Effect of zinc oxide sources and dosages on intestinal Enterobacteriaceae and gut integrity of weaned piglets

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Conclusion: compared to regular ZnO at pharmacological dosage, the potentiated ZnO at low dosage showed equal reductions of Enterobacteriaceae counts, and improved gut epithelial barrier function and alkaline phosphatase mRNA expression in a similar or even better way

Background

Zn is an essential trace element for piglets
- Requirements NRC 2012; 100 and 80 mg Zn per kg feed for piglets of 5-11 and 11-25 kg BW, respectively
- Maximum levels are established to reduce output to environment and risk for co-selection for antibiotic resistance (e.g. in EU, 150 mg total Zn per kg feed for piglets)

ZnO is an antimicrobial growth promoter for weaned piglets
- Pharmacological doses: 2000-4000 mg Zn per kg feed
- Mode of action: improved performance, reduced incidence of diarrhoea, reduced inflammation, improved histomorphology, improved light junction integrity, reduced secretory responses, antimicrobial by free Zn2+ > radical inside the bacterium (Lactobacillus spp ↓, heterofermentative species ↑, clostridia ↑, diversity of Enterobacteriaceae ↑, E. coli in 1st week PW ↓), reduced adhesion of ETEC
- Drawbacks in practice: high buffering capacity, reduced feed intake, delayed

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- Requirements NRC 2012; 100 and 80 mg Zn per kg feed for piglets of 5-11 and 11-25 kg BW, respectively

ZnO at low dosage
- Non-significant increase of gastric pH with pharmacological dose of regular ZnO
- Bacteria by plating: regular ZnO at pharmacological dose and HiZox at both 110 and 220 mg Zn/kg reduced numbers of E. coli and coliforms by ≥ 1 logCFU/g in distal small intestine (Fig. 1), smaller and non-significant reductions in proximal small intestine
- Bacteria by qPCR: data suggest no effect on Lactobacilli, reduced E. coli and coliforms numbers (log10 CFU/g)

Materials and Methods

Animals and diets
- 48 weaned piglets, 19 d, 6.04±0.77 kg
- 24 pens with 2 piglets each (balanced for litter and gender)
- Animals were fed for 15 days, and then euthanised and sampled
- Basal diet was free of supplemental Zn, low Cu, no organic acids
- 2400 mg/kg of Zn per kg feed
- High iron level was used to induce gastro-intestinal disturbances
- Animals were fed for 15 days, and then euthanised and sampled for gut health parameters

Dietary treatments

<table>
<thead>
<tr>
<th>Source of ZnO</th>
<th>regular ZnO</th>
<th>HiZox</th>
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<tbody>
<tr>
<td>ZnO (mg/kg) 110</td>
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<tr>
<td>ZnO (mg/kg) 2400</td>
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Measurements

- Animal performance (n=4) and health (including fecal scores) (n=8)
- Digesta from stomach, proximal (first 25% of length) and distal (last 25% of length) small intestine (n=8)
  - pH
  - Dry matter
  - E. coli and coliform counts by plating on selective media
  - Lactobacilli, Clostridium XIVa, Enterobacteriaceae by qPCR (only T3-T6)
  - Diversity indices (Richness and Shannon) (only T3-T6)
  - Small intestinal mucosa at 75% of length (n=8)
  - Ex vivo paracellular with FITC-4kDa as marker
  - Transepithelial electrical resistance (TEER)
  - Basal short-circuit current (Isc) and Iec upon stimulation with the secretagogues serotonin and theophylline
  - Gene expression for (only T3-T6):
    - Tight junction proteins: occludin, claudin 1, claudin 5, claudin 7 and zona occludens
    - Toll like receptor 4
    - Inflammatory cytokines: TNFα, IFNγ and Il1β
  - Intestinal alkaline phosphatase

Statistics

Mixed model with factor treatment as fixed factor and factor block as random factor and their interaction (SAS Enterprise Guide 8.0). Means are separated by the Tukey-test (P<0.05)

Results

- In contrast to anticipated, the high Fe level did not deteriorate performances; here below only results of T3-T6 are then given
- HiZox at 220 mg Zn/kg improved performance in a almost similar way as pharmacological dose of regular ZnO
- Non-significant increase of gastric pH with pharmacological dose of regular ZnO
- Bacteria by plating: regular ZnO at pharmacological dose and HiZox at both 110 and 220 mg Zn/kg reduced numbers of E. coli and coliforms by ≥ 1 logCFU/g in distal small intestine (Fig. 1), smaller and non-significant reductions in proximal small intestine
- Bacteria by qPCR: data suggest no effect on Lactobacilli, reduced E. coli and coliforms numbers (log10 CFU/g)

Fig 1. Effect of zinc sources and dosages on distal small intestinal Enterobacteriaceae and E. coli and coliforms numbers (log10 CFU/g)

Fig 2. Effect of zinc sources and dosages on distal small intestinal enteropathogenic E. coli and coliform barrier function by measuring transepithelial resistance (TEER)

Fig 3. Effect of zinc sources and dosages on distal small intestinal mucosal mRNA level of intestinal alkaline phosphatase