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Reduced Severity of Disease Associated With Feeding a Pharmacologic Amount of Zinc in a Laboratory Mouse Model of Swine Dysentery

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Swine dysentery is a highly contagious diarrheal disease of growing and finishing pigs which continues to cost an estimated $115.2 million to the United States' pork producers each year. The disease is caused by the spiral-shaped bacterium, *Serpulina (Treponema) hyodysenteriae* and is characterized by severe bloody diarrhea, reduced weight gain and death of susceptible pigs. When introduced in an uninfected herd, the disease quickly becomes established, requiring continuous medication at a cost of more than $8.00 per pig going to market. Although the cause of the disease has been known since the early 1970s, disease control strategies have essentially remained the same; medication of animals with expensive residue-causing antimicrobials and sanitation of premises.

Serpulina hyodysenteriae produces a toxin capable of destroying red blood cells and killing white blood cells that are involved in the pig's immune defense. Production of intestinal damage in animals inoculated with partially-purified toxin suggests that the toxin is involved in the disease. We have shown previously that the production of the toxin by *S. hyodysenteriae*

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can be greatly reduced or completely eliminated by adding various amounts of zinc sulfate ($ZnSO_4$) to culture medium used in the laboratory. Based on that result, we hypothesized that dietary zinc compounds could affect the severity of intestinal infection by *S. hyodysenteriae*. Indeed, a group of researchers had noted reduced severity of dysentery in swine fed zinc-supplemented diets, but the benefit of this approach was not evaluated under well-controlled laboratory conditions. The purpose of the present investigation was to assess the prophylactic effect of a pharmacologic amount of different, feed-grade zinc compounds on infection and production of intestinal damage in laboratory mice inoculated with *S. hyodysenteriae*. Laboratory mice were chosen because they have been extensively used as a model to evaluate intestinal damage caused by *S. hyodysenteriae* infection.

Study Design

A basal diet with or without added zinc oxide (ZnO), $ZnSO_4$, or Zn-methionine to a final concentration of approximately 6,000 ppm of Zn^{2+} was fed to a total of 156 mice randomly allocated to 4 treatment groups consisting of 39 mice each. The control and the zinc-supplemented diets were fed for 10 days before oral inoculation of half of the mice in each group either with *S. hyodysenteriae* or sterile medium. Diets were continued for 42 days, while at weekly intervals, the body weights, liver zinc concentrations, presence of *S. hyodysenteriae* in the intestines, and assessment of intestinal damage were determined in 3 mice per group.

Procedures and Statistical Analysis

The concentrations of Zn^{2+} in each feed formulation and in each mouse liver were determined using inductively coupled argon plasma atomic emission spectrometry (ICAP-AES). Results were reported as means $\pm$ one standard error of the mean (SEM) for each feed formulation and for mice from the same treatment group examined on the same post-inoculation day (PID). After determining the body weight of each mouse, a portion of the intestines was processed for histological examination and determination of the length of the intestinal crypts (longitudinal crypt length = LCL).

From these measurements, the mean $\pm$ SEM of body weights and LCL of mice from the same treatment group examined on the same PID were calculated. To determine if supplementation of the basal diet with each zinc compound had an effect on the growth of the mice and the development of intestinal damage associated with *S. hyodysenteriae* infection, the means of the body weights and LCL of mice fed with the basal diet and with the basal diet supplemented with each zinc compound and inoculated either with *S. hyodysenteriae* or medium were subjected to analysis of variance for each weekly sampling from PID 0 to 42. Differences between groups were considered significant if the *P* value of the statistical test was less than 0.05 ($P < 0.05$).

Results

The basal diet contained 42.5 ppm of Zn^{2+}. The mean concentrations of Zn^{2+} were 6,010 $\pm$ 509.8, 6,075 $\pm$ 123.7, and 6,135 $\pm$ 384.7 ppm in the basal diets supplemented with ZnO, $ZnSO_4$, and Zn-methionine, respectively. From PID 0 to 42, the liver zinc concentrations of mice fed the zinc-supplemented diets were approximately twice that of the mice fed the basal diet, irrespective of the source of zinc. Although no significant difference in the mean body weight gain (BWG) was found between mice in the *S. hyodysenteriae*- and the medium-inoculated groups fed with the same diet, a statistically significant difference in the mean BWG was found between mice fed with different diets ($P < 0.05$). The mean BWG of mice fed the basal diet from day 0 to 42 was 9.0 $\pm$ 0.9 g compared with 4.8 $\pm$ 0.6 g and 3.0 $\pm$ 0.3 g for mice fed the basal diet supplemented with Zn-methionine and ZnO, respectively. The mean body weight of mice fed the basal diet supplemented with $ZnSO_4$ did not change between day 0 and day 42 of the experiment. Overall, the mice fed the basal diet had significantly greater mean BWG than did mice fed the zinc-supplemented diets ($P < 0.05$). From PID 7 through 42, *S. hyodysenteriae* was isolated from the intestines of 77.8% of the infected mice fed the basal diet. In contrast, *S. hyodysenteriae* was not isolated from any of the *S. hyodysenteriae*-inoculated mice fed the diets supplemented with either ZnO or Zn-methionine. Of the *S. hyodysenteriae*-inoculated mice fed the $ZnSO_4$-supplemented diet, *S. hyodysenteriae* was isolated from the intestines of 3 of 3 mice examined on PID 7. The overall percentage of *S. hyodysenteriae* isolation from the $ZnSO_4$-supplemented group from PID 7 through 42 was 16.7%.

On PID 14 and 21, mice inoculated with *S. hyodysenteriae* and fed the basal diet had intestinal damage typical of *S. hyodysenteriae* infection. On PID 14, no intestinal damage was present in 3 of 3 mice fed the ZnO-, 2 of 3 mice fed the $ZnSO_4$-, and 1 of 3 mice fed the Zn-methionine-supplemented diet and inoculated with *S. hyodysenteriae*. The remaining mice had mild to moderate intestinal damage similar to that present in mice fed the basal diet. No significant changes were present in the intestines obtained from *S. hyodysenteriae*-inoculated mice fed the zinc-supplemented diets after PID 14, except in one mouse fed the $ZnSO_4$-supplemented diet on PID 35. This mouse had changes similar to those present in mice fed the basal diet. Marked increase in the mean LCL occurred in the intestines of infected mice fed with the basal diet on PID 14 and 21 (Figure 1). Conversely, the mean LCL in the intestines of infected mice fed the zinc-supplemented diets were significantly reduced on PID 14 and 21 ($P < 0.05$).

Discussion

The isolation of *S. hyodysenteriae* from mice fed the basal diet was similar to that reported in previous experiments. In contrast, isolation of *S. hyodysenteriae* and severity of intestinal damage in *S. hyodysenteriae*-inoculated mice fed a pharmacologic amount of dietary zinc were strikingly less than that of mice fed the basal diet

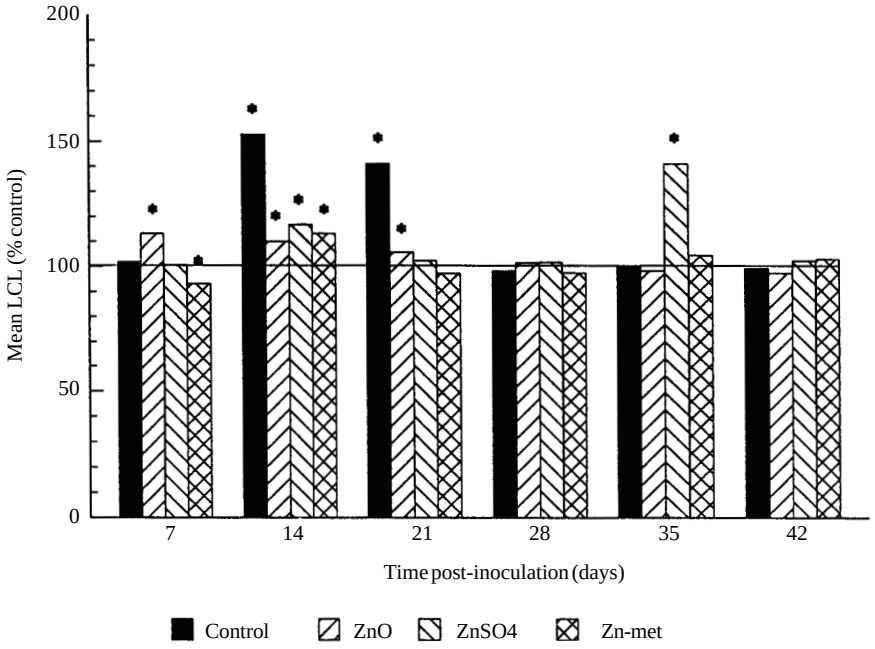

Figure 1. Mean longitudinal crypt length (LCL) in intestine of *Serpulina hyodysenteriae*-inoculated mice expressed as a percent of the mean LCL of the medium-inoculated control mice fed with either the basal diet (control) or the basal diet supplemented with 6,000 ppm of either ZnO, $ZnSO_4$, or Zn-methionine (Zn-met). Each bar represents the percent mean LCL of 3 *S. hyodysenteriae*-inoculated mice against 3 medium-inoculated mice with * indicating statistically significant differences between each group ($P < 0.05$).

alone, irrespective of the source of zinc ($P < 0.05$). However, feeding diets supplemented with approximately 6,000 ppm of zinc had a deleterious effect on the growth of the mice. Because of its antimicrobial activity, $ZnSO_4$ was widely used for treatment of gastroenteritis, diarrhea, and dysentery in human beings before the advent of antibiotics.

Production of toxins by bacteria is an adaptive process which can be induced or repressed in response to changing environments. We have shown previously that approximately 15 ppm of $ZnSO_4$ can inhibit the production of toxin by *S. hyodysenteriae*. Also, addition of approximately 800 ppm of $ZnSO_4$ to culture medium significantly reduces both the production of toxin and the growth of the spirochetes. It is conceivable that reduced isolations of *S. hyodysenteriae* and reduced severity of intestinal damage were due to zinc-induced inhibition of toxin production by the *S. hyodysenteriae*.

Intestinal damage in mice fed the basal diet and infected with *S. hyodysenteriae* were similar to those described previously. Subjective histologic assessment of intestinal damage and measurements of LCL as a quantitative evaluation of intestinal damage confirmed the prophylactic effect of dietary zinc against the development of intestinal damage associated with *S. hyodysenteriae* infection. Of the different zinc compounds, Zn-methionine appeared to have the most significant prophylactic effect. Recurrence of intestinal damage in the absence of positive isolation of *S. hyodysenteriae* in one mouse fed the $ZnSO_4$-supplemented diet on PID 35 suggests that low numbers of spirochetes might have been present in the intestines of mice fed the zinc supplemented diets.

Feeding over 100 times the recommended concentration of dietary zinc for 52 days had a deleterious effect on the BWG of the mice. The effect on the BWG was most pronounced with $ZnSO_4$, but mice fed ZnO and Zn-methionine also gained only about half the weight of mice fed with the basal diet. Based on elevated liver Zn^{2+} and Fe^{2+} concentrations (approximately double that of mice fed with the basal diet, $P < 0.05$) combined with decreased liver Cu^{2+} concentrations (approximately half that of mice fed with the basal diet, $P < 0.05$), we attributed the reduced BWG of mice fed pharmacologic amount of zinc to excessive zinc exposure. Signs of excessive zinc exposure in animals are thought to be those of induced copper deficiency and interference with absorption and utilization of iron.

Different zinc formulations are absorbed by different pathways, and this can result in differences in the bioavailability of the zinc cation. Although very little is known about zinc absorption in bacteria, reduced isolations of *S. hyodysenteriae* and reduced intestinal damage in infected mice fed with Zn-methionine may indicate a greater bioavailability of the zinc-chelate for the spirochetes.

Conclusions

Although the cause of swine dysentery is well known, the disease continues to result in considerable economic losses to commercial swine producers. A vaccine for swine dysentery has been commercially available for many years; but it has not provided the protection anticipated. Researchers have shown that if pigs are allowed to recover naturally from swine dysentery, without medication, they are protected against the disease. Immunity following natural recovery thus can provide adequate protection against swine dysentery. It is conceivable then that protection against swine dysentery can be achieved by inhibiting production of toxin by the spirochetes while the pig's immune system is allowed to build up resistance. Inhibition of *S. hyodysenteriae* toxin by a dietary element such as zinc offers an attractive alternative to continuous medication of animals with expensive, residue-causing antimicrobials.

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