**Reduction of Campylobacter and Salmonella in pigs treated with a select nitrocompound**


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**Summary:** The aim of this study was to test the effectiveness of administering a select nitrocompound (S-NO) on reducing naturally colonized Campylobacter and experimentally infected Salmonella in the weaned pig gut. Pigs were divided into four groups; control (0 g S-NO/pig), 1X (0.2 g S-NO/pig), 5X (1 g S-NO/pig), and 10X (2 g S-NO/pig). Treatments were administered via oral gavage 24 h before sacrifice. Mean ± SD populations (log10 cfu/g) of Campylobacter in the cecum were reduced (P < 0.05) in pigs receiving the 10X dose when compared with untreated controls (1.64 ± 1.30 vs 5.31 ± 0.58, respectively). Campylobacter concentrations in rectal contents from pigs administered the 5X dose were reduced (P < 0.05) compared to control (2.65 ± 2.86 vs 5.90 ± 0.94, respectively). Rectal Salmonella concentrations were reduced (P < 0.05) in all of the S-NO-treated groups. Adverse effects of S-NO on pig health were not observed. These results demonstrate that S-NO may have potential as an intervention to reduce pig colonization by Campylobacter and Salmonella.

**Keywords:** antimicrobial, pre-harvest, food safety, intervention, swine.

**Introduction:** Campylobacter and Salmonella spp. continue to cause human infections and are implicated as two of the most leading etiological agents of foodborne diseases. Pigs are known to be a major natural reservoir for these pathogens (Weijtens et al., 1999; Duijkeren et al., 2002). Some nitroalkanes have been reported as alternative electron acceptors by Denitrobacterium detoxificans (Anderson et al., 2000). Hence, these compounds have an antimicrobial activity against Campylobacter jejuni (unpublished) and are also effective in inhibiting ruminal methanogenesis in vitro (Anderson et al., 2003). The aim of this study was to determine the bactericidal activity of one of these nitrocompounds, S-NO against Campylobacter and Salmonella in the cecum and rectum of weaned pigs. We also determined the concentrations of total culturable anaerobes, total coliforms as well as generic Escherichia coli.

**Materials and Methods:** An experiment was conducted using weaned pigs (21-28 d-old) obtained from a local producer and housed in concrete floor pens. Piglets were provided ad libitum access to an unmedicated corn-soybean meal and whey starter diet during entire experimental period. Upon arrival, rectal swabs were collected from each piglet and cultured for the presence of Salmonella and Campylobacter. Piglets were challenged via oral gavage with 5 ml of a novobiocin and nalidixic-resistant S. Typhimurium that had been grown overnight in tryptic soy broth. The concentrations of the challenge dose of S. Typhimurium were approximately 2.0 x 10⁹ CFU/ml. Pigs were divided into four groups with unbalanced design; control (0 g S-NO /pig; n = 10), 1 X (0.2 g S-NO/pig; n = 10), 5 X (1 g S-NO/pig; n = 6), and 10 X (2 g S-NO/pig; n = 3). Treatments (S-NO) were administered via oral gavage 24 h before sacrifice. Cecal and rectal contents (1 g) were serially diluted (1:10) in phosphate buffer (PB; pH 6.5) spread plated onto brilliant green agar (BGA) supplemented with 25 µg novobiocin ml⁻¹ only (N) or with novobiocin and 20 µg nalidixic acid ml⁻¹ (NN), and to Campy-Cephex agar for enumerating S. Typhimurium, Salmonella, and Campylobacter, respectively. Escherichia coli and total coliforms were enumerated using E. coli/coliform Petrifilm plates. All plates were incubated at 37°C for 24 h except Campy-Cephex plates which were incubated in a microaerobic environment (5 % O₂, 10 % CO₂, 85 % N₂) at 42°C for 48 hours. For qualitative enrichment, cecal and rectal contents were enriched in tetrathionate broth for 24 h and then streaked on BGA for Salmonella. For Campylobacter, contents were enriched in bolton broth at 37 °C for 4 h and then 42 °C for 44 h. Suspected colonies on BGA and
Campy-Cephex plates showing typical salmonellae and *Campylobacter* morphology were picked and confirmed biochemically and serologically. Total culturable anaerobes were determined via direct plating on anoxic brucellar agar under CO₂ (5%). The concentrations of volatile fatty acid (VFA; acetic, butyric, propionic, isobutyric, valeric, and isovaleric) were determined by gas chromatography (Hinton, et al., 1990). The bacterial populations recovered on plates were transformed to log₁₀ CFU for statistical analysis. Qualitative enrichments resulting no growth on BGA plate were assigned a value of 0.1 log₁₀ CFU/g and enrichment yielding *Salmonella* positive plates were assigned a value of 9 CFU/g. Data were analyzed using the general linear model of Statistical Analysis Systems (SAS, 1999).

**Results and Discussion:** Although we did not observe any biologically adverse effects after perorally administrating S-NO, there were a few piglets showing diarrhea among all groups including untreated control group but this was probably due to the high dose of *Salmonella* administration. Table 1 shows the effect of S-NO on naturally occurring *Campylobacter* populations in the cecum and rectum. More than 3 log unit reductions of *Campylobacter* populations occurred (P < 0.05) in cecal and rectal contents from piglets treated with 10 X and 5 X doses of S-NO, respectively. The resurgent increase of *Campylobacter* populations in rectum at 10 X dose-treated group is unclear but may suggest that *Campylobacter* recovered from this group was relatively more resistant to the S-NO compound than those isolates from the other group. Alternatively, environmental factors in the gut such as pH, redox potential or other unknown factors may have diminished the bactericidal activity of S-NO or have perturbed its pharmacokinetic properties in situ. Preliminary in vitro results have shown an enhanced bactericidal activity of S-NO against *C. jejuni* under slightly alkaline conditions (pH 8.0) (data not shown). The wildtype *Salmonella* population was reduced (P < 0.05) upon all S-NO treated groups in rectum (Fig 1) while we observed no effect on *S. Typhimurium* concentrations (data not shown). This latter result is probably due to an insufficient challenge. In contrast, results from recovered populations of total coliform, *E. coli* and total culturable anaerobes reveal that these bacteria were not affected (P < 0.05) by this compound (data not shown). In addition, analysis of acetate, propionate, and butyrate revealed no S-NO effect (P < 0.05) on concentrations of these volatile fatty acids in the cecum and rectum of treated pigs (data not shown). These findings suggest that the S-NO treatment did not influence the fermentation profiles within the pig gut. More studies including optimization of concentrations for effective doses, determining the spectrum of activity of this compound, and elucidation of mechanism(s) of action are in progress in our laboratory.

**Conclusion:** The scope of this study shows that S-NO may have potential applicability as an intervention to reduce pig colonization by *Campylobacter* and *Salmonella*.

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**References:**


Table 1. Effect of select nitrocompound (S-NO) treatment on cecal and rectal Campylobacter populations following natural exposure in weaned pigs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cecal</th>
<th>Rectal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.31 (0.58) A</td>
<td>5.90 (0.94) A</td>
</tr>
<tr>
<td>1 X S-NO</td>
<td>4.95 (1.02) A</td>
<td>4.68 (1.71) A</td>
</tr>
<tr>
<td>5 X S-NO</td>
<td>5.30 (0.43) A</td>
<td>2.65 (2.86) B</td>
</tr>
<tr>
<td>10 X S-NO</td>
<td>1.64 (1.30) B</td>
<td>5.65 (0.85) A</td>
</tr>
</tbody>
</table>

*a* Weaned piglets (21 to 28 days of age) were used in this experiment; *n* = 10 for all treatment groups at each cecal and rectal concentration except 5 X (*n* = 6) and 10 X (*n* = 3). Means within columns with different letters differ (*P* < 0.05). There was a significant (*P* < 0.05) interaction between treatment and sampling location (data not shown).

*b* Treatments, 0 (control), 0.2 (1 X), 1 (5 X) and 2 g (10 X) S-NO per pig were administered via oral gavage 16 h post challenge. Cecal and rectal contents were collected 24 h afterwards.

Fig. 1. The effect of select nitrocompound (S-NO) on wildtype Salmonella concentrations in weaned pigs. Weaned piglets (21 to 28 days of age) were used in this experiment; *n* = 10 for all treatment groups at each cecal and rectal concentration except 5X (*n* = 6) and 10 X (*n* = 3). Asterisks indicate means differ from 0 X control means (*P* < 0.05).