Application of Acidified Sodium Chlorite in the Drinking Water to Control *Salmonella* serotype Typhimurium and *Campylobacter jejuni* in Commercial Broilers[1]

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Primary Audience: Veterinarians, Quality Assurance Personnel, Microbiology Laboratory Personnel, Production Managers, Flock Supervisors

SUMMARY

The effect of acidified sodium chlorite (ASC), produced by the combination of sodium chlorite (SC) with citric acid (CA) or sodium acid sulfate (SAS), on *Salmonella* and *Campylobacter* reduction in market age broilers was investigated. In the first experiment, the tolerance to increasing concentrations of SC (0, 100, 300, 600, 1,200, 3,000, and 6,000 ppm) and CA (0, 0.003, 0.01, 0.02, 0.04, 0.08, 0.18, and 0.40%) in the drinking water was assessed. In the second experiment, broilers were fasted for 2 h and orally gavaged with 10^5 cfu of *Salmonella enterica* serotype Typhimurium 1 h before the initiation of drinking water treatments involving 5 concentrations of SC (0, 150, 300, 600, and 1,200 ppm), acidified to pH of 2.6, either with CA or SAS. In the third experiment, 8-d-old chicks were orally challenged with 10^5 cfu of *Salmonella* and 10^5 cfu of *Campylobacter jejuni.* On d 29, birds were provided 3 concentrations of SC (0, 300, and 600 ppm) acidified to pH 2.6 ± 0.1 with only water, CA, or SAS for 5 d. In experiment 2 and 3, the challenge organisms were enumerated in the upper, middle, and lower segments of the digestive tract. Water consumption was depressed significantly at levels of SC above 600 ppm and levels of CA above 0.18%. In experiment 2, SC levels above 600 ppm negatively affected water consumption regardless of the acid used. A level of 600 ppm of ASC was adequate to reduce the transient crop *Salmonella*, whereas 1,200 ppm was required for a significant reduction in the lower digestive tract. In experiment 3, six hundred parts per million of ASC reduced *Salmonella* only in the upper digestive tract. *Campylobacter* counts were not affected by SC treatments in experiment 3 ($P > 0.05$). Preslaughter use of acidified SC in the drinking water may be an effective way to reduce *Salmonella* in the crop, including those that may be picked up through litter consumption and caprophagy.

Key words: broiler, acidified sodium chlorite, *Campylobacter*, *Salmonella*

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DESCRIPTION OF PROBLEM

Foodborne diseases due to *Salmonella* and *Campylobacter* continue to account for a significant portion of human enteritis cases in the United States [1, 2]. Poultry products are important sources of human salmonellosis and campylobacteriosis [3, 4, 5]. Intestinal carriage

[1]The mention of trade names in this publication does not imply endorsement of the products mentioned or exclusion of the products not mentioned.

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of foodborne pathogens by food animals is the most important factor contributing to carcass contamination during processing [6]. The best site for *Salmonella* colonization is the ceca [6, 7], although some studies have also reported a high number of *Salmonella* spp. present in the crop [8]. Different intervention strategies have been recently investigated as possible hurdles to control *Salmonella* and *Campylobacter* in broiler chickens. The addition of fermented liquid feed has been shown to hinder the introduction of *Salmonella* in broiler chickens [4]. In addition, organic acids and their salts have been studied for their antimicrobial properties to control foodborne pathogens in red meat and poultry carcasses. The undissociated portion of the organic acid molecule is believed to be responsible for the antimicrobial effect [9], especially in the upper part of the gastrointestinal tract [10]. However, a recent study described a significant reduction in *Salmonella* counts in the ceca of chickens treated with sodium chlorate in drinking water for 24 to 48 h during feed withdrawal [11]. Reducing or limiting pathogen load in the digestive tract before slaughter will reduce the likelihood of broiler carcass contamination during processing and will result in safer food products [12].

Acidified sodium chlorite (**ASC**; SANOVA) [13] is an antimicrobial agent generated by mixing sodium chlorite (**SC**) at concentrations from 500 to 1,200 ppm with a generally recognized as safe acid, such as citric acid (**CA**). When used in a spray or dip solution, ASC can be used in combination with any generally recognized as safe acid at a level sufficient to achieve a solution pH of 2.3 to 2.9 [14]. Acidified SC has been approved by the US Food and Drug Administration for use in poultry, red meat, comminuted meat products, seafood, and processed fruits and vegetables to reduce bacterial contamination [15, 16]. Acidified SC is currently used in poultry-processing operations to decrease microbial load on carcasses [16].

The purpose of this study was to evaluate the efficacy of providing ASC in the drinking water, before slaughter, to control *Salmonella* and *Campylobacter* in the digestive tract of broiler chickens.

MATERIALS AND METHODS

Experimental Design

Experiment 1. All experiments were performed at the Auburn University Poultry Research Unit. Three hundred, 35-d-old broilers were individually weighed and randomly placed in 60 floor pens (5 birds per pen), which were equipped with 1-gal drinkers. The birds were allowed to acclimate to the new environment for 1 wk before the initiation of the study. The experiment involved 15 randomized treatments (4 replicate pens per treatment) consisting of 7 concentrations (0, 100, 300, 600, 1,200, 3,000, and 6,000 ppm) of SC [17] and 8 concentrations (0, 0.003, 0.01, 0.02, 0.04, 0.08, 0.18, and 0.40%) of CA [18]. The pens were 1.70 × 2.30 m in dimension and were lined with new pine shavings litter. All solutions were prepared with fresh tap water and replaced twice a day. Water intake, feed consumption, weight gain, and mortality were monitored on a pen basis for the 7-d experimental period [19].

Experiment 2. Two hundred forty 35-d-old broilers were individually weighed and placed in Petersime batteries [20] (5 birds per drawer, 12 drawers per battery). Birds were allowed to acclimate to the battery cages for 48 h before the initiation of the experiment. After this acclimation period, all birds were fasted for 2 h, individually weighed, and inoculated (oral gavage) with 1 mL of buffered peptone water [21] containing 10^5 cfu/mL of nalidixic acid-resistant *Salmonella enterica* serotype Typhimurium, provided by N. A. Cox, USDA, Athens, GA. Clean pans were placed under each drawer for the collection of fecal droppings and for visual examination of the excreta. The experiment consisted of 12 drinking water treatments assigned randomly, with 4 replicate battery drawers per treatment. Five concentrations of ASC (0, 150, 300, 600, and 1,200 ppm) were prepared in tap water by the addition of either CA or sodium acid sulfate (**SAS**) as an acidifier [22] to lower the pH of the solution to 2.6 ± 0.1. Two additional treatments of uninoculated (no challenge; plain water) and inoculated (challenged; plain water) groups served as negative and positive controls, respectively. Because ASC solutions are sensitive to light and chemical reactions, treatment solutions were prepared and replaced

every 6 h. Birds were provided the drinking water treatments and a nonmedicated feed ad libitum during the experiment. A water trough was filled with a known amount of water and placed on the floor of the battery room to quantify evaporative losses for each measurement period. Water consumption was measured and corrected for evaporation. At the end of the 24-h experimental period, all birds were euthanized, individually weighed, and the digestive tract aseptically removed. Samples were placed in sterile Whirl-Pak bags [23]. The digestive tract was split into 3 segments, consisting of upper (crop to gizzard), middle (duodenum to ileocecal junction), and lower digestive tract (ceca to cloaca), for the enumeration of *Salmonella*. The excreta from each treatment were visually examined.

Experiment 3. Two hundred 1-d-old chicks were placed in floor pens, as described above, and provided a nonmedicated feed and water ad libitum. Starting on d 8, chicks were provided with drinking water containing 10^5 cfu/mL of *Salmonella* Typhimurium and 10^6 cfu/mL of *Campylobacter jejuni* isolated from broiler carcasses at the Microbiology Laboratory, Department of Poultry Science, Auburn University [24], for a 3-d period. On d 13, cloacal swabs were taken from 20 randomly selected birds to confirm *Salmonella* and *Campylobacter* shedding. On d 29, birds were individually weighed, wing-banded, and assigned to 1 of 9 water treatments in Petersime battery cages (n = 180), with 5 drawers per treatment and 4 birds per pen (Table 1). Treatments consisted of a 3×3 factorial arrangement of 3 concentrations of SC (0, 300, and 600 ppm) and 3 levels of acidification: either no acidification (mixed directly with water) or acidified to a pH of 2.6 ± 0.1 with CA or SAS. After 48 h of acclimation in the batteries, birds were provided treatment solutions for a 5-d experimental period. During this period, a nonmedicated feed was provided ad libitum, and the lighting program was set at 12 h of light per day. Treatment solutions were prepared fresh and replaced every 6 h during the light period. Daily water consumption was calculated by taking into account the amount lost to evaporation. At the end of the experimental period, feed was withheld for 12 h, with water available, to simulate preslaughter feed withdrawal. All birds were then euthanized, individually weighed, and the 3 segments of the digestive tract were aseptically removed as described in experiment 2.

Microbiological Analysis

The digestive tract segments of the birds sampled were pooled in Whirl-Pak stomacher bags [25] on a pen basis. Pooled segments were then weighed, diluted (10×) with buffered peptone water, and then stomached for 1 min to homogenize the tissues. *Salmonella* Typhimurium was semiquantified in homogenates of each digestive tract segment by using a modification of a swab-plate method [26, 27]. *Campylobacter* spp. were enumerated by serial dilution of homogenates in phosphate buffer solution and by transferring 0.1 mL of the dilutions onto *Campylobacter* blood-free agar base plates [21] in duplicates. Plates were then incubated at 42°C under microaerophilic conditions for 24 h. For enrichment, 30 mL of the homogenized sample was mixed in a bag with 30 mL (2×) of Preston broth [28] and then incubated for 24 h at 42°C under microaerophilic conditions. Enumeration was done by direct counting of the presumptive positive colonies and by observation of the colonies under phase-contrast microscopy for confirmation. The countable colonies were transformed into base-10 logarithm colony forming units per gram of cecal content. Negative samples for direct plating that became positive after enrichment were assigned a value of 10 cfu/mL [24].

Statistical Analysis

Bacterial counts (cfu/g of digestive tract material) were transformed to base-10 logarithm colony-forming units per gram before analysis. All data were subjected to ANOVA using the GLM procedure of SAS [29] to test the main effects and their interactions. Means were separated, when significant ($P \leq 0.05$), using Tukey's Studentized Range test. Orthogonal polynomials were used to analyze the concentration effects.

RESULTS AND DISCUSSION

In experiment 1, significant ($P < 0.05$) linear and quadratic responses to increasing concentrations of SC and CA, respectively, were observed for water consumption. Sodium chlorite levels

Table 1. Concentrations of citric acid and sodium chlorite in the drinking water (experiment 1)

Item	BW (g)			Feed consumption[1] (g)	Water consumption[1] (mL)
	Initial	Final	Gain		
Citric acid (%)					
0.000	936[a]	1,148[a]	212[a]	497[a]	563[ab]
0.003	927[a]	1,148[a]	221[a]	492[a]	534[ab]
0.010	965[a]	1,209[a]	244[a]	514[a]	544[ab]
0.020	965[a]	1,187[a]	222[a]	512[a]	643[a]
0.040	963[a]	1,187[a]	224[a]	535[a]	614[a]
0.080	985[a]	1,197[a]	212[a]	528[a]	560[ab]
0.180	1,022[a]	1,240[a]	218[a]	535[a]	503[ab]
0.400	938[a]	1,127[a]	189[a]	477[a]	383[b]
SEM	25	33	16	23	40
Sodium chlorite (ppm)					
0	985[a]	1,184[ab]	199[a]	518[a]	683[a]
100	961[a]	1,154[ab]	193[a]	489[a]	581[ab]
300	979[a]	1,158[ab]	179[a]	456[a]	648[a]
600	1,080[a]	1,201[a]	121[abc]	485[a]	518[ab]
1,200	952[a]	1,100[abc]	148[ab]	417[a]	381[b]
3,000	985[a]	926[bc]	–59[bc]	282[b]	154[c]
6,000	953[a]	872[c]	–81[bc]	190[b]	98[c]
SEM	39	58	45	59	47

[a–c]Means within a column with different superscripts differ significantly ($P < 0.05$).
[1]Total feed or water consumption per bird for the 7 d period.

above 1,200 ppm reduced water consumption and weight gain significantly ($P < 0.05$) during the experimental period (Table 1). Birds provided 3,000 and 6,000 ppm SC actually lost weight during the experiment. Water consumption was significantly reduced with 0.40% CA, but no significant effect on weight gain was detected. There was no mortality encountered during the experiment.

In experiment 2, all negative control birds were negative for *Salmonella*. Positive control birds showed 2.84, 2.31, and 2.56 log cfu/g of *Salmonella* Typhimurium in the upper, middle, and lower digestive tract segments, respectively. There was no effect of acidifier (CA or SAS) detected ($P > 0.05$); therefore, the data for the 2 acids were combined. The water consumption decreased linearly ($P < 0.05$) with increasing concentrations of ASC, but was significantly reduced only when the ASC concentration exceeded 600 ppm (Table 2). *Salmonella* Typhimurium counts (log cfu/g) in the upper, middle, and lower digestive tract are presented in Table 2. A significant reduction ($P < 0.05$) of about 1 log cfu/g in *Salmonella* Typhimurium counts in the upper digestive tract segment was observed in the chickens that received ASC at a level of 600 ppm. The same level of ASC also reduced the *Salmonella* Typhimurium counts in the middle digestive tract. An ASC concentration of 1,200 ppm produced a significant reduction in the lower digestive tract. No observable differences in the excreta appearance and color were detected among the treatments.

The main effects of SC and acidifiers in experiment 3 are presented in Table 3. There was no interaction of the main effects ($P > 0.05$). Sodium chlorite, at a level of 600 ppm, significantly suppressed water intake, regardless of the acidifier used. No significant differences were detected ($P > 0.05$) in total water consumption as affected by the acids, although water consumption was numerically depressed in birds receiving SAS (Table 3).

A significant reduction ($P < 0.05$) in *Salmonella* was only detected with 600 ppm of SC in the upper digestive tract. No significant differences in *Salmonella* were observed for the middle and lower digestive tract. Water administration of ASC at levels up to 600 ppm for a 5-d period did not affect *C. jejuni* levels in the digestive tract. This lack of effect may be attributed to the large numbers of *C. jejuni* that usually

Table 2. *Salmonella* Typhimurium counts (log_{10} cfu/g) in 3 segments of the digestive tract of broilers (experiment 2)

Acidified sodium chlorite[1] (ppm)	Water consumption (mL)	*Salmonella* counts (log_{10} cfu/g)		
		Upper[2]	Middle[3]	Lower[4]
0	160[a]	3.02[a]	2.43[a]	2.92[a]
150	134[ab]	3.05[a]	1.84[a]	2.49[a]
300	130[ab]	2.36[a]	1.79[a]	2.96[a]
600	126[ab]	1.32[b]	1.29[b]	2.47[a]
1,200	104[b]	0.43[b]	1.00[b]	1.51[b]
SEM	10	0.66	0.73	0.74
P-value	0.045	0.036	0.691	0.650

[a,b]Means within a column with different superscripts differ significantly ($P < 0.05$) .
[1]Acidified to a pH of 2.6 ± 0.1 by either citric acid or sodium acid sulfate. Acidifier effect was not significant ($P > 0.05$) and was combined.
[2]Segment of the digestive tract from the crop to gizzard.
[3]Segment of the digestive tract from duodenum to ileocecal junction.
[4]Segment of the digestive tract from ceca to cloaca.

colonize the lower gastrointestinal tract of chickens (Table 3).

Effect of ASC was assessed against *Salmonella* that were transient in the digestive tract (experiment 2) or against digestive tract colonized with *Salmonella* and *Campylobacter* (experiment 3). Acidified SC was administered in the drinking water either for short (24 h in experiment 2) or extended (5 d in experiment 3) periods of time to market age broilers in this study. Efficacy of ASC was also evaluated in 3 distinct regions of the digestive tract. Acidified SC concentrations of above 600 ppm consistently resulted in the depression of water intake by the birds. However, at a 600-ppm level, ASC was effective against the transient and colonized *Salmonella* in the upper digestive tract. It is unlikely that lack of water could have played a role in this effect. Withholding feed and water from market age broilers typically increases colonization and shedding of *Salmonella* [30] and *Campylobacter* [31] in market age broilers. Lack of feed and water alters the normal protective microflora and increases the pH of crop through a reduction in lactic acid produced by *Lactobacilli* [32]. Crop has been shown to be a critical source of *Salmonella* before slaughter and during evisceration [8, 11, 12]. Acidified SC is a

Table 3. *Salmonella* Typhimurium and *Campylobacter jejuni* counts (log cfu/g) in the 3 segments of the digestive tract of broilers (experiment 3)

Treatments	Water consumption[1] (mL)	*Salmonella* (log cfu/g)			*Campylobacter* (log cfu/g)		
		Upper[2]	Middle[3]	Lower[4]	Upper[2]	Middle[3]	Lower[4]
Sodium chlorite (ppm)							
0	1,466[a]	3.52[a]	2.34[a]	0.89[a]	4.76[a]	4.72[a]	5.02[a]
300	1,398[ab]	3.09[ab]	1.90[a]	1.37[a]	4.52[a]	4.19[a]	4.74[a]
600	1,312[b]	2.61[b]	2.34[a]	1.39[a]	4.32[a]	4.47[a]	5.36[a]
Acidifier							
None	1,456[a]	3.69[a]	2.45[a]	1.29[a]	4.52[a]	3.94[a]	4.22[a]
Citric acid	1,467[a]	3.08[a]	1.88[a]	1.46[a]	4.38[a]	4.44[a]	4.78[a]
Sodium acid sulfate	1,326[a]	3.00[a]	2.46[a]	1.03[a]	4.59[a]	4.16[a]	5.12[a]
SEM	166	1.07	1.00	1.06	1.23	1.28	1.04
Interaction	NS	NS	NS	NS	NS	NS	NS

[a,b]Means within a column and treatment group with different superscripts differ significantly ($P < 0.05$).
[1]Total amount for the 5-d experimental period.
[2]Segment of the digestive tract from the crop to gizzard.
[3]Segment of the digestive tract from duodenum to ileocecal junction.
[4]Segment of the digestive tract from ceca to cloaca.

potent antimicrobial, and its use in the drinking water may be a significant preslaughter intervention tool. The failure to consume high concentrations of ASC and potential neutralization by the microflora may have contributed to a lack of antimicrobial effect against *Salmonella* in the middle and lower digestive tract. Microencapsulation in a lipid shell may increase efficacy by providing high concentration of the ASC through the digestive tract [33]. Acidified SC was not effective as a preslaughter treatment of controlling *C. jejuni* in this study.

CONCLUSIONS AND APPLICATIONS

1. When ASC (SANOVA) was added in the drinking water at levels beyond 600 ppm, water consumption was significantly reduced.
2. Acidified SC, when provided for 24 h or 5 d at a level of 600 ppm in the drinking water, significantly reduced *Salmonella* load in the upper digestive tract.
3. Acidified SC did not have an effect on *C. jejuni* in the digestive tract of market age broilers.
4. Preslaughter inclusion of ASC in the drinking water may be an effective way to reduce *Salmonella* contamination during evisceration.

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