RESEARCH REPORT

PORK SAFETY

Title: Effect of sodium chlorate on *Salmonella* Typhimurium in the pig gut
NPB #00-136

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I. Abstract: *Salmonella* cause disease and compromise food safety. Consequently, strategies are sought to reduce their concentration in pigs immediately before processing. Respiratory nitrate reductase activity possessed by *Salmonella* coincidentally catalyzes the intracellular reduction of chlorate to chlorite, a consequence that kills the microbe. Since most beneficial gut bacteria lack respiratory nitrate reductase, we conducted several studies to see if chlorate may selectively kill *Salmonella*, but not beneficial microbes, within the pig gut. In the first study, weaned pigs orally infected with *Salmonella* Typhimurium were treated 8 and 16 h later via oral gavage (10 ml) with 0 or 100 mM sodium chlorate. The pigs were euthanized at 8 h intervals after receiving the last treatment and samples collected by necropsy were cultured for *Salmonella*. A significant chlorate treatment effect ($P < 0.05$) on cecal *Salmonella* concentrations was observed, although a treatment x time after treatment interaction was also observed which suggests that the chlorate effect was concentration dependent. In follow up studies, 0, 15 or 30 mM sodium chlorate solutions were administered via drinking water to weaned or finished pigs experimentally challenged 24 h earlier with *Salmonella* Typhimurium. These pigs were euthanized 12 h or 24 h after being allowed ad libitum access to their respective treatments and gut samples were again cultured for the challenge *Salmonella* strain. In these studies, *Salmonella* concentrations in gut contents collected from chlorate treated pigs were reduced up to 1000-fold compared to concentrations from control pigs. These results demonstrate that chlorate administration may be a practical procedure for reducing gastrointestinal concentrations of *Salmonella* in pigs immediately prior to processing.

II. Introduction: Many *Salmonella* species are human pathogens. Because these may reside within the gastrointestinal tract of pigs and other food producing animals they pose a risk to human health (8). Subsequently, strategies are sought to rid these pathogens from the pig gut prior to slaughter in order to minimize the risk of contamination of pork products (9). As members of the family *Enterobacteriaceae*, *Salmonella* possess a respiratory nitrate reductase enzyme that catalyzes the reduction of nitrate to nitrite (10). Characteristically, respiratory nitrate reductases also reduce chlorate intracellularly to the lethal chlorite ion (12). An intriguing feature of this characteristic is that bacteria possessing respiratory nitrate reductases, such as *Salmonella* and *E. coli*, are consequently killed by the chlorite, but bacteria not

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possessing the respiratory nitrate reductase, i.e., many commensal and mutualist (beneficial) bacteria, are not affected (5, 12). Chlorate is only mildly toxic to most animals, with a lethal dose of 1 or more g chlorate per kg body wt (11). Thus, we hypothesized that it may be possible to orally administer low concentrations of chlorate to animals to selectively kill *Salmonella*, but not beneficial bacteria, within the lumen of the gut and that this may be useful for the preharvest control of *Salmonella* in swine.

III. Objectives: The objectives of the proposed research were to test and evaluate the practicality of orally administering chlorate ion to pigs at different stages in the production cycle as a means of reducing gut concentrations of *Salmonella*. The ultimate goal of the proposed research is to develop a practical procedure for reducing gastrointestinal concentrations of *Salmonella* in pigs immediately prior to processing.

IV. Procedures: *Study one.* Two separate experiments were conducted with a total of 90 weaned 26 to 29 day old pigs. Each pig was infected orally with 8×10^7 colony forming units (CFU) of a novobiocin and naladixic acid resistant *Salmonella* Typhimurium strain (NVSL 95-1776). The pigs were randomly placed into respective treatment groups (15 pigs per treatment group per experiment) with each group kept in separate concrete floored pens. The pigs in each group were then treated via oral gavage (10 ml volumes), at 8 and again at 16 h post *Salmonella* challenge, with solutions containing 0, 100 or 200 mM sodium chlorate, corresponding to 0X, 1X or 2X treatment, respectively. Treatments also contained 2.5 mM sodium nitrate as an inducer of nitrate reductase activity and to ensure at least a small amount of available reductant, 20 mM sodium lactate. Piglets were euthanized at 8 h intervals (five per group per experiment) following the last treatment and ileocolic lymph tissue and cecal contents (1 to 2 g) were collected by necropsy. The specimens were cultured qualitatively and quantitatively for the challenge *Salmonella* strain.

Studies two and three. Weaned and finished pigs experimentally infected with *Salmonella* Typhimurium as above were provided ad libitum access 24 h later to drinking water containing 0, 15 or 30 mM sodium chlorate. A separate placebo treatment containing 15 mM sodium chloride was also provide to a group of the weaned pigs. Again, each treatment also contained 2.5 mM sodium nitrate and 20 mM sodium lactate. Water intake was measured to assess any potential aversions to chlorate. Pigs were euthanized 36 to 48 h post challenge (corresponding to 12 or 24 h after initial access to treatments, respectively) and specimens collected by necropsy were cultured qualitatively and quantitatively for *Salmonella* as described earlier. Animals in all studies were cared for according to typical animal husbandry practices and all protocols were approved by the USDA/ARS SPARC Animal Care and Use Committee, which abides by recognized guidelines for the humane care and use of animals.

Analytical. Specimens were cultured qualitatively for the challenge *Salmonella* strain via initial 24 h enrichment in 20 ml tetrathionate broth and further enrichment (24 h) of 100 µl in 5 ml Rappaport-Vassiliadis broth. Selective differentiation was accomplished after overnight incubation on brilliant green agar containing 25 µg novobiocin ml^{-1} and 20 µg naladixic acid ml^{-1}. Cecal concentrations of *Salmonella* were determined via direct plating of serial ten-fold dilutions on the antibiotic supplemented brilliant green agar. Concentrations of total culturable anaerobes were estimated via a three-tube most probable number method (5, 7) and cecal contents (diluted 1:10 in phosphate buffered saline) were analyzed for volatile fatty acid concentrations by gas chromatography. All incubations were done at 37°C.

V. Results: The bactericidal activity of sodium chlorate against *Salmonella* Typhimurium DT104 and *Escherichia coli* O157:H7 had been demonstrated earlier in vitro but not in vivo (5). Results obtained from our first study now clearly demonstrate that sodium chlorate killed *Salmonella* Typhimurium in the gut of experimentally infected weaned pigs (Table 1),

Table 1. Effect of oral chlorate administration on recovery of *Salmonella* serovar Typhimurium from experimentally weaned pigs[a]

	Qualitative recovery (% culture positive)								
	Ileocolic lymph tissue			Cecal contents			Quantitative recovery (Mean $\log_{10}$ CFU/g)[a]		
Treatment	Hours after last chlorate treatment						Hours after last chlorate treatment		
	8	16	24	8	16	24	8	16	24
0X	10	50	50	90	90	70	3.2	3.2	1.5
1X	10	10	30	90	40	70	2.0	0.5	2.3
2X	10	10	10	50	70	50	0.8	1.8	1.2
Chlorate effect	($P > 0.05$)			($P > 0.05$)			($P < 0.05$)		
Time effect	($P > 0.05$)			($P > 0.05$)			($P > 0.05$)		
Interaction	---			---			($P < 0.05$)		
SEM	---			---			0.50		

[a]Pigs were challenged with 10^7 CFU *Salmonella* Typhimurium and were treated with 0, 100 or 200 mM sodium chlorate (0X, 1X or 2X, respectfully) via oral gavage (10 ml) at 8 h and again at 16 h post challenge. All treatments also contained 2.5 mM sodium nitrate as an inducer of nitrate reductase activity and 20 mM sodium lactate. Treatments were tested for differences in proportions of *Salmonella*-positive specimens ($n = 10$) using a Fisher's exact test. *Salmonella* concentrations were analyzed for treatment or time after treatment differences using an analysis of variance and LSD comparison of means, $n = 10$ for all except 0X treatment at 24 h, in which case $n = 9$.

Although a significant treatment x time after treatment interaction on cecal *Salmonella* concentrations suggests that other pharmacokinetic properties of chlorate remain to be investigated. In this first study, the total dose following the collective administration of the 1X or 2X treatment was 213 or 426 mg sodium chlorate, respectively. Based on an estimated average body wt of 10 kg, these dose levels correspond to 0.02 or 0.04 g per kg body wt. Neither chlorate treatment caused observable adverse effects in any of the pigs. The cause of the interaction is not readily apparent from the present data but it is probable that the observed interaction reflects gut concentrations of chlorate, which were not measured. For instance, significant reductions did not occur in contents collected 8 h after administration of the last 1X treatment (16 h after administration of the first treatment) and this suggests that effective chlorate concentrations had not yet accumulated in the lower gut. Regarding the lack of effect observed in contents collected 24 h after the last treatment, it is reasonable to expect that chlorate concentrations would have been diminished due to microbial reduction of chlorate to chlorite and(or) absorption. These data thus indicate a need to develop practical administration procedures that optimize not only delivery, but also maintenance, of effective concentrations of chlorate to the lower gut.

Chlorate treatment did not reduce concentrations of total culturable anaerobes which ranged from 10.6 to 11.5 $\log_{10}$ cells/g thus supporting the concept that the bactericidal effect of chlorate is specific to those bacteria possessing respiratory nitrate reductase activity. A significant treatment, time after treatment effects and their interaction were also observed with respect to cecal propionate and total VFA concentrations (Table 2), which suggests that chlorate may have affected propionate producing bacteria such as *Selenomonas* and *Propionibacterium*.

Table 2. Effect of oral chlorate administration on volatile fatty acid concentrations in cecal contents of experimentally infected weaned pigs[a]

	Acetate (µmol/ml)			Propionate (µmol/ml)			Butyrate (µmol/ml)		
	Hours following last chlorate treatment			Hours following last chlorate treatment			Hours following last chlorate treatment		
Treatment	8	16	24	8	16	24	8	16	24
0X	62.6	65.3	91.9	38.4	43.6	67.7	10.5	9.5	16.4
1X	65.5	63.2	68.3	34.5	34.6	32.3	13.8	11.4	10.9
2X	56.5	49.8	63.8	38.6	26.6	35.0	15.3	8.0	14.3
Time effect	($P > 0.05$)			($P > 0.05$)			($P > 0.05$)		
Dose effect	($P > 0.05$)			($P < 0.05$)			($P > 0.05$)		
Interaction	($P > 0.05$)			($P < 0.05$)			($P > 0.05$)		
SEM	----- 19.0 -----			----- 13.0 -----			----- 9.7 -----		

[a]Pigs were challenged with 10^7 CFU *Salmonella* Typhimurium and were treated with 0, 100 or 200 mM sodium chlorate (0X, 1X or 2X, respectfully) via oral gavage (10 ml) at 8 h and again at 16 h post challenge. All treatments also contained 2.5 mM sodium nitrate as an inducer of nitrate reductase activity and 20 mM sodium lactate. Concentrations were analyzed for treatment or time after treatment differences using an analysis of variance and LSD comparison of means, $n = 5$ for all except 0X treatment at 24 h, in which case $n = 4$.

These species are among the few anaerobes possessing respiratory nitrate reductase activity (1, 2) but are not necessarily critical for optimal gut function.

Results from our follow up studies clearly demonstrate the practicality of administering sodium chlorate as a drinking water supplement to reduce gut concentrations of *Salmonella* in weaned and finished pigs (Tables 3 and 4). In both studies, the bactericidal effect of sodium chlorate against the challenge *Salmonella* strain is evident. Again, none of the pigs exhibited ill effects and water consumption was not diminished due to chlorate (Table 4).

Results obtained during these studies have been presented in abstract form at various meetings (3, 6) and(or) have been published (4).

Table 3. Effect of administering sodium chlorate in the drinking water on gut concentrations of *Salmonella* Typhimurium in experimentally challenged weaned pigs.

	Qualitative recovery (% *Salmonella* culture-positive)			*Salmonella* conc'n (Mean ±SD $\log_{10}$ CFU/g)	
Treatment	Ileocolic lymph tissue	Cecal contents	Rectal contents	Cecal contents	Rectal contents
Water only	29	100	100	4.36[b] ± 0.92	1.59[b] ± 1.46
15 $NaClO_3$	0	71	29	2.63[c] ± 2.29	0.67[c] ± 1.49
30 $NaClO_3$	0	43	14	1.26[c] ± 1.95	0.14[c] ± 0.38
30 NaCl	43	100	86	4.19[b] ± 1.56	1.97[b] ± 1.23

[a]Pigs were allowed ad libitum access 24 h post *Salmonella* challenge to drinking water containing 0, 15 or 30 mM sodium chlorate or 15 mM sodium chloride. Pigs were euthanized 36 h post challenge. Treatments were tested for differences in proportions of *Salmonella*-positive specimens ($n = 7$) using Fisher's exact test; no differences were found ($P > 0.05$). *Salmonella* concentrations were analyzed for treatment or time after treatment differences using an analysis of variance.

[b, c]Means ($n = 7$) within same column differ ($P < 0.10$).

Table 4. Effect of administering sodium chlorate in the drinking water on gut concentrations of *Salmonella* Typhimurium in experimentally challenged finished pigs[a].

	Salmonella conc'n ($\log_{10}$ CFU/g)		Water consumption		
Treatment	Cecal contents	Rectal contents	(mL/kg BW)	Cecal pH	Rectal pH
Water only	3.8 ± 0.7	1.9 ± 1.6	40.0 ± 22.9	5.7 ± 0.2	6.6 ± 0.2
15 $NaClO_3$	1.8[**] ± 1.5	1.3 ± 1.7	34.2 ± 21.0	5.7 ± 0.2	6.2[**] ± 0.2
30 $NaClO_3$	2.4[**] ± 1.3	0.6[*] ± 0.3	40.0 ± 30.0	6.0[**] ± 0.2	6.4 ± 0.4

[a]Pigs were allowed ad libitum access 24 h post *Salmonella* challenge to drinking water containing 0, 15 or 30 mM sodium chlorate or 15 mM sodium chloride. Pigs were euthanized 48 h post challenge. Treatment means were compared to water only control means using a T-test. All values are reported as the mean ± SD ($n = 6$ for all except 30 $NaClO_3$ treatment group in which case $n = 5$).

[*]Treatment means differ from those of water only control ($P < 0.10$).

[**]Treatment means differ from those of water only control ($P < 0.05$).

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