ORIGINAL RESEARCH

Huang SX, McFall M, Cegielski AC, et al. Effect of dietary zinc supplementation on *Escherichia coli* septicemia in weaned pigs. *Swine Health Prod.* 1999;7(3):109–111.

Effect of dietary zinc supplementation on *Escherichia coli* septicemia in weaned pigs

Suxi X. Huang, PhD; Margaret McFall; Artur C. Cegielski, DVM; Roy N. Kirkwood DVM, PhD

Summary

Objective: To determine the effect of 3000 ppm dietary zinc oxide (ZnO) on bacterial counts in the mesenteric lymph nodes and systemic circulation.

Methods: Eighteen piglets were fed a commercial starter diet with (ZnO treatment) or without (controls) supplementation of 3000 ppm zinc oxide. After a 6-day adaptation to the diets, the pigs were intramuscularly injected with lipopolysaccharide (LPS) (derived from E. coli 026:B6) at 150 µg per kg bodyweight. The pigs were monitored throughout the experimental period. Twenty-four hours after LPS injection, pigs were euthanized and samples were collected.

Results: Bacteria could be cultured from ileal mesenteric lymph nodes in fewer ZnO-treated (three of nine) than control (eight of nine) piglets (P<.05). In ZnO-treated pigs, there tended to be fewer colony forming units (CFU) of bacteria per g of tissue from the ileal mesenteric lymph nodes than in the pigs fed the control diet (P<.1). Treatment had no effect on the species of bacteria cultured. Treatment had no effect on bacteremia.

Implications: Supplementing starter diets with 3000 ppm ZnO reduced the level of bacterial translocation from the small intestine to the ileal mesenteric lymph node. However, the mechanism whereby this effect was achieved could not be determined.

Keywords: pigs, zinc, *Escherichia coli* septicemia

Received: September 23, 1998
Accepted: December 4, 1998

Acute postweaning mortality due to toxigenic *Escherichia coli* is emerging as a more common disease problem in high-health swine herds. Health technologies such as segregated early weaning will not eliminate *E. coli* disease and it remains a significant cause of mortality in the nursing and early grower phases of production (Winkelman. *AD Leman Swine Conf Proc*. 1995; 22:59–60). *Escherichia coli* toxemia disrupts the intestinal mucosal barrier,[1,2] potentially increasing the opportunity for bacteria to translocate from the intestines to the blood and internal organs, causing septicemia.

Dietary supplementation with 3000 ppm zinc oxide (ZnO) has been reported to reduce the prevalence of postweaning diarrhea and mortality attributed to toxigenic *E. coli* infection in piglets, although the mechanisms involved are not fully understood. Concentrations of 3000 ppm dietary zinc, while very high, are reported to have no toxic effect in pigs.[1] The objective of this study was to determine whether high concentrations of dietary zinc would protect against septicemia due to toxigenic *E. coli*.

Materials and methods

Animals

Eighteen piglets (Camborough × Canabrid), weaned at 21 days of age without access to creep feed, were obtained from the University of Alberta swine unit. Pigs were housed in pairs in metabolism crates, with the room temperature controlled between 28–30°C. The piglets were fed a commercial starter diet ad libitum, formulated to provide 149 ppm zinc (as fed). Half of the piglets (n = 9) were randomly allocated into a ZnO-treated group. The diet of the ZnO-treated group was supplemented with 3000 ppm ZnO. The other nine pigs served as controls. The pigs were visually monitored throughout the experimental period at hourly intervals to ensure that animal welfare was not unduly compromised.

After adapting to the diets for 6 days, pigs were intramuscularly (IM) injected with lipopolysaccharide (LPS from *E. coli* 026:B6, Sigma, St. Louis, Missouri) at 150 mg per kg bodyweight. The LPS was dissolved in saline to a concentration of 1.5 mg per mL.

Sampling

All samples were collected 24 hours after the injection. The pigs were anesthetized under halothane in a biological containment hood, and blood was obtained aseptically by cardiac puncture. The abdominal area was disinfected and a laparotomy performed. The ileal mesenteric lymph nodes and those corresponding to the large intestines were removed and samples of liver and spleen were obtained. All specimens were put in sterile containers and immediately transported to the laboratory.

For lymph node, liver, and spleen samples, 1 g of tissue was placed into a 50-mL sterile centrifuge tube containing 4 mL of sterile PBS and homogenized for 10 seconds. We pipetted 0.2 mL of homogenized sample onto each of five blood agar plates (BAPs) and spread for confluent growth. Also, 1 mL of the sample was pipetted into tryptic

SXH, MM, ACC: Alberta Pork Research Centre, AAFRD. Reprint requests to RNK: Farm Animal and Equine Medicine and Surgery, Royal Veterinary College, Hawkshead Lane, Hatfield, Herts AL9 7TA England; rkirkwoo@rvc.ac.uk

This article is available online at http://www.aasp.org/shap.html.

soya broth (TSB). Three BAPs and the TSB were incubated aerobically at 35°C in an O_2 incubator for 24 hours. Two BAPs were incubated anaerobically at 35°C for 48 hours.

For mesenteric lymph nodes, a 1:10 dilution was also performed by transferring 1 mL of homogenized sample into 9 mL of PBS for five dilutions. One tenth mL of each dilution was pipetted onto four BAPs and spread for confluent growth. Two BAPs from each dilution were incubated aerobically at 35°C for 24 hours and the other two anaerobically for 48 hours. Colony counts were performed on aerobic plates at 24 and 48 hours and anaerobic plates at 48 hours.

For blood samples, 0.2 mL of blood was pipetted onto five BAPs and spread for confluent growth, and 1 mL into TSB.

Statistical analysis

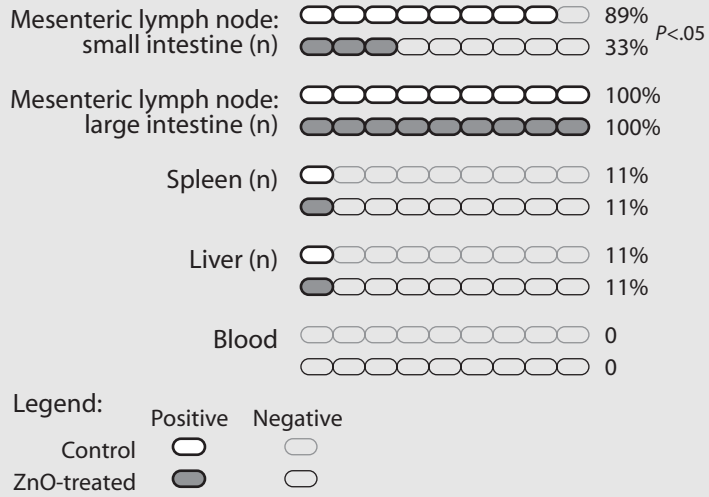
Treatment effects on the number of pigs in which bacteria could be cultured from samples were analyzed by the Fisher's exact test, and numbers of colony-forming units (CFUs) compared by t-test (Number Cruncher Statistical System, Kaysville, Utah).

Results

Bacteria were cultured from ileal lymph nodes of fewer ($P<.05$) of the pigs fed supplemental ZnO (three of nine) than control pigs (eight of nine) (Figure 1). The numbers of bacteria in the ileal lymph nodes tended to be lower in ZnO-treated pigs (15±7 CFU per g of tissue; range: 6–29) than in control pigs (387±194 CFU per g of tissue, range: 5–1500) ($P<.1$). There was no treatment effect on the numbers of bacteria in mesenteric lymph nodes corresponding to the large intestine, or to the spleen and liver. No bacteria were cultured from the blood.

The bacteria cultured from the lymph nodes associated with the small and large intestines were primarily *E. coli* and *Enterococcus* spp. (Table 1). No anaerobes were isolated from the ileal lymph nodes, but *Bacteroides* spp. and *Clostridium* spp. were isolated from the lymph nodes of the large intestine from one pig and anaerobic Gram-positive bacilli from another pig.

Figure 1



Influence of dietary zinc on the incidence of enteric bacterial translocation and numbers of bacteria translocating in pigs

Table 1

Numbers of pigs (n=18) with translocated bacteria in mesenteric lymph nodes, liver, and spleen

	MLN-S		MLN-L		Liver		Spleen	
Treatment	C	ZnO	C	ZnO	C	ZnO	C	ZnO
Aerobes:								
E. coli	5	2	9	9	1	0	0	0
Enterococcus spp.	2	1	2	3	1	0	1	0
Diphtheroids	1	0	0	1	0	0	0	0
Pseudomonas spp.	0	0	2	0	0	0	0	0
Viridans grp *Streptococcus*	1	0	0	1	0	0	1	1
Staphylococcus	0	0	0	1	0	0	0	1
Streptococcus	0	0	0	0	0	1	0	0
Anaerobes:								
Bacteroides spp.	0	0	0	1	0	1	0	0
Clostridium spp.	0	0	0	1	0	1	0	0
Gram-positive bacilli	0	0	0	1	0	0	0	0

MLN-S Mesenteric lymph node corresponding to small intestine
MLN-L Mesenteric lymph node corresponding to large intestine
C pigs fed a commercial starter diet
ZnO pigs fed the commercial starter diet supplemented with 3000 ppm ZnO

Discussion

The present study demonstrated that supplementing the nursery diet with 3000 ppm ZnO reduced bacterial translocation from the small intestine to the corresponding lymph nodes in weaned pigs. However, the mechanism involved in achieving this effect was not determined. It has been postulated that intestinal villus atrophy, such as occurs at weaning, may lead to increased intestinal permeability and systemic infection secondary to bacterial translocation.[3] Therefore, it is possible that the effect of zinc was mediated by an improvement in the structural and/or immunologic integrity of the mucosal barrier. Alternatively, since bacterial translocation is also promoted by a disruption of

the enteric microflora populations, it is possible that the effect of zinc was mediated by changes in microfloral populations (Melin, et al. *Proc 14th IPVS Cong*, 1996; 465).

The lack of an observable treatment effect on the percentage of pigs from which bacteria was cultured from the mesenteric lymph nodes associated with the large intestine is consistent with the observation of Steffen and Berg,[4] who observed a direct relationship between the numbers of *E. coli, Proteus mirabilis*, and *Klebsiella pneumoniae* in the cecum and the numbers of these strains translocating to the mesenteric lymph node complex. Steffen and Berg suggested that the population size could be a factor promoting bacterial translocation. As the bacterial population is much larger in the large intestine than in the small intestine, it is possible that the large population of bacteria might have overwhelmed the effect of ZnO in the large intestine in the present study. More research is required to investigate these mechanisms.

Zinc has a primary effect on tissues with a high turnover rate, as in those of the gastrointestinal tract and immune system. Zinc is needed in these tissues for DNA and protein synthesis. Zinc stabilizes the membrane structure and may modify membrane functions,[5] protects membranes from the effects of infectious agents,[3] and may act at the tight junction to prevent the increase of intestinal permeability associated with malnutrition or tumor necrosis factor α.[6,7] Zinc may also stimulate immune defenses while minimizing the adverse effect of immune cell activation by bacterial translocation on the epithelial layer.[8] In weaned pigs, the feeding of high concentrations of zinc oxide (3000 ppm) has been shown to have efficacy in controlling *E. coli* diarrhea[1] and for increasing their daily voluntary feed intake and weight.[9] The growth response to dietary zinc concentrations that are 20 times higher than the normal requirement implies that the function of zinc is more than a simple nutritional need.

Implications

- Supplementing the starter diet with 3000 ppm zinc oxide for pigs during the first week after weaning may reduce the numbers of bacteria reaching the ileal mesenteric lymph nodes.
- The model used in this study did not result in septicemia. Further work is required to understand how ZnO reduces losses due to postweaning *E. coli* infection.

Acknowledgments

We are very grateful to Shirley Rawluk, Carol Goertz, Arlene Otto, Louise Hawker, and Vicki Maitland for their technical support, and our students Averall Forbes and Tara Foy for their assistance in the management of this study, and to the Alberta Pork Producers Development Corporation and the Alberta Agricultural Research Institute for financial support.

References

1. Holm A, Poulsen HD. Zinc oxide in treating *E. coli* diarrhea in pigs after weaning. *Compend Cont Ed Prac Vet*. 1996; 18:S26-S29.

2. Deitch EA, Berg G, Specian R. Endotoxin promotes the translocation of bacteria from the gut. *Arch Surg*. 1987; 122:185–190.

3. Mahadevan D, Ndirika A, Vincent J, Bashford L, Chambers T, Pasternak C. Protection against membrane-mediated cytotoxicity by calcium and zinc. *Am J Pathol*. 1990; 136:513–520.

4. Steffen EK, Berg RD. Relationship between cecal population levels of indigenous bacteria and translocation to the mesenteric lymph nodes. *Infect Immun*. 1983; 39:1252–1259.

5. Bray TM, Bettger WJ. The physiological role of zinc as an antioxidant. *Free Radic Biol Med*. 1990; 8:281–291.

6. Rodriguez P, Darmon N, Chappuis P, Candalh C, Blaton MA, Bouchaud C, Heyman M. Intestinal paracellular permeability during malnutrition in guinea pigs: Effect of high dietary zinc. *Gut*. 1996; 39:416–422.

7. Hennig B, Wang Y, Ramasamy S, McClain CJ. Zinc protects against tumor necrosis factor-induced disruption of porcine endothelial cell monolayer integrity. *J Nutr*. 1993; 123:1003–1009.

8. King LE, Osati-Ashtiani F, Fraker PJ. Depletion of cells of the B lineage in the bone marrow of zinc-deficient mice. *Immunology*. 1995; 85:69–73.

9. Hahn JD, Baker DH. Growth and plasma zinc response of young pigs fed pharmacologic levels of zinc. *J Anim Sci.* 1993; 71:3020–3024.