RESEARCH REPORT
PORK SAFETY

Title: Pre-nitrate adaptation and chlorate supplementation to reduce *Salmonella*, *Escherichia coli*, and *Yersinia enterocolitica* in swine - **NPB #03-136**

Investigators: Robin C. Anderson and Yong Soo Jung

Institution: U. S. Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, Food & Feed Safety Research Unit, College Station, TX

Date Received: June 15, 2004

Abstract: An experimental chlorate product that targets the respiratory nitrate reductase enzyme of bacteria such as *Salmonella* and *Escherichia coli* has shown promising results in reducing concentrations of these bacteria in the gut of food animals. Because expression of the target enzyme is induced by nitrate, we conducted laboratory and animal studies to see if short-duration, low level nitrate or nitrocompound preconditioning would enhance the ability of an experimental chlorate product to kill these bacteria. Results from these studies showed that preconditioning the gut microflora of swine with low levels of nitrate or nitrocompounds markedly enhanced the ability of the chlorate product to kill *Salmonella* and *E. coli*. Moreover, results from laboratory studies showed that the nitrocompounds had the ability to kill *Yersinia,* another important pathogen of swine. Further studies are needed before these compounds can be fed as feed additives to animals, although it is likely that nitrate preconditioning may be more near to market than the nitrocompounds, which may require more comprehensive review by FDA.

Introduction: In the United States, foodborne pathogens are estimated to cause nearly 3.9 million human infections annually (Mead et al., 1999). *Salmonella* and *Campylobacter* are leading etiologic agents among foodborne pathogens. In addition, pigs are also known as natural carriers of *Yersinia enterocolitica* (Robins-Browne, 1997). The tongue, tonsil, gastrointestinal tract and its contents are recognized as major sites for harboring these foodborne pathogens (Hosek et al., 1997, Gray et al., 1996) and have been implicated as important sources contributing to the contamination of carcasses during slaughter (Morgan et al., 1987, Swanenburg et al., 2001, Nesbakken et al., 2003).

Although numerous interventions are being developed to reduce these pathogen at various stages of production (pre and post-harvest), none of these methods are completely satisfactory. Considerable interest exists, therefore, in the development of strategies that can reduce concentrations of these bacteria in swine before processing, especially since quantitative risk assessments indicate that such interventions may significantly reduce human exposures to the pathogens (Hynes and Wachsmuth, 2000; Vugia et al., 2003).

Recently, the USDA has developed an experimental chlorate product that exploits the fact that *Enterobacteriaceae,* including *E. coli* and *Salmonella,* use an inducible respiratory nitrate reductase enzyme that also converts chlorate to cytotoxic chlorite, a consequence that is lethal to the bacteria (Anderson et al., 2000). Because this strategy selectively targets bacteria possessing a nitrate reductase enzyme, bacteria such as *E. coli* and *Salmonella* are killed but not beneficial anaerobes in the gut, some that may be able to help exclude pathogens. Considerable evidence supports the conceptual use of an experimental chlorate product as a feed additive to reduce gut concentrations of foodborne pathogens both in ruminants (Anderson et al., 2002; Callaway et al., 2002; Edrington et al., 2003) and monogastrics (Anderson et al., 2001a,b; Byrd et al., 2003). Recently, research with broilers has shown that the bactericidal effect of the chlorate treatment was even further enhanced by as much as 100-fold if the birds' gastro-intestinal microflora was first adapted to low dietary concentrations of nitrate (Jung et al., 2003a). Results presented here extend our previous research by demonstrating that a low level nitrate preconditioning period enhanced the bactericidal activity of an experimental chlorate product fed to swine. This research addresses the industry's need for the development of practical, cost-effective multi-hurdle pathogen control strategies.

Objectives: The primary objective of the proposed research was to develop a nitrate adaptation strategy for swine to maximize the bactericidal effect of experimental chlorate supplementation against *Salmonella.* Secondary objectives were to evaluate the effect of such a nitrate adaptation strategy on *E. coli* and *Yersinia enterocolitica*.

Materials and methods:

In vitro experiments: Freshly collected porcine pig feces (100 g) was mixed 1 to 5 with anaerobic buffer and then inoculated to achieve 10^6 CFU/ml of a novobiocin and naladixic acid resistant *Salmonella* Typhimurium (NVSL 95-1776). Ten ml volumes of this fresh fecal suspension were transferred to 18 x 150 mm crimp top tubes that had been preloaded to achieve 16 mM formate and concentrations of the select nitrocompounds as indicated. The experimental chlorate product was included to achieve 10 mM active ion. The tubes (n = 3/treatment) were then sealed and incubated at 37^{o}C under CO_2 and sampled at 0, 3, 6 and 24 h for traditional quantitative and

qualitative cultivation of the novobiocin and naladixic acid resistant *Salmonella* (Jung et al., 2003a). Data were analyzed by general analysis of variance and means were separated using Tukey's method.

The effect of chlorate treatment, with or without added nitrate or 2-nitropropanol, on the growth of pure cultures of *Yersinia enterocolitica* was assessed during growth in Brain Heart Infusion supplemented with sodium nitrate, 2-nitropropanol (2NPOH) and(or) sodium chlorate as indicated.

In vivo study: Finished pigs, averaging 47.6 ± 5.4 (SD) kg body weight were randomly allocated to one of eight treatments; basal diet control (*n* = 6), 1 day nitrate preconditioning (*n* = 4), 2 day nitrate preconditioning (*n* = 6), 2 day nitroethane preconditioning (*n* = 6), terminal experimental chlorate treatment (*n* = 6), 1 day nitrate preconditioning plus a terminal experimental chlorate treatment (*n* =4), 2 day nitrate preconditioning plus a terminal experimental chlorate treatment (*n* = 6) or a 2 day nitroethane preconditioning plus a terminal experimental chlorate treatment (*n* = 6). Sodium nitrate (0.01% wt/wt) and nitroethane (0.4% wt/wt) were mixed in the feed immediately prior to each meal's feeding, which were offered twice a day at 08:00 and 16:00. Preconditioning treatments were fed prior to the last day's provision of feed supplemented with an experimental chlorate product (1% wt/wt) containing 30% active ion. The experimental chlorate product was fed in two meals as above. The dose level of the experimental chlorate product was ½ the minimum efficacious dose. Nitroethane was added as the sodium salt which was prepared fresh each day. All pigs were orally challenged with 2.0 x 10^{10} CFU of a novobiocin and naladixic acid resistant *Salmonella* Typhimurium 6 days before initiation of the preconditioning. Pigs were euthanized one day following the experimental chlorate treatment and gut contents were collected for cultivation of *E. coli* on 3M Petrifilm (Minneapolis, MN) and the challenge *Salmonella* as traditionally done (Jung et al., 2003a). Gut contents were also cultured qualitatively for wildtype *Yersinia* on Yersinia Selective Agar (Difco, Detroit, MI) and samples from pigs fed nitroethane with or without the experimental chlorate product were quantitatively cultured for *Campylobacter* on Cephex Agar. Data were analyzed by analysis of variance and means were separated using the Least Significant Difference method.

Results: *Effect of nitrate and nitrocompound supplementation on the bactericidal effect of chlorate against Salmonella Typhimurium in laboratory models*

The bactericidal effect of chlorate against *Salmonella* Typhimurium in pig fecal suspensions was markedly enhanced by the inclusion of 5 mM nitrate (Fig 1-a). There was a temporary enrichment of *Salmonella* in suspensions incubated with nitrate only suggesting that long term pre-adaptation with nitrate alone may be undesirable. In subsequent studies, we determined that nitrate pre-adaptation can be safely managed by limiting the duration and amount of nitrate supplementation, as concentrations < 2.5 mM did not enrich populations of *Salmonella* or *E. coli* yet upon subsequent addition of an experimental chlorate product achieved enhancement its bactericidal activity (not shown).

Additional tests with different electro-negative nitrocompounds showed that substitution of these compounds for nitrate into the fecal suspensions further enhanced the bactericidal activity of chlorate against *Salmonella* Typhimurium (Fig. 1-b, c, d). The nitrocompounds by themselves did not enrich for *Salmonella* but rather exhibited in their own right a more persistent bactericidal activity against *Salmonella* than did chlorate alone. After incubating these compounds and chlorate, *Salmonella* populations were reduced by approximately 6 log units (1,000,000-fold) after 24 h.

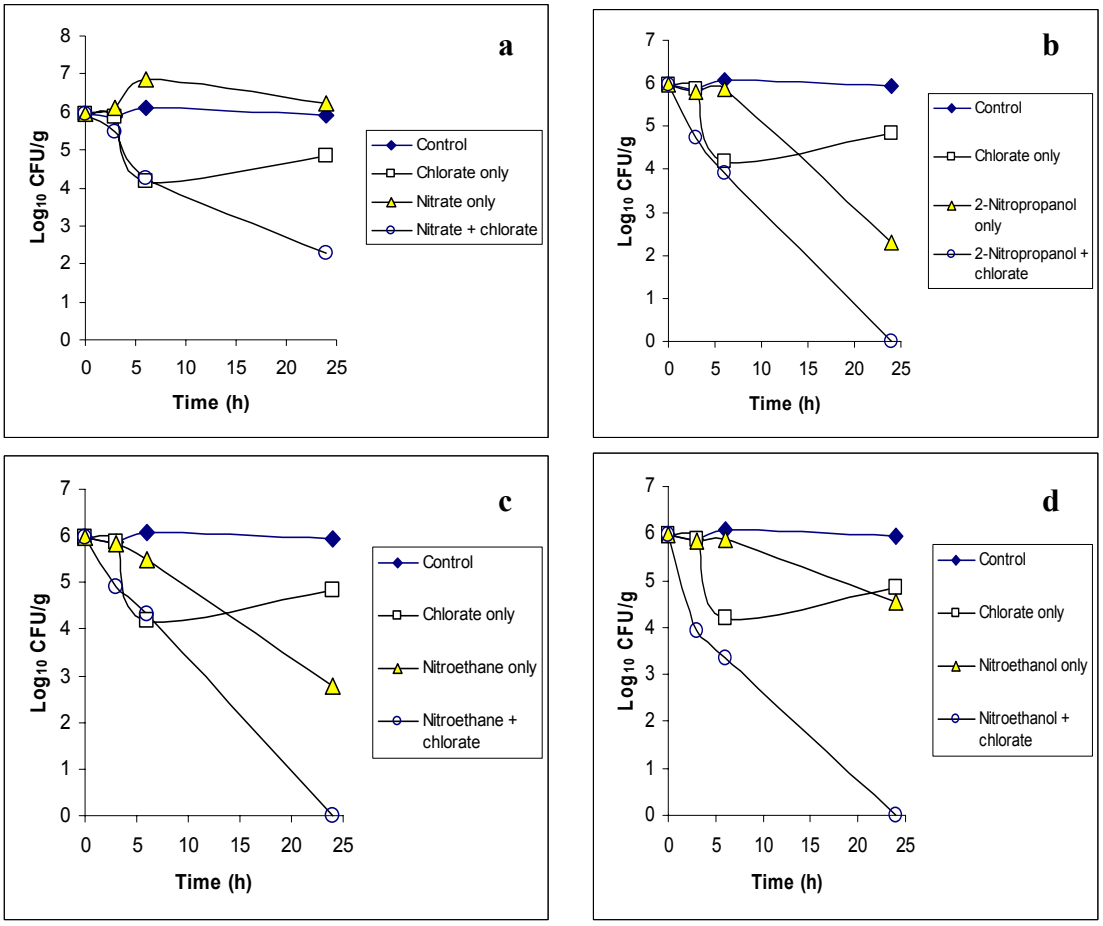

Figure 1. Effect of chlorate treatment, with or without nitrate or nitrocompound supplementation, on *Salmonella* Typhimurium in fecal fluid. Data points are mean value (n=3). The concentration of chlorate used in this study was 10 mM final. Other nitrocompounds were at 5 mM except sodium salt of nitroethane which was added at 4 mg nitroethane equivalents/ml suspension.

Effect of nitrate, 2-nitropropanol and chlorate treatment on the growth of Yersinia enterocolitica in pure culture

Figure 2 shows the effect of chlorate treatment, with or without nitrate or 2-nitropropanol supplementation, on the growth of *Yersinia enterocolitica*. The growth rate was markedly inhibited with 5 mM 2-nitropropanol treatment and inhibited further with co-administration of 10 mM chlorate treatment.

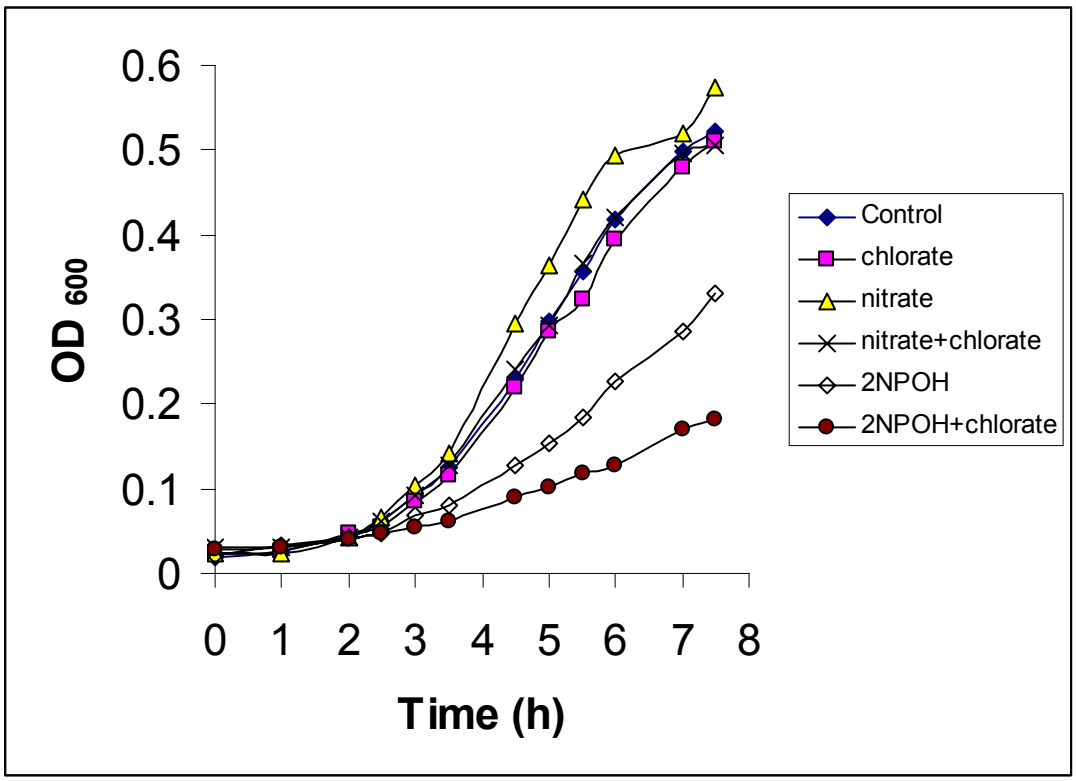

Figure 2. The mean growth rate of *Yersinia enterocolitica* in BHI broth as affected by nitrate or 2-nitropropanol (2NPOH), each at 5 mM, and 10 mM chlorate.

Effect of nitrate or nitroethane pretreatment and a terminal chlorate treatment on the gut concentrations of enteropathogens in a live animal model

In our live animal experiment, we chose to administer ½ the minimum efficacious dose of the experimental chlorate product so as to allow opportunity to measure effects of the nitrate and nitroethane preconditioning. Consequently, the experimental chlorate product by itself did not cause significant reductions of enterobacteria. The effects of a short 2-day or 1-day nitrate preconditioning treatment, with or without a terminal treatment with an experimental chlorate product are shown in Tables 1 and 2.

Unlike that observed in our laboratory models, the low level nitrate preconditioning by itself did not significantly increase numbers of wildtype *E. coli* or our challenge *Salmonella* strain. When combined with the terminal chlorate treatment, both the 1- and 2-day preconditioning period enhanced the killing of wildtype *E.coli* in the cecum and rectum and the 2-day preconditioning period enhanced the killing of wildtype *E.coli* in the ileum (Table 1). Concentrations of *Salmonella* were also reduced by the 2-day nitrate preconditioning plus experimental chlorate treatment in the cecum and rectum (Table 2). Similarly, 2-day nitroethane preconditioning by itself had little effect on gut concentrations of *E. coli* or *Salmonella* but concentrations were at least 1 log unit (10-

fold) lower in all gut compartments when combined with the terminal experimental chlorate treatment although significance was achieved only for cecal *E.coli* concentrations (Table 1) and rectal *Salmonella* concentrations (Table 2).

Wildtype *Yersinia* were not recovered from any of the gut samples. Nitroethane preconditioning, with or without a terminal feeding with an experimental chlorate product, had no effect on wildtype *Campylobacter* (Table 3).

Table 1. Effect of a low level nitrate or nitroethane preconditioning feeding period on the bactericidal activity of an experimental chlorate treatment (ECP) on gut concentrations of wildtype *E. coli*.

	E. coli conc'n (mean ± SD $\log_{10}$ CFU)		
Treatment[a]	Ileal	Cecal	Rectal
Control (basal only)	$3.7 \pm 2.0^{b,c}$	5.5 ± 1.1^{b}	$5.3 \pm 1.2^{b,c}$
Nitrate preconditioning (1 d)	$2.8 \pm 2.7^{b,c,d}$	$4.9 \pm 2.5^{b,c,d}$	$5.5 \pm 2.6^{b,c}$
Nitrate preconditioning (2 d)	4.5 ± 2.0^{b}	5.7 ± 0.9^{b}	6.2 ± 0.9^{b}
Nitroethane preconditioning (2 d)	$3.2 \pm 3.1^{b,c,d}$	$5.2 \pm 1.4^{b,c}$	$5.6 \pm 1.0^{b,c}$
ECP alone	$2.5 \pm 1.9^{b,c,d}$	$4.6 \pm 1.8^{b,c,d,e}$	$5.1 \pm 1.6^{b,c}$
Nitrate preconditioning (1 d) + ECP	$1.3 \pm 1.0^{c,d}$	$3.6 \pm 0.9^{c,d,e}$	3.2 ± 1.1^{d}
Nitrate preconditioning (2 d) + ECP	1.1 ± 0.9^{d}	3.1 ± 0.5^{e}	3.1 ± 0.9^{d}
Nitroethane preconditioning (2 d) + ECP	$2.2 \pm 2.2^{b,c,d}$	$3.5 \pm 0.9^{d,e}$	$4.3 \pm 1.3^{c,d}$

[a]Sodium nitrate (0.01% wt/wt) and nitroethane (0.4% wt/wt) were mixed in the feed immediately prior to each meal's feeding, which were offered twice a day at 08:00 and 16:00, and were fed for 1 or 2 days, as indicated, prior to the last day's provision of feed supplemented with an experimental chlorate product (ECP; 1% wt/wt) containing 30% active ion. The ECP dose was approximately ½ the minimum efficacious dose. Nitroethane was added as the sodium salt which was prepared fresh each day. Pigs were orally challenged with 2.0×10^{10} CFU *Salmonella* Typhimurium 6 days before feeding of treatment diets. [b,c,d,e]Means were separated using a Least Significant Difference method. Values within columns with unlike superscripts differ ($P < 0.05$).

Table 2. Effect of a low level nitrate or nitroethane preconditioning feeding period on the bactericidal activity of an experimental chlorate treatment (ECP) on gut concentrations of *Salmonella* Typhimurium

	Salmonella conc'n (mean ± SD $\log_{10}$ CFU)		
Treatment[a]	Ileal	Cecal	Rectal
Control (basal only)	1.6 ± 1.3^{b}	$2.3 \pm 1.0^{b,c}$	2.0 ± 1.1^{b}
Nitrate preconditioning (1 d)	0.7 ± 0.7^{b}	$2.3 \pm 1.3^{b,c}$	$1.7 \pm 1.5^{b,c}$
Nitrate preconditioning (2 d)	1.6 ± 1.5^{b}	2.6 ± 1.0^{b}	$1.6+ 0.6^{b,c}$
Nitroethane preconditioning (2 d)	0.8 ± 0.6^{b}	$2.1 \pm 1.1^{b,c}$	$1.7 \pm 0.97^{b,c}$
ECP alone	1.1 ± 1.5^{b}	$2.3 \pm 1.0^{b,c}$	2.0 ± 1.1^{b}
Nitrate preconditioning (1 d) + ECP	0.8 ± 0.4^{b}	$1.0 \pm 0.9^{c,d}$	$1.3 \pm 0.5^{b,c,d}$
Nitrate preconditioning (2 d) + ECP	0.6 ± 0.9^{b}	0.9 ± 0.7^{d}	0.4 ± 0.5^{d}
Nitroethane preconditioning (2 d) + ECP	0.7 ± 0.7^{b}	$1.2 \pm 0.8^{c,d}$	$0.9 \pm 0.7^{c,d}$

[a]Sodium nitrate (0.01% wt/wt) and nitroethane (0.4% wt/wt) were mixed in the feed immediately prior to each meal's feeding, which were offered twice a day at 08:00 and 16:00, and were fed for 1 or 2 days, as indicated, prior to the last day's provision of feed supplemented with an experimental chlorate product (ECP; 1% wt/wt) containing 30% active ion. The ECP dose was approximately ½ the minimum efficacious dose. Nitroethane was added as the sodium salt which was prepared fresh each day. Pigs were orally challenged with 2.0×10^{10} CFU *Salmonella* Typhimurium 6 days before feeding of treatment diets. [b,c,d]Means were separated using a Least Significant Difference method. Values within columns with unlike superscripts differ ($P < 0.05$).

Table 3. Effect of a low level nitroethane preconditioning feeding period on the bactericidal activity of an experimental chlorate treatment (ECP) on gut concentrations of wildtype *Campylobacter*.[a]

	Campylobacter conc'n (mean ± SD $\log_{10}$ CFU)

Treatment	Ileal	Cecal	Rectal
None	2.0 ± 1.2	4.8 ± 1.2	3.5 ± 1.9
Nitroethane preconditioning (2 d)	1.7 ± 1.5	4.8 ± 1.1	3.7 ± 1.4
ECP alone	2.2 ± 2.7	4.9 ± 1.4	3.0 ± 2.5
Nitroethane preconditioning (2 d) + ECP	1.8 ± 2.0	5.0 ± 1.0	3.6 ± 1.8

[a]Nitroethane (0.4% wt/wt) was mixed in the feed immediately prior to each meal's feeding, which were offered twice a day at 08:00 and 16:00, and were fed for 1 or 2 days, as indicated, prior to the last day's provision of feed supplemented with an experimental chlorate product (ECP; 1% wt/wt) containing 30% active ion. The ECP dose was approximately ½ the minimum efficacious dose. Nitroethane was added as the sodium salt which was prepared fresh each day. All pigs were orally challenged with 2.0 x 10^{10} CFU *Salmonella* Typhimurium 6 days before feeding of treatment diets.

Discussion: In agreement with earlier research (Jung et al., 2003a), nitrate preconditioning of the pig gut microflora with nitrate enhanced the bactericidal activity of chlorate against enteropathogens. Results from our laboratory studies further show that the nitrocompounds may be preferable to nitrate as preconditioning agents as these exhibited bactericidal activity against *Salmonella* and *Yersinia* in their own right and also showed potential to enhance the bactericidal effect of chlorate in our animal studies. In support of this, 2-nitropropanol and nitroethane have been shown to reduce *Salmonella* concentrations in the gut of broilers and pigs (Jung et al., 2003b, 2004). A disadvantage of the nitrocompounds; however, is that they may require more comprehensive review for approval by FDA than nitrate. With respect to nitrate preconditioning, our laboratory studies suggested that this compound may potentially result in an undesirable increase in numbers of *Salmonella* before treatment with chlorate but the increase in the test tube cultures was slight and nitrate preconditioning did not cause an increase in numbers of *Salmonella* in gut contents in our animal experiments. Contrary to results from an earlier animal study (Jung et al., 2003a), nitroethane treatment had no effect on *Campylobacter* concentrations in the study conducted here. Laboratory results have indicated that the ability of nitroethane to inhibit the growth of pure cultures of *Campylobacter jejuni* is enhanced at pH values > 8.0 (unpublished). Therefore, it is possible that the pH of cecal and fecal contents in the present studies were too acidic (pH < 7.0) to allow the nitroethane to be effective. Research is underway to more fully examine the inhibitory activity of the nitrocompounds against these important pathogens.

Lay Summary: *Salmonella, E. coli* and *Yersinia* are bacteria that can cause foodborne illness in humans. These bacteria can be found in the intestinal tract of pigs, therefore methods are sought to rid these bacteria from pigs before slaughter. We previously demonstrated that an experimental chlorate product could reduce *Salmonella* and *E. coli* in pigs. In the present study, certain nitrocompounds were able to kill *Salmonella*, *E. coli* and *Yersinia* in the laboratory and when fed to swine, markedly enhanced the bacterial-killing capacity of the chlorate product. Subject to regulatory approval, it is possible that these compounds may be developed into feed additives that can be fed prior to slaughter to rid bacterial pathogens from swine.

Literature Cited:

Anderson, R. C., S. A. Buckley, T. R. Callaway, K. J. Genovese, L. F. Kubena, R. B. Harvey and D. J. Nisbet. 2001a. Effect of sodium chlorate on *Salmonella* Typhimurium concentrations in the weaned pig gut. J. Food Prot. 64:255-258.

Anderson, R. C., S. A. Buckley, L. F. Kubena, L. H. Stanker, R. B. Harvey and D. J. Nisbet. 2000. Bactericidal effect of sodium chlorate on *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT104 in rumen contents in vitro. J. Food Prot. 63:1038-1042.

Anderson, R. C., T. R. Callaway, T. J. Anderson, L. F. Kubena, N. K. Keith and D. J. Nisbet. 2002. Bactericidal effect of sodium chlorate on *Esherichia coli* concentrations in bovine ruminal and fecal contents in vivo. Microb. Ecol. Dis. 14:24-29.

Anderson, R. C., T. R. Callaway, S. A. Buckley, T. J. Anderson, K. J. Genovese, C. L. Sheffield and D. J. Nisbet. 2001b. Effect of oral sodium chlorate administration on *Escherichia coli* O157:H7 in the gut of experimentally infected pigs. Int. J. Food Microbiol. 71: 125-130.

Byrd, J. A., R. C. Anderson, T. R. Callaway, R. W. Moore, K. D. Knape, L. F. Kubena, R. L. Ziprin and D. J. Nisbet. 2003. Effect of experimental chlorate product administration in the drinking water on *Salmonella* Typhimurium contamination of broilers. Poultry Sci. 82:1403-1406.

Callaway, T. R., R. C. Anderson, K. J. Genovese, T. L. Poole, T. J. Anderson, J. A. Byrd, L. F. Kubena and D. J. Nisbet. 2002. Sodium chlorate supplementation reduces *E. coli* O157:H7 populations in cattle. J. Animal. Sci. 80:1683-1689.

Edrington, T. S., T. R. Callaway, R. C. Anderson, K. J. Genovese, Y. S. Jung, J. L. McReynolds, K. M. Bischoff and D. J. Nisbet. 2003. Reduction of *E. coli* O157:H7 populations in sheep by supplementation of an experimental sodium chlorate product. Small Rumin. Res. 49:173-181.

Gray, J. T., P. J. Fedorka-Cray, T. J. Stabel, and T. T. Kramer. 1996. Natural transmission of *Salmonella choleraesuis* in swine. Appl. Environ. Microbiol. 62:141-146.

Hosek, G., D. Leschinsky, S. Irons, and T. J. Safranek. 1997. Multidrug-resistant *Salmonella* serotype Typhimurium-United States, 1996. Morbid. Mortal. Wkly. Rep. 46:308-310.

Hynes, N. A. and I. K. Wachsmuth. 2000. *Escherichia coli* O157:H7 risk assessment in ground beef: a public health tool. 4th Int. Symp. and Workshop on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections, Kyoto, Japan, October 29-November 2, p.46.

Jung, Y. S., R. C. Anderson, J. A. Byrd, T. S. Edrington, R. W. Moore, T. R. Callaway, J. McReynolds, and D. J. Nisbet. 2003a. Reduction of *Salmonella* Typhimurium in experimentally challenged broilers by nitrate adaptation and chlorate supplementation in drinking water. J. Food Prot. 66:660-663.

Jung, Y. S., R. C. Anderson, T. S. Edrington, K. J. Genovese, J. A. Byrd, T. R. Callaway and D. J. Nisbet. 2004. Experimental use of 2-nitropropanol for reduction of *Salmonella* Typhimurium in the ceca of broiler chicks. J. Food Prot. (*In press*).

Jung, Y. S., R. C. Anderson, K. J. Genovese, T. S. Edrington, J. A. Byrd, K. M. Bischoff, T. R. Callaway, J. McReynolds, R. B. Harvey, and D. J. Nisbet. 2003b. Use of sodium salt of nitroethane to reduce *Salmonella* Typhimurium in weaned pigs. Proc. Allen D. Leman Swine Conference. St. Paul, MN. Sep. 14-16, p. 27.

Jung, Y. S., R. C. Anderson, K. J. Genovese, T. S. Edrington, T. R. Callaway, J. A. Byrd, K. M. Bishoff, R. B. Harvey, J. MacReynolds and D. J. Nisbet. 2003c. Reduction of *Campylobacter* and *Salmonella* in pigs treated with a select nitrocompound. Proc. 5th Intl. Symp. Epidemiol. and Control of Foodborne Pathogens in Pork, Hersonissos, Crete-Greece. p. 205-207.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerg. Inf. Dis. 5:607-625.

Morgan, I. R., F. L. Krautil, and J. A. Craven. 1987. Effect of time in lairage on caecal and carcass salmonellae contamination of slaughter pigs. Epidemiol. Infect. 98:323-330.

Nesbakken, T., K. Eckner, H. K. Høidal, and O.-J. Røtterud. 2001. Occurrence of *Yersinia enterocolitica* and *Campylobacter* spp. in slaughter pigs and consequence for meat inspection, slaughtering, and dressing procedures. Int. J. Food Microbiol. 80:231-240.

Robins-Browne, R. M. 1997. Yersinia enterocolitica. *In* Doyle, M. P., L. R. Beuchat, and T. J. Montville (ed) Food Microbiology: Fundamentals and Frontiers, pp. 192-194. American Society for Microbiology, Washington, DC.

Swanenburg, M., H. A. Urlings, J. M. Snijders, D. A. Keuzenkamp, and F. van Knapen. 2001. *Salmonella* in slaughter pigs: prevalence serotypes and critical control points during slaughter in two slaughterhouse. Int. J. Food Microbiol. 70:243-54.

Vugia, D., J. Hadler, S. Chaves, D. Blythe, K. Smith, D. Morse, P. Cieslak, T. Jones, A. Cronquist, D. Goldman, J. Guzewich, F. Angulo, P. Griffin, R. Tauxe. 2003. Preliminary FoodNet Data on the Incidence of Foodborne Illnesses -Selected Sites, United States, 2002. MMWR. 52:340-343.