ENVIRONMENT, WELL-BEING, AND BEHAVIOR

The effect of sodium bisulfate on *Salmonella* viability in broiler litter

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ABSTRACT Controlling *Salmonella* populations on commercial broiler grow out farms is a crucial step in reducing *Salmonella* contamination in processing plants. Broiler litter harbors many species of pathogenic bacteria including *Salmonella*. Sodium bisulfate has been shown to reduce concentration of bacteria in broiler litter. In experiments 1 and 2, sodium bisulfate was applied to broiler litter at rates that are comparable to what is commonly used by the poultry industry: 22.7, 45.4, and 68.0 kg/92.9 m^2. After application, sodium bisulfate was mixed into the litter. In experiments 3 and 4, sodium bisulfate was applied at 45.4 kg/92.9 m^2 to the surface of the litter. For all experiments, a cocktail of 5 *Salmonella* serovars was applied to the litter. Ammonia, pH, moisture, and water activity measurements were taken; additionally, total aerobic, anaerobic, enteric, and *Salmonella* concentrations were determined at 0, 24, and 96 h. In experiments 1 and 2, *Salmonella* concentrations were higher for treated litter than the control at 24 and 96 h ($P < 0.001$). In experiments 1 and 2, litter pH was lower for treated litter at 24 and 96 h; lowest pH was observed with the 68.0 kg/92.9 m^2, with a pH of 5.95 ($P < 0.001$). In experiments 3 and 4, litter pH was lowered for treated litter to 2.1 ($P < 0.001$). Even this lower pH did not reduce *Salmonella* concentrations compared with the control ($P = 0.05$). The decreased litter pH appeared to be responsible for increased viability of *Salmonella*. This research shows that the lowering of litter pH, which decreases litter ammonia production, could actually lead to an increased survivability of certain bacteria, such as *Salmonella*.

Key words: sodium bisulfate, broiler litter, *Salmonella*, management

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INTRODUCTION

Salmonella spp. account for an estimated 1.4 million cases of food-borne illnesses in the United States per year, with approximately 95% being food-borne in origin (Mead et al., 1999). *Salmonella* recovery in broiler litter is sporadic, given that it is not normally in the litter. Lu et al. (2003) detected no *Salmonella* by culture methods and PCR in broiler litter. In contrast, *Salmonella* was detected in 83% of farms that reuse litter and 68% of farms that dispose of litter after a single flock of broilers on Australian broiler farms (Chinivasagam et al., 2010). The concentrations of *Salmonella* in each litter sampled varied, with almost a 5-log difference between some litter sampled; the researchers attributed this to several factors, including transmission from parent flocks and environmental conditions (Chinivasagam et al., 2010). Comparison of soil characteristics, such as pH, and their effects on *Salmonella* incidence in broiler litter yielded 29% of litter samples being positive, with higher *Salmonella* concentrations observed with lower soil pH (Volkova et al., 2009).

In a survey of bacteria in poultry litter, it was found that samples with lower pH had lower bacterial counts (Terzich et al., 2000). The use of litter amendments to reduce ammonia emissions is a common practice in broiler houses (Terzich et al., 1998). These amendments reduce ammonia by direct chemical interactions, reduction in litter pH, and reduced numbers of ammonia-producing bacteria (Terzich et al., 1998).

Although litter amendment effects on overall bacterial numbers have been shown, its use against *Salmonella* has not been fully characterized. Payne et al. (2007) found the greatest reduction in *Salmonella* populations with litter pH of 4 or less and no reduction at pH of 7 and 9. When birds were raised on litter treated with varying levels of aluminum sulfate (3.63 or 7.26 kg/4.6 m^2) or sodium bisulfate (1.13 or 1.81 kg/4.6 m^2), which lowered litter pH to less than 5, there was no reduction in *Salmonella* concentrations or frequency for these birds' whole-carcass rinses or ceca samples (Line, 2002). Commercially available sodium bisulfate applied at the manufacturer's guidelines (2.27 kg/9.29 m^2) also yielded no effect on *Salmonella* populations in broiler litter, even though a pH of 1.2 was achieved in the treated litter (Pope and Cherry, 2000). Payne et al. (2002) used granulated sulfuric acid and sodium bisulfate at various application levels on litter that had been

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placed in baking pans and inoculated with *Salmonella*. High levels of both granulated sulfuric acid and sodium bisulfate (45.3 kg/92.9 m^2) were needed to obtain litter pH low enough to reduce *Salmonella* concentration (Payne et al., 2002).

Salmonella has various mechanisms by which it can survive acidic environments, such as in the stomach (Foster and Hall, 1990; Lee et al., 1994; Perez and Groisman, 2007). Acid tolerance of *Salmonella* has been linked to synthesis of several proteins that protect cells in acidic environments. These mechanisms when activated in such environments allow *Salmonella* to survive in pH as low as 3.3 (Foster and Hall, 1990; Lee et al., 1994). Virulence of *Salmonella* has been linked to acid resistance (Foster and Hall, 1990). The researchers found that virulent strains of *Salmonella* were more resistant to acidic environments than avirulent strains.

The goal of this research was to determine any differences for varying application rates and application methods of sodium bisulfate to broiler litter and to observe any differences in *Salmonella* concentrations.

MATERIALS AND METHODS

Experiments 1 and 2 were performed in May and June of 2010. Experiments 3 and 4 were performed in February and April of 2011. All experiments were identical, except that a different litter source was used in each trial. Each experiment was carried out for 96 h from initial addition of *Salmonella* spp. and treatment with sodium bisulfate.

Five *Salmonella* species used in these experiments were *Salmonella enterica* serovar Enteritidis, *Salmonella* Montevideo, *Salmonella* Heidelberg, *Salmonella* Typhimurium, and *Salmonella* Kentucky. These *Salmonella* were isolated from either commercial broiler farms or processing plants. *Salmonella* were isolated from carcass rinses, cloacal swabs, or environmental samples and enriched in tetrathionate broth at 37°C for 48 h. After enrichment, XLT4 plates were streaked for isolation, using standard protocols, and then incubated at 37°C for 24 h. Suspect *Salmonella* colonies were identified using a bioMerieux API-20E Microbial Identification Kit (bioMerieux, Durham, NC). Cultures were stored frozen at −80°C until needed. When needed, frozen cultures were streaked onto tryptic soy agar containing 5% SRBC and incubated at 37°C for 24 h. A single colony was picked at 24 h and streaked onto brain heart infusion agar slants and then incubated at 37°C for 24 h. Slants were kept for future use. Brain heart infusion broth cultures were made from streaking a colony from the brain heart infusion agar slants and incubating them at 37°C for 24 h with constant shaking. For each of the 5 serovars, 6 mL of brain heart infusion broth was taken and combined into a 30-mL cocktail that would be applied to litter.

Sufficient quantity of pine-shavings broiler litter that had been used for at least 2 grow-out periods on the Auburn Poultry Research Farm was obtained, weighed, and stored in a heated room (27°C). Litter moisture was determined at least 5 d before initiation of each experiment by taking 3 samples from the litter and drying as described below. From the percent moisture obtained, it was possible to determine the amount of water to add to equilibrate the litter moisture to 25 to 30%. This water was added 72 h before initiation of the experiment. As water was added, the pile was turned to allow even dispersal of water. The litter was kept in a heated (27°C) room for the duration of the experiment to facilitate ammonia production.

Initially, 3 kg of litter was placed into one of twelve 0.53 × 0.39 m (0.2 m^2) Rubbermaid plastic tubs. Litter was allowed to equilibrate for 1 h before any measurements were taken or treatments added. After equilibration, initial samples were taken and the *Salmonella* cocktail and then sodium bisulfate were applied to the litter. Each litter replication received 30 mL of *Salmonella* cocktail at 10^9 cfu/mL.

For experiments 1 and 2, sodium bisulfate was applied to the litter in 3 replicate tubs at a rate of either 22.7, 45.4, or 68.0 kg/92.9 m^2. Each application rate was applied to 3 boxes, with 3 untreated replications serving as controls. Sodium bisulfate was applied to the litter by evenly distributing it on top of the litter and mixing it into the litter by hand; *Salmonella* cultures were added similarly. Grab samples were taken in which approximately 50 g of litter was collected by hand from each of the 4 corners and the center of the plastic tub. For each tub, the 5 samples were thoroughly mixed before analysis. After sample removal, the remaining litter was evenly spread in the container to maintain a flat surface area.

Sodium bisulfate in experiments 3 and 4 was applied at a rate of 45.4 kg/92.9 m^2 for all treatments. For treatment 1, litter was allowed to equilibrate for 1 h in a plastic tub, and then the *Salmonella* cocktail and sodium bisulfate were added and mixed into the litter as described above. For treatments 2 and 3, litter was placed into tubs, and *Salmonella* was added and then allowed to sit for 24 h. After 24 h, sodium bisulfate was applied to the litter surface and not mixed into the litter. For the control, litter was allowed to equilibrate for 1 h, after which the *Salmonella* cocktail was added. Samples from treatments 1 and 3, and the control were taken by the grab method described for trials 1 and 2. Samples from treatment 2 were taken by scraping the heel of the hand across the surface of the litter. Treatments and sampling methodology are given in Table 1.

Litter pH was obtained by taking 5 g of litter to which 45 mL of distilled water was added after being allowed to equilibrate for 1 h; pH readings were taken with a Fisher Scientific Accumet pH meter 50 (Denver Instrument Company, Bohemia, NY). Litter moisture was obtained by taking 10 g of litter and drying for 48 h at 100°C. Using the weight loss between initial and dried litter, the percentage of moisture was calculated.

Ammonia measurements were taken at 0, 24, 48, 72, and 96 h posttreatment. Dräger Chip Measuring Sys-

Table 1. Description of treatments used in experiments 3 and 4

Item	Treatment			
	1	2	3	4
Sodium bisulfate application method	Thoroughly mixed into litter by hand	Surface applied	Surface applied	None
Hours between *Salmonella* application and sodium bisulfate application	0	24	24	0
Sampling technique	Grab sampling from the 4 corners and center of box	Surface scrape with heel of hand	Grab sampling from the 4 corners and center of box	Grab sampling

tem was used with a plastic rectangular tub (17 × 25 × 12 mm) attached to a pump (Dräger Safety Inc., Lübeck, Germany). The tub was placed directly onto the litter and the pump was allowed to run for 60 s before a measurement was taken. After each measurement, the collecting tub was purged with fresh air by moving the tub with the pump on up and down 4 to 5 times.

For bacterial recovery, 10 g of litter from 0-, 24-, and 96-h samples was obtained. This litter was placed in sterile filter bags with 90 mL of PBS and stomached for 60 s. Additional serial dilutions were made by taking 1 mL and adding it to 9 mL of PBS, after which the dilution was vortexed for 5 s. This was carried out for sequential dilutions, until the appropriate dilutions had been achieved. For each dilution, 0.1 mL was spread-plated, in duplicate, onto appropriate media types for each specific bacterium. These plates were inverted and incubated at 37°C for 24 h; aerobic bacteria, *Salmonella,* and *E. coli* were incubated in a normal atmosphere, whereas anaerobic bacteria were incubated in an atmosphere consisting of 5% CO_2, 5% H_2, and the balance in N_2. Because bacterial recovery methods were not performed strictly anaerobically, any anaerobic bacteria recovered were classified as either facultative anaerobes or anaerobic spore formers.

Media used were plate count agar (Difco, Becton, Dickinson and Company, Franklin Lakes, NJ) for total aerobic bacteria, anaerobic agar (Difco) for total anaerobic bacteria, and MacConkey's agar (Difco) for *E. coli*. *Salmonella* recovery was performed by 2 methods, direct plating and enrichment. Direct plating was performed as described onto xylose-lysine-tergitol 4 (**XLT4**) agar (Difco). Enrichments were performed in tetrathionate broth (Difco) for 48 h and then streaked onto XLT4 to ensure that any sample with *Salmonella* concentrations below enumeration limits were not falsely identified as negative.

Data were analyzed using SAS 9.2 using the GLM at a 0.05 level of significance (SAS Institute, 2009). Litter moisture percentages were arcsine-transformed and colony-forming units per gram of litter were transformed to $\log_{10}$. Means were separated using Tukey's honestly significant difference test. Data were analyzed for effects attributable to sodium bisulfate level or application method, sampling time, trial, and the interaction between sodium bisulfate level or application level and sampling time. If no difference was found due to main effects or interactions between experiments, data were combined.

RESULTS

Experiments 1 and 2

Total aerobic counts were not affected by level of sodium bisulfate added to the litter ($P > 0.05$). There was an increase in total aerobes between 24 and 96 h: 8.57 and 9.44 $\log_{10}$ cfu/g of litter, respectively ($P < 0.001$). Total facultative anaerobes were reduced by the 22.7 kg/92.9 m^2 treatment ($P = 0.037$).

Escherichia coli were affected by sodium bisulfate level ($P = 0.02$) and trial ($P < 0.001$). Sodium bisulfate applied at 45.4 kg/92.9 m^2 had higher levels of *E. coli* than the control, 6.4 and 4.8 $\log_{10}$ cfu/g of litter, respectively. *Escherichia coli* levels were only affected by trial ($P < 0.001$), where in the second trial there was no recoverable *E. coli* (data not shown).

Salmonella concentrations before the addition of the *Salmonella* cocktail (0 h sampling) were below detectable limits, by both plating and enrichment. At 24 h after the initial treatment, *Salmonella* concentration for the control was 3.0 $\log_{10}$ cfu/g of litter, whereas concentrations for 22.7, 45.4, 68.0 kg/92.9 m^2 application rates were 5.7, 5.9, and 5.0 $\log_{10}$ cfu/g of litter, respectively. At 96 h, control *Salmonella* concentrations were 0.83 $\log_{10}$ cfu/g of litter, whereas concentrations for 22.7, 45.4, 68.0 kg/92.9 m^2 were 3.57, 4.41, 4.35 $\log_{10}$ cfu/g of litter.

An interaction for *Salmonella* was observed between treatment level and sampling time, as shown in Figure 1A ($P = < 0.001$). At 24 h after the sodium bisulfate treatment and *Salmonella* addition, *Salmonella* concentrations were higher in sodium bisulfate-treated litter, regardless of application rate, than in control litter by at least 2 log. At 96 h, *Salmonella* in control, 22.7, and 45.4 kg/92.9 m^2 treatments had decreased from 24 h. The highest application rate (68.0 kg) had decreased *Salmonella* concentrations but not statistically lower than other treated groups. In the untreated control, *Salmonella* levels had been reduced to less than 1 log. This implies that the sodium bisulfate at any level of application can prolong *Salmonella* survival and viability in litter.

Litter pH was affected by the interaction of sodium bisulfate level and time, as shown in Figure 1B ($P <$

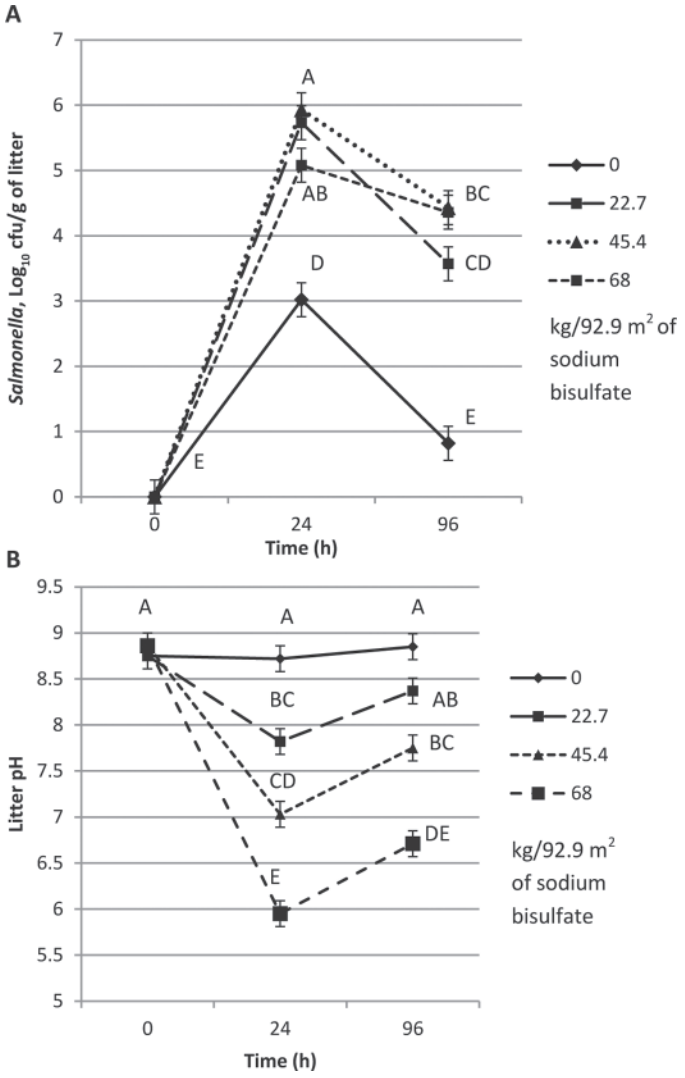

Figure 1. *Salmonella* concentrations and litter pH for experiments 1 and 2. Time posttreatment given in hours on the horizontal axis and $\log_{10}$ cfu/g of litter (panel A) or litter pH (panel B) given on the vertical axis. A) *Salmonella* concentration was affected by a 2-way interaction between sampling time and sodium bisulfate application rate ($P < 0.001$). Populations decreased to barely detectable levels, whereas *Salmonella* concentrations in sodium bisulfate-treated litter remained high for the duration of the experiment. B) Litter pH was affected by a 2-way interaction between sampling time and sodium bisulfate supplication rate ($P < 0.001$). An increased amount of sodium bisulfate applied led to a decrease in litter pH at both sampling times. Litter pH decreased from 0 to 24 h and increased from 24 to 96 h. $^{A–E}$Numbers with different letters are different ($P < 0.05$).

0.001). No decrease was found in the control litter. For the 22.7 and 45.5 kg application rates, pH was less than the control and higher than the 68.0 kg rate for all time periods. These 2 treatments were never different from each other. A decrease was observed for 22.7, 45.4, 68.0 kg/92.9 m^2 application rates from 0 h to 24 h, and a numerical increase was observed from 24 to 96 h.

At 0 h, no differences were observed between sodium bisulfate application rates for ammonia levels ($P = 0.968$). At 24, 48, 72, and 96 h, the control litter had higher ammonia levels than the treated litter ($P < 0.001$). At 24 h, no differences were observed for the treated litter. At 48 h, the 68 kg/92.9 m^2 application was lower than the 22.7 kg/92.9 m^2 rate: 22.4 and 67.5 ppm, respectively ($P < 0.001$). At 72 h, both the 45.4 and 68 kg/92.9 m^2 were lower than the 22.7 kg/92.9 m^2 rate: 52.9, 27.0, and 85.8, respectively ($P < 0.001$). At 96 h, ammonia level for the 68 kg/92.9 m^2 application rate was lower than the 22.7 kg/92.9 m^2 rate: 37.0 and 86.0 ($P < 0.001$; data shown in Table 2).

Experiments 3 and 4

In experiments 3 and 4, ammonia levels were reduced 24 h after *Salmonella* addition for all treatments, and the control treatments had higher levels of ammonia when compared with treatments 1, 2, and 3 ($P < 0.001$). There was no difference in ammonia between any of the sodium bisulfate treatments at each sampling time (data not shown).

For both experiments 3 and 4, initially there were no differences between treatments for total aerobes and anaerobes, also no *Salmonella* was recovered (aerobes $P = 0.80$, anaerobes $P = 0.801$).

In experiment 3, at 24 h posttreatment, no difference was observed for aerobic bacteria ($P = 0.25$) and *Salmonella* ($P = 0.088$). A difference was observed for anaerobic bacteria ($P = 0.004$) and *E. coli* ($P < 0.001$). Anaerobic bacteria were higher for treatments 1 and 4, 7.1 and 6.9 $\log_{10}$ cfu/g of litter, than treatment 2, 5.2 $\log_{10}$ cfu/g of litter. No *E. coli* was recovered for treatment 2, whereas treatments 1, 3, and 4 had *E. coli* concentrations of 5.1, 4.2, and 4.9 $\log_{10}$ cfu/g of litter, respectively.

Table 2. Average ammonia levels (ppm) for each treatment at 0, 24, 48, 72, and 96 h for experiments 1 and 2

	Time (h) posttreatment				
Item	0	24	48	72	96
Sodium bisulfate (kg/92.9 m^2)					
0 (control)	426.6	199.8[a]	207.0[a]	158.1[a]	136.5[a]
22.7	411.3	41.6[b]	67.5[b]	85.8[b]	86.0[b]
45.4	422.3	27.0[b]	47.7[bc]	52.9[c]	54.97[bc]
68	436.6	16.6[b]	22.4[c]	27.9[c]	37.0[c]
SEM	36.6	7.6	8.0	7.4	8.1
P-value	0.968	<0.001	<0.001	<0.001	<0.001

$^{a–c}$Numbers with different superscripts are different ($P < 0.05$).

Table 3. Difference in *Salmonella* concentrations ($\log_{10}$ cfu/g of litter) and litter pH for experiments 3 and 4

	Experiment 3			Experiment 4		
Item	0 h	24 h	96 h	0 h	24 h	96 h
Treatment						
1	0	4.0	4.5[a]	0	1.3[b]	4.0[a]
2	0	1.1	0.7[b]	0	2.5[ab]	2.4[b]
3	0	2.7	2.0[ab]	0	3.8[a]	2.5[b]
4	0	3.7	2.9[ab]	0	3.2[ab]	2.0[b]
SEM	0	0.42	0.47	0	0.26	0.12
P-value	1	0.09	0.05	1	0.027	0.015

[a,b]Numbers with different superscripts at each time are different ($P < 0.05$).

In experiment 3, at 96 h, differences were observed for aerobic bacteria ($P = 0.03$), anaerobic bacteria ($P = 0.029$), *E. coli* ($P = 0.002$), and *Salmonella* ($P = 0.05$). Total aerobic and anaerobic bacteria were higher in treatment 4 than treatment 2, 9.6 and 7.0 $\log_{10}$ cfu/g of litter for aerobes and 6.6 and 4.6 $\log_{10}$ cfu/g of litter for anaerobic bacteria. Again at 96 h, no *E. coli* was recovered from treatment 2, whereas treatment 3 had 1.2 $\log_{10}$ cfu/g of litter; treatment 1 had 3.3 $\log_{10}$ cfu/g of litter and treatment 4 was the highest with 4.9 $\log_{10}$ cfu/g of litter. *Salmonella* concentrations in treatment 1 were higher than in treatment 2: 4.5 and 0.6 $\log_{10}$ cfu/g of litter, respectively.

In experiment 4, at 24 h posttreatment, there were differences attributable to treatment for aerobic bacteria ($P = 0.018$) and *Salmonella* ($P = 0.027$), but no difference was observed for anaerobic bacteria ($P = 0.614$). Treatment 4 had a higher concentration of total aerobic bacteria than treatment 2: 8.9 and 8.4 $\log_{10}$ cfu/g of litter, respectively. *Salmonella* concentration was higher in treatment 3 than treatment 1: 3.7 and 1.3 $\log_{10}$ cfu/g of litter, respectively.

At 96 h, a difference was observed for aerobic bacteria ($P = 0.009$), anaerobic bacteria ($P = 0.031$), and *Salmonella* ($P = 0.015$). Total aerobic bacteria were highest for treatment 1: 9.2 $\log_{10}$ cfu/g of litter. No difference was observed between treatments 2, 3, and 4 with 8.5, 8.7, and 8.4 $\log_{10}$ cfu/g of litter, respectively. Total anaerobic bacteria were higher in treatment 2 than treatment 4: 6.0 and 5.1 $\log_{10}$ cfu/g of litter. *Salmonella* were higher in treatment 1 than treatments 2 and 4: 4.0 and 2.4 and 2.0 $\log_{10}$ cfu/g of litter. *Salmonella* data for experiments 3 and 4 given in Table 3.

There was a 2-factor interaction between treatment and time for experiments 3 and 4 with regard to litter pH ($P < 0.001$). In both experiments, litter pH was reduced from 0 to 24 h in litter that had been treated with sodium bisulfate. Regardless of sampling time, litter pH in sodium bisulfate-treated litter was less than in the control litter. In experiment 3, treatments 2 and 3 had lower litter pH than in treatment 1. In experiment 4, treatment 2 had lower litter pH than both treatments 1 and 3, which were not different from each other. At 96 h, litter pH in experiment 3 was lower in treatments 2 and 3 than in treatment 1. In experiment 3, litter pH was lower in treatment 2 than treatment 1. Litter pH for experiments 3 and 4 is shown in Table 4.

No differences were observed between treatments for moisture content for any experiment (experiments 1 and 2, $P = 0.5288$; experiment 3 and 4 data not shown).

DISCUSSION

As observed in experiments 1 and 2, as litter pH was decreased because of the application of sodium bisulfate, the survivability of *Salmonella* increased. Past studies have shown application of sodium bisulfate to decrease the pH of litter to much lower levels than found in this study (Payne et al., 2002, 2007). Payne et al. (2002) found that after 24 h at the application rate of 45.4 kg/92.9 m^2, litter pH was reduced to 3.4. This application rate was also found to reduce *Salmonella* concentrations; this reduction in pH and *Salmonella* is in disagreement with the findings presented here. The 45.4 kg/92.9 m^2 application rate for experiments 1 and 2 did not reduce litter pH as much, to only 7.03 at 24 h.

Table 4. Two-factor interaction between time and treatment for pH for experiments 3 and 4

	Experiment 3			Experiment 4		
Item	0 h	24 h	96 h	0 h	24 h	96 h
Treatment						
1	8.5[a]	5.9[b]	5.6[b]	8.9[a]	6.8[bc]	7.5[ab]
2	8.5[a]	2.7[cd]	2.1[d]	8.9[a]	3.7[d]	5.3[cd]
3	8.5[a]	3.4[c]	2.6[cd]	8.9[a]	5.9[bc]	6.5[bc]
4	8.5[a]	7.9[a]	7.9[a]	8.9[a]	8.9[a]	8.8[a]
SEM		0.117			0.181	
P-value		<0.001			<0.001	

[a–d]Numbers with different superscripts at each time are different ($P < 0.05$).

A pH of 7.03 is a physiologically neutral pH and would benefit *Salmonella.* Payne et al. (2002) attributed the observed decrease in *Salmonella* to acidified litter, with higher sodium bisulfate application rates. In agreement with Payne et al. (2002), this study found that when litter pH is reduced to a neutral pH, *Salmonella* viability is not reduced but may actually be increased. Line (2002) found no difference in *Salmonella* colonization in broilers reared on litter treated with sodium bisulfate at the application rates of 22.9 and 36.2 kg/92.9 m^2. These researchers also found a reduction in litter pH but no reduction in *Salmonella* colonization. Why the litter pH observed here was so much higher than other reported litter pH with the same or very similar sodium bisulfate application rates is due to application and sampling method. Sodium bisulfate was mixed into the litter and grab samples were taken. In previous studies, sodium bisulfate was surface applied and surface samples were taken (Payne et al., 2002).

In experiments 3 and 4, application method of sodium bisulfate, either surface applied or mixed into litter, decreased ammonia levels after initial treatment, but surprisingly, there was no difference between the 2 methods. It was expected that surface-applied sodium bisulfate would reduce ammonia emissions more effectively. Application method did affect *Salmonella* concentrations at 24 and 96 h after treatment. In experiment 4, at 24 h, treatment 1 had lower concentrations of *Salmonella* compared with treatment 3. In experiments 3 and 4, the situation was reversed at 96 h, with treatment 1 having higher concentrations of *Salmonella*. In experiment 3, the difference in *Salmonella* levels between treatment 1 and treatment 2 can be attributed to sampling method, as there was no difference in *Salmonella* between surface-applied sodium bisulfate with grab sampling and mixed in sodium bisulfate or surface application with the heel scrape sampling technique. This demonstrates a potential bias due to sampling technique. In experiment 4, despite differences in pH at 24 h between treatments, there was no difference for *Salmonella*. An increase in *Salmonella* was observed between 24 h and 96 h when sodium bisulfate was mixed into the litter; however, no treatment had lower *Salmonella* levels than the control.

Salmonella's acidic pH survival mechanisms have been observed to allow it to survive in acidic environments as low as pH 3.3 (Foster and Hall, 1990; Lee et al., 1994). *Salmonella* that has been subjected to acidic pH levels have been observed to exhibit increased virulence (Foster and Hall, 1990) and increased antibiotic resistance (Perez and Groisman, 2007). The pH values observed in experiments 1 and 2 did not reach acidic levels but remained at a neutral level; the highest application rate of 68.0 kg/92.9 m^2 had the lowest pH at 5.9, a slightly acidic but still neutral pH. In experiments 3 and 4, litter pH reached an acidic level, the lowest at 2.11, but even this pH still did not reduce *Salmonella* concentration. The genetic defense mechanisms of *Salmonella* should have been able to resist this pH change and allow the bacterium to survive in litter as observed. Data presented here show that in the complex environment of broiler litter, a combination of treatments or approaches is needed to reduce ammonia as well as potentially pathogenic bacteria.

Commercially available sodium bisulfate is commonly used in broiler grow-out houses to reduce ammonia volatilization from litter; the data in this paper show that in this regard the chemical works very well. What the data presented in this paper dispute is the ability of sodium bisulfate to reduce *Salmonella* in broiler litter. Previous research has indicated application of sodium bisulfate to substantially reduce or eliminate *Salmonella*; however, the observations made here showed no reduction in *Salmonella* regardless of application method or application rate. This research suggests the need for further research into the efficacy of sodium bisulfate to control *Salmonella* in broiler litter.

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