigs were weaned at 14 d (4.5 kg BW), individually housed in plastic metabolic cages, and randomly assigned to two dietary Zn concentrations. Dietary treatments consisted of a basal diet supplemented with 0 or 3000 ppm zinc as ZnSO4. The basal diet was a commercially prepared milk replacer. Dietary treatments were mixed with distilled water (12 g diet/100 mL water) and fed to the pigs at 8 a.m., 12 p.m., 5 p.m., and midnight. After 2 d on the basal diet, pigs were fed the experimental diets for 14 d. On d 14, pigs were deprived of food for 10 h and injected intraperitoneally with saline or 25 g/kg body weight endotoxin (Lipopolysaccharide from E.coli sterotype 0111:B4). Rectal body temperature measurements and blood were collected before (0 h) and at 1.5, 3, 6 and 24 h post injection (PI). In general, pigs fed the diet containing 0 ppm Zn had lower white blood cell counts, cortisol, creatinine, urea nitrogen, and triglycerides than pigs fed 3000 ppm Zn. Concentrations of C-reactive protein, albumin, and total protein were greater in pigs fed 0 ppm zinc vs pigs fed 3000 ppm. Endotoxin challenge increased body temperature, serum triglycerides, creatinine, C-reactive protein, and white blood cells. The results indicate that supplementation of 3000 ppm zinc/kg diet to the weanling pigs appeared to be beneficial in protecting against infection induced by the E.Coli endotoxin.


Key Words: Pigs, Zinc, Endotoxin, Immunity, Temperature

## Introduction

Feeding 2000 to 3000 ppm zinc as ZnO has been shown to increase the performance of weanling pigs (Hahn and Baker, 1993; Mahan et al., 2000). However, the mechanism responsible for the increase in growth performance is not known. Zinc is known to play a central role in growth, the immune system, and sexual maturity. As early as in the 1960s, studies on zinc deficiency in pigs reported a reduction in the size of the thymus, the central organ for T lymphocyte development (Miller et al., 1968; Shanklin et al., 1968). Limited research is available on the effects of pharmocological concentrations of zinc fed to the early weaned pigs on immune function.

Endotoxin lipopolysaccharide (LPS), the principle component of gram negative bacteria, is the major contributing factor to the pathogenesis of bacterial infections. Lymphocyte immuno-suppression was observed in pigs infected with *Salmonella Chloraesus* (Gray et al., 1996). Disease stress causes profound metabolic changes, characterized by shifts in nutrient use away from growth process towards support of immune system function. Decreased growth performance has been observed in pigs injected with *Escherichia coli lipopolysaccharide* (van Heugten et al., 1996; van Heugten et al., 1994). Body temperature and plasma cortisol increase, and cytokines such as TNF- and IL-6 are secreted by the macrophages and monocytes in response to endotoxin challenge (Parrot et al., 1997; Roth et al., 1994). These cytokines together with cortisol not only induce acute phase protein response such as C-reactive proteins, but also hepatic metabolism which could alter serum chemistry.

The purpose of this study was to investigate the effects of deficient and excess levels of dietary zinc on clinical chemistry parameters and immune function in endotoxin-challenged

weanling pigs.

## Materials and Methods

Twenty-four weanling boar pigs, 14 d old, were assigned randomly to four dietary treatments in a randomized complete block design. Dietary treatments consisted of two concentrations of dietary zinc (0 vs 3000 ppm). The diets were prepared by addition of trace minerals to a commercially prepared milk replacer containing no added trace mineral source. Diets were mixed before every feeding with 12 g of diet in every 100 ml of de-ionized water. Pigs were fed four times a day; 8:00 a.m., 12:00 p.m., 5:00 p.m., and 12:00 a.m. The amounts fed increased daily from 200 to 1200 ml by d 16 of the treatment period. Pigs were housed individually in metabolism chambers (.75 x 1.0 m) with plastic coated wire mesh floors. Adaptation diet (no added zinc) was fed for 2 d followed by the experimental diets for 14 d. On d 14 of the experiment, the pigs were injected intraperitoneally with either saline or endotoxin (*E.Coli*, 0111B:4) in a saline carrier (25 ug LPS / kg BW). Blood was collected from the jugular vein and rectal body temperature was recorded before (0 h) and at 1.5, 3, 6 and 24 h post injection. Blood was collected in three vaccutainers with different anticoagulants to perform the appropriate lab tests for each time period.

***Analysis***. Whole blood was analyzed for complete blood counts. Plasma cortisol was analyzed by RIA. Serum was analyzed for creatinine, glucose, urea nitrogen, albumin, total protein, and triglycerides using Roche Reagents.

***Statistical Analysis****:* Data were analyzed as 2 x 2 factorial array in a randomized complete block design using analysis of variance procedures (Steel et al., 1997). Orthogonal contrasts were used to test the effects of zinc (0 vs 3000 ppm), endotoxin challenge (0 vs 25 ug/kg), and the zinc x endotoxin interaction. Pig was considered the experimental unit.

## Results and Discussion

***Clinical Chemistry***: At time 0 h, serum albumin was increased (P<.10) and serum urea nitrogen and creatinine were decreased (P<.10) in pigs fed 0 ppm Zn compared to pigs fed 3000 ppm (Table 1). Triglycerides and total protein were numerically greater in pigs fed 3000 ppm vs 0 ppm Zn; however these differences were not significant (P<.10). Plasma urea nitrogen remained higher (P<.10) in pigs fed 3000 ppm vs pigs fed 0 ppm zinc throughout the blood sampling period.

At 6 h post-injection, endotoxin (P<.10) increased serum creatinine and hematocrit. Higher concentrations (P<.10) of serum triglycerides and serum urea nitrogen were observed in pigs fed 3000 ppm Zn at 6 h post injection (PI). No effects (P<.10) of diet or endotoxin were observed on albumin, glucose, total protein, or hemoglobin at 6 h PI (Table 1).

At 24 h PI, a diet x endotoxin interaction (P<.10) was observed for creatinine and total protein (Table 1). A decrease (P<.10) in serum glucose and urea nitrogen were observed with endotoxin and diet at 24 h PI. An increase (P<.10) in serum triglycerides was observed with endotoxin challenge. Albumin, hemoglobin and hematocrit did not differ significantly in the treatment groups at 24 h PI.

***Immune Response***: Endotoxin challenge increased (P<.10) body temperature at 1.5, 3, and 6 h PI (Table 2). An endotoxin x diet interaction (P<.10) was observed for temperature (Table 2) at 6 h PI. A similar trend was observed at 0 and 24 h PI, but no statistical difference was observed at this time period between the treatment groups. In concert with the increase in temperature, endotoxin challenge increased (P<.10) white blood cells at 1.5,

3, and 6 h PI. The increase in white blood cells with endotoxin challenge tended to be greater in pigs fed 0 ppm zinc compared with pigs fed 3000 ppm at 3 h PI.

Cortisol was lower (P<.05) in pigs fed 0 ppm compared to pigs fed 3000 ppm zinc at 0 h. Endotoxin challenge increased plasma cortisol numerically at 3 h PI. C-reactive protein (CRP), an acute phase protein, was reduced (P<.01) in pigs fed 3000 ppm zinc compared with pigs fed 0 ppm at 3 h PI. At 6 h PI, CRP concentrations were similar (P>.10) across treatments. At 24 h PI, pigs fed 0 ppm Zn had greater (P<.10) CRP concentrations than pigs fed 3000 ppm. Also, CRP was increased (P<.10) in pigs given endotoxin compared with pigs given saline.

These results suggest that dietary zinc may influence the response of weanling pigs to a disease challenge. At 3 h PI, the increase in white blood cells, cortisol, and CRP associated with endotoxin challenge was less pronounced in pigs fed 3000 ppm compared with pigs fed 0 ppm zinc. At 6 h PI, the concentrations in white blood cells, cortisol, and CRP were not different in pigs fed 0 ppm or 3000 ppm Zn; however, pigs fed 3000 ppm Zn fed had lower concentrations at 0 h. Thus, the increases in growth performance observed in pigs fed 2000-3000 ppm zinc may, to some degree, be associated with a lowered, or more efficient, response to disease challenge.

A better response, that is a resistance to the effects of endotoxin, as shown by lowered white blood cells, cortisol, and CRP, may allow more nutrients to be available for growth processes. Higher white blood cells, CRP, and albumin in the pigs fed 0 ppm zinc may indicate an elevated response to stress as compared to pig fed 3000 ppm zinc. These data suggest that feeding 3000 ppm zinc was not detrimental and indeed, appeared to be beneficial in weanling pigs.

## Implications

The observed response to dietary zinc supplementation and subsequent endotoxin-induced immune challenge in weanling pigs are intriguing. Pigs fed 3000 ppm Zn tended to have lower concentrations of acute phase proteins and white blood cells as compared with pigs fed 0 ppm Zn. Also, a delayed response to endotoxin challenge was observed in pigs fed 3000 ppm Zn. These results may help to explain the beneficial effects of pharmacological concentrations of zinc on the growth performance of weanling pigs.

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**Table 1. Effect of dietary zinc and endotoxin treatment on serum metabolites in weanling pigs[a].**

| | | Added zinc, ppm | | | | |
|---|---|---|---|---|---|---|
| | | 0 | | 3000 | | |
| | | Endotoxin (0 vs 25 ug/kg) | | | | |
| Item | Time, h | - | + | - | + | SE |
| Creatinine | 0[b] | 1.06 | 1.23 | 1.26 | 1.75 | .66 |
| mg/dL | 6[c] | 1.25 | 1.31 | 1.28 | 1.42 | .56 |
| | 24[d] | 1.10 | 1.21 | 1.27 | 1.45 | .70 |
| | | | | | | |
| Glucose | 0 | 110.3 | 111.5 | 110.5 | 110.8 | 8.17 |
| mg/dL | 6 | 116.0 | 108.5 | 93.7 | 105.8 | 7.27 |
| | 24[bc] | 125.6 | 116.2 | 115.0 | 99.5 | 6.38 |
| | | | | | | |
| Urea | 0 [b] | 7.80 | 9.96 | 12.88 | 15.41 | 2.03 |
| Nitrogen | 6 [b] | 10.68 | 10.06 | 14.16 | 13.63 | 1.75 |
| mg/dL | 24 [b] | 6.28 | 8.98 | 14.31 | 12.50 | 1.68 |
| | | | | | | |
| Albumin | 0[b] | 3.26 | 3.63 | 2.90 | 3.18 | .15 |
| mg/dL | 6 | 3.18 | 3.23 | 3.03 | 2.95 | .14 |
| | 24 | 3.25 | 3.21 | 2.82 | 3.21 | .21 |
| | | | | | | |
| Protein | 0 | 5.25 | 5.86 | 5.02 | 5.20 | .27 |
| mg/dL | 6 | 4.98 | 5.10 | 4.77 | 4.93 | .13 |
| | 24[d] | 4.98 | 5.21 | 5.28 | 4.46 | .22 |
| | | | | | | |
| Trigly- | 0 | 29.83 | 34.83 | 32.83 | 42.83 | 4.54 |
| cerides | 6[b] | 32.50 | 32.90 | 45.16 | 43.16 | 4.75 |
| mg/dL[e] | 24[c] | 22.16 | 43.00 | 32.60 | 39.56 | 7.59 |
| | | | | | | |
| Hemo- | 0 | 11.30 | 12.10 | 10.76 | 11.56 | .40 |

| | | | | | | |
|---|---|---|---|---|---|---|
| globin | 6 | 10.51 | 10.85 | 9.33 | 10.33 | .48 |
| g/dL | 24 | 9.53 | 10.06 | 9.14 | 9.28 | .41 |
| | | | | | | |
| Hematocrit | 0 | 33.04 | 35.14 | 30.76 | 33.60 | 1.02 |
| % | 6[c] | 29.94 | 31.82 | 26.27 | 30.30 | 1.39 |
| | 24 | 27.80 | 29.02 | 26.25 | 26.84 | 1.27 |

[a]Least squares means of six pigs per treatment combination.

[b]Diet effect (P<.10).

[c]Endotoxin effect (P<.10).

[d]Endotoxin x diet effect (P<.10).

[e]Endotoxin effect (P<0.10) over all time periods.

**Table 2. Effect of dietary zinc and endotoxin treatment on immune function in weanling pigs[a].**

| | | Added zinc, ppm | | | | |
|---|---|---|---|---|---|---|
| | | 0 | | 3000 | | |
| | | Endotoxin (0 vs 25 ug/kg) | | | | |
| Item | Time, h | - | + | - | + | SE |
| Temper- | 0 | 102.0 | 101.9 | 101.9 | 101.7 | .33 |
| ature | 1.5[c] | 102.0 | 102.5 | 101.2 | 103.0 | .43 |
| $^0$F | 3.0[c] | 102.0 | 103.7 | 101.1 | 103.8 | .38 |
| | 6.0[cd] | 102.1 | 103.2 | 101.2 | 103.8 | .46 |
| | 24.0 | 102.1 | 102.1 | 101.4 | 101.9 | |
| | | | | | | |
| Cortisol | 0 [b] | 107.0 | 99.0 | 160.5 | 124.5 | 16.9 |
| nmole/L | 3.0 | 189.0 | 409.0 | 249.9 | 208.1 | 118.0 |
| | 6.0 | 198.0 | 207.0 | 205.5 | 218.8 | 45.1 |
| | 24.0 | 124.0 | 95.0 | 79.5 | 108.2 | 18.7 |
| | | | | | | |
| C-Reactive | 0 [b] | 6.62 | 7.06 | 2.70 | 2.89 | .83 |
| protein | 6.0 | 4.42 | 5.67 | 4.53 | 5.67 | 1.01 |
| mg/dL | 24.0[bc] | 5.19 | 8.97 | 2.22 | 5.45 | .66 |
| | | | | | | |
| White | 0 | 15.86 | 16.03 | 15.29 | 14.61 | 1.9 |
| blood cells | 1.5[c] | 15.78 | 12.93 | 14.78 | 10.86 | 1.8 |
| $10^3$ cells/ | 3.0[c] | 14.57 | 22.17 | 12.72 | 14.76 | 2.5 |
| $mm^3$ | 6.0[c] | 14.10 | 25.96 | 13.50 | 24.93 | 3.1 |
| | 24.0 | 13.40 | 15.40 | 13.90 | 14.50 | |

[a]Least squares means of six pigs per treatment combination.

[b]Diet effect (P<.10).

[c]Endotoxin effect (P<.10).

[d]Diet x endotoxin (P<.10).

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