

Effect of Experimental Chlorate Product Administration in the Drinking Water on *Salmonella* Typhimurium Contamination of Broilers

J. A. Byrd,^{*1} R. C. Anderson,^{*} T. R. Callaway,^{*} R. W. Moore,^{*} K. D. Knape,[†]
L. F. Kubena,^{*} R. L. Ziprin,^{*} and D. J. Nisbet^{*}

^{*}USDA-ARS, Southern Plains Agricultural Research Center, College Station, Texas 77845; [†]Texas A&M University, Veterinary Pathobiology and Poultry Science, Texas Agricultural Experiment Station, College Station, Texas 77843

ABSTRACT The crop is a known source of *Salmonella* and *Campylobacter* contamination. Previously, we evaluated lactic acid in the drinking water during a simulated pretransport feed withdrawal (FW) and reported 0.44% lactic acid significantly ($P < 0.05$) reduced the number of *Salmonella* recovered in market-age broiler crops. However, total consumption of the organic acid-treated drinking water was reduced. Presently, we evaluated the effect of experimental chlorate product (ECP; 1× ECP is equivalent to a 15 mM chlorate ion concentration) during a 10-h pretransport FW. Market-age broilers were obtained from a commercial processing plant and randomly assigned to ECP-treated or control (nontreated) groups. Broilers were challenged by crop gavage with 10^8 *Salmo-*

nella Typhimurium (ST) immediately upon arrival and 1 d prior to termination of the experiment. One day later, broilers were killed for ST enumeration (cfu) in the crop and ceca. Broilers provided ECP 24 h prior to slaughter consumed slightly more ECP water than broilers provided distilled water. Treatment with ECP caused a significant decrease ($P < 0.05$) in the incidence of ST in crop contents (2%) as compared to the controls (36.7%). Similarly, ECP treatment caused a significant decrease ($P < 0.05$) in number of ST ($0.96 \log_{10}$ ST/g cecal content) detected in the ceca when compared to controls ($2.52 \log_{10}$ ST). This study suggested that incorporation of ECP in the drinking water 24 to 48 h prior to slaughter could reduce *Salmonella* contamination in broilers.

(Key words: salmonella, crop, ceca, chicken, chlorate)

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INTRODUCTION

Foodborne illness continues to be a significant worldwide health problem. Nontyphoid *Salmonella* continues to be the leading foodborne pathogen worldwide, and poultry and poultry products have been reported as one of the prevailing vehicles for salmonellosis (Bean and Griffin, 1990; Persson and Jendteg, 1992).

Several intervention strategies that have been used to control *Salmonella* antemortem contamination of poultry include chemical litter treatments (Huff et al., 1996; Moore and Miller, 1994; Moore et al., 1996; Terzich et al., 1998), medications (Goodnough and Johnson, 1991; Muirhead, 1994), competitive exclusion (Nurmi and Rantala, 1973; Schoeni and Wong, 1994; Corrier et al., 1998; Nisbet et al., 1998; Cox et al., 2001; Stern et al., 2001), vaccinations (Hasan and Curtiss, 1997; Zhang-Barber et al., 1999; Sydenham et al., 2000), and acidification of the drinking water during feed withdrawal (Byrd et al., 2001). Federal government

officials, industry personnel, and consumers continue to demand cost-efficient nonmedicated approaches to control foodborne illness.

Recently, our laboratory investigated an approach that uses a specific intracellular bacterial metabolic pathway to reduce the number of foodborne pathogens in food-producing animals. This approach uses a cost-efficient compound, chlorate ion, that is metabolized within specific bacteria to produce an intracellularly ion (chlorite) that is toxic to the individual cell (Anderson et al., 2000).

Salmonella and *Escherichia coli* O157:H7 are members of the family *Enterobacteriaceae* that also includes some of the most pathogenic bacteria most often encountered in human disease outbreaks including *Shigella*, *Klebsiella*, and *Yersinia* (Brenner, 1984). Most members of the family *Enterobacteriaceae* have respiratory nitrate reductase activity (Brenner, 1984; Stewart, 1988). Respiratory nitrate reductase functions to conserve electrons via electron transport phosphorylation (Cole, 1988; Stewart, 1988). One interesting fact that has been reported is that respiratory nitrate reductase also cometabolizes chlorate to chlorite. Chlorite is cytotoxic to

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¹To whom correspondence should be addressed: byrd@ffsr.tamu.edu.

Abbreviation Key: BGA = brilliant green agar; ECP = experimental chlorate product; FW = feed withdrawal; NA = nalidixic acid; NO = novobiocin; ST = *Salmonella* Typhimurium.

the bacterium. The nitrate reductase bacteria build up toxic levels of chlorite and eventually die (Stewart, 1988). In vitro and in vivo studies in cattle rumen contents indicate that chlorate significantly reduced *Escherichia coli* O157:H7 populations and did not alter the total anaerobic bacterial counts (Anderson et al., 2000, 2002; Callaway et al., 2001). Similarly, sodium chlorate significantly reduced cecal concentrations of *Salmonella* Typhimurium (ST) and *E. coli* orally administered in swine but not potentially beneficial anaerobes (Anderson et al., 2001a,b). Because sodium chlorate effectively reduced *E. coli* O157:H7 and ST in bovine and swine species, we conducted a study to determine whether chlorate would selectively inhibit *Salmonella* in market-age poultry.

MATERIALS AND METHODS

Experimental Design

Experiment 1. The effect of experimental chlorate product (ECP; 1× ECP is equivalent to a 15 mM chlorate ion concentration) on *Salmonella* contamination of crop and ceca contents was evaluated in three replicates. For each flock, 5-wk-old broilers obtained from a commercial processing plant were divided into four groups of 16 birds each and were placed in floor pens on new pine shavings. Free access to water and a balanced unmedicated ration based on corn-soybean meal were provided to birds for 1 wk prior to the start of the experiment. To determine whether or not the broilers were colonized with *Salmonella*, cloacal swabs were taken from each bird and cultured by selective enrichment procedures described below.

Broilers were provided distilled water, 0.5×, 1.0×, or 2.0× ECP. Each bird was challenged with 10⁸ to 10⁹ cfu novobiocin (NO)- and nalidixic acid (NA)-resistant ST 1 d prior to termination of the experiment (d 41). Crops and ceca were collected aseptically from all broilers from each group after 10 h of feed withdrawal (FW; d 42). Crop and a cecum from each sampled broiler were incised aseptically and placed in separate Whirl-pac bags.² Ten milliliters of sterile water was added to each bag, and the sample was stomached for 30 s.³ Samples were then cultured for NO- and NA-resistant ST by selective enrichment procedures and serial dilution and spread plating described below.

To determine if cloacal swabs from broilers were positive for salmonellae other than NA- or NO-resistant isolates used for experimental challenge, 10 mL of tetrathionate broth was added to each swab and incubated for 24 h at 37°C. After incubation, the broth was streaked onto BGA plates free of NA and examined for the presence of typical *Salmonella* colonies. *Salmonella* were not detected in the cloacal samples.

Experiment 2. The effect of ECP on *Salmonella* contamination of crop and ceca contents was evaluated in three replicates. For each flock, 5-wk-old broilers were divided into four groups of 20 birds each and were placed in floor pens on new pine shavings. Free access to water and a balanced, unmedicated ration based on corn-soybean meal were provided to birds for 1 wk prior to introduction of the experiment.

Broilers were provided distilled water or 1× ECP for 10, 24, and 48 h prior to termination of the experiment. Each bird was challenged with 10⁸ to 10⁹ cfu NO- and NA-resistant ST 1 d prior to termination of the experiment. Crops and ceca were collected aseptically from 20 broilers from each group after 10 h of FW (d 42) and sampled as stated in experiment 1.

Salmonella challenge inoculates for experiments 1 and 2 were prepared from a primary poultry isolate of ST obtained from the National Veterinary Services Laboratory, Ames, Iowa. The isolate was selected for resistance to NO and NA and maintained in media containing 20 µg/mL NA and 25 µg/mL NO. Challenge inocula were prepared from an overnight soy broth culture⁴ serially diluted in sterile phosphate-buffered saline. The optical density of the cell dilution was measured with a spectrophotometer⁵ at 625 nm, and the number of cells for each inoculum was determined using a standard curve. The viable cell concentration of the challenge inocula was confirmed by colony counts on BGA plates.⁶

Salmonella Culture Procedures

Crop samples collected during experiments 1 and 2 were cultured by direct plating and serial-dilution plating. After incubation, the broth was streaked onto NA/NO-BGA plates, and typical *Salmonella* colonies from each plate were confirmed biochemically on triple sugar iron⁴ and lysine iron agar⁷ slants (Andrews et al., 1992). Serology was performed on *Salmonella* colonies using *Salmonella* O (Group B; factors 1, 4, 5, and 12) antisera⁶ to confirm ST colonies.

In experiments 1 and 2, an additional 1.0-mL sample of the blended crop contents was serially diluted through three tubes containing 9 mL each of sterile Butterfield's buffer⁸ (1:10, 1:100, and 1:1,000 dilutions). A portion (0.10 mL) was removed from the undiluted crop contents and from each dilution tube and spread-plated onto NA/NO brilliant green agar (BGA) plates to produce final dilutions of 1:10, 1:100, 1:1,000, and 1:10,000. The plates were incubated for 24 h at 37°C, and ST colony-forming units were enumerated.

Statistical Analysis

In experiment 1, all three replicates were combined, and differences among groups in crop and cecal log₁₀ cfu *Salmonella* were determined by one-way ANOVA (Snedecor and Cochran, 1967) using the general linear model procedure in the SAS software (SAS Institute, 1987). Means for each treatment showing significant differences in the ANOVA were further separated using Duncan's multiple range test.

²Nasco, Fort Atkinson, WI.

³Teckmar Stomacher 80, Laboratory Blender, Cincinnati, OH.

⁴Becton Dickinson Company, Sparks, MD.

⁵Spec 20D, Milton Roy, Analytical Products Division, Rochester, NY.

⁶Oxoid, Unipath Ltd., Basingstoke, Hampshire, England.

⁷Difco Laboratories, Detroit, MI.

⁸Sigma Chemical Company, St. Louis, MO.

TABLE 1. Effect of an experimental chlorate compound (ECP) provided in the drinking water during 10 h of feed withdrawal on *Salmonella* Typhimurium crop and cecal colonization in broilers (three trials)^{1,2}

	Fluid consumed (mL/bird per h)	Crop incidence (+/-)	Crop contents (log ₁₀ <i>Salmonella</i> /g)	Cecal incidence (+/-)	Cecal contents (log ₁₀ <i>Salmonella</i> /g)
Control	15.41 + 15.58	24/46 (52.1%)	1.56 + 1.67 ^A	38/46 (82.6%)	3.38 + 1.79
0.5× ECP	36.92 + 22.4	13/47 (27.7%)**	0.87 + 1.56 ^B	35/47 (74.5%)	2.88 + 1.89
1.0× ECP	29.07 + 20.13	13/46 (28.3%)**	0.65 + 1.17 ^B	36/46 (78.3%)	2.89 + 1.59
2.0× ECP	44.4 + 27.82	5/46 (10.9%)***	0.35 + 0.96 ^B	41/46 (89.1%)	3.57 + 1.86

^{A,B}Mean values within the same column with no common superscripts differ significantly ($P \leq 0.05$).

¹(1× ECP is equivalent to a 15 mM chlorate ion concentration).

²A significant difference was found between the number of positive controls and positive, treated crops or ceca (** $P \leq 0.025$; *** $P \leq 0.001$).

Salmonella colonies were logarithmically transformed prior to analysis in order to achieve homogeneity of variance and were expressed as log₁₀ cfu. In experiments 1 and 2, differences among groups in the incidence of *Salmonella* crop and cecal contamination were analyzed with chi square. All analyses were conducted using commercial statistical analysis software (Luginbuke and Schlotzhauer, 1987). All statements of significance are based on the $P < 0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

In broilers given selected concentrations of chlorate product (0.5×, 1×, and 2× ECP) in the drinking water during 10 h of FW, crop *Salmonella* incidence and the number of *Salmonella* colony-forming units recovered from crops of birds administered ECP were statistically ($P < 0.05$) lower than the controls (Table 1). No significant differences were observed in the ceca in experiment 1.

In experiment 2, ECP provided for 24 and 48 h reduced ($P < 0.05$) crop and cecal ST concentrations in market-age broilers (Table 2). These data suggest that ECP treatment may be a practical preharvest intervention strategy. Previously, Davies et al. (1999) suggested that preharvest *Salmonella* control might have the greatest effect when applied in the final period before harvesting. Because poultry have access to water during the FW period and ECP can be administered in the drinking water, ECP could have an impact on the pathogen load that would be transported to

the processing plant. The reduction of foodborne pathogens entering the processing plant should reduce pathogen contamination on the final product.

The use of ECP in a preharvest program will need to be investigated further to maximize its effectiveness in a commercial setting. Chlorate has been used in low concentrations in veterinary and human medicine and has reportedly been approved for use in toothpaste in Europe (Cosmetic Ingredient Review Panel, 1995). The lethal dose of chlorate in humans has been determined to be 1 g/kg BW (Cosmetic Ingredient Review Panel, 1995).

Recent studies in our laboratory have demonstrated that chlorate supplementation effectively decreased *E. coli* O157:H7 and ST in cattle and pigs prior to harvest (Anderson et al., 2001b, 2002; Callaway et al., 2002). These studies demonstrated that chlorate can significantly reduce *E. coli* O157:H7 and ST in gastrointestinal contents while not significantly altering normal total culturable anaerobic bacterial numbers and further demonstrate that chlorate supplementation may be a viable strategy to reduce foodborne pathogens that have the nitrate reductase enzyme.

Government regulations (USDA-Food Safety and Inspection Service, 1996) have brought a need for cost-efficient approaches to reducing foodborne pathogens without dramatically altering present management techniques. The results of the present study suggest a cost-efficient means to reduce food borne pathogens that can be incorporated into existing commercial management procedures.

TABLE 2. Effect of an 1× experimental chlorate compound (ECP) provided in the drinking water for 10, 24, or 48 h on *Salmonella* Typhimurium (ST) crop and cecal colonization in broilers (three trials)^{1,2,3}

	Fluid consumed (mL/bird per h)	Time of consumption (h)	Crop <i>Salmonella</i> incidence (+/-)	Crop <i>Salmonella</i> (log ₁₀ ST/g of crop contents)	Cecal <i>Salmonella</i> incidence	Cecal <i>Salmonella</i> (log ₁₀ ST/g of cecal contents)
Control	9.26	48	22/60 (36.7)	0.92 + 1.43 ^A	32/60 (53.3)	2.52 + 1.71 ^A
Chlorate						
10 h	2.99	10	10/60* (16.7)	0.45 + 1.05 ^{BC}	32/60 (53.3)	2.41 + 1.92 ^A
24 h	7.19	24	3/60*** (5)	0.17 + 0.87 ^C	32/60 (53.3)	1.47 + 1.53 ^B
48 h	10.87	48	1/60*** (1.7)	0.07 + 0.40 ^C	19/60** (31.7)	0.96 + 1.51 ^B

^{A-C}Mean values within the same column with no common superscripts differ significantly ($P \leq 0.05$).

¹(1× ECP is equivalent to a 15 mM chlorate ion concentration).

²Broilers had no access to feed during the last 10 h of the study to simulate commercial feed withdrawal conditions.

³A significant difference was found between the number of positive controls and positive, treated crops or ceca (* $P \leq 0.05$; *** $P \leq 0.001$).

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