ENVIRONMENT AND HEALTH

Campylobacter and *Salmonella* Populations Associated with Chickens Raised on Acidified Litter

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ABSTRACT Two commercially available acidifying litter treatments, aluminum sulfate (alum) and sodium bisulfate, were tested to determine their effects on *Campylobacter* and *Salmonella* colonization frequencies and populations associated with broilers raised on treated pine litter. To produce contaminated litter, broiler chicks were inoculated with two bacterial cocktails (multistrain mixtures of campylobacters and salmonellae) and were allowed to shed on the litter for about 6 wk. Upon bird removal, litter in duplicate pens was immediately treated with two levels of aluminum sulfate [3.63 or 7.26 kg/4.6 m^2 (8 or 16 lb/50 ft^2)] or sodium bisulfate 1.13 or 1.81 kg/4.6 m^2 (2.5 or 4 lb/50 ft^2)]; untreated pens served as controls. Immediately after treatment, day-of-hatch chicks were released in the pens. Frequency and populations of *Campylobacter* and *Salmonella* associated with ceca and whole carcass rinse (WCR) samples were determined for each duplicate pen at Weeks 1, 4, and 6. Both levels of the aluminum sulfate and sodium bisulfate litter treatments tested significantly ($P < 0.05$) reduced *Campylobacter* colonization frequency and populations in the ceca. Significantly, no *Campylobacter* was recovered from WCR samples associated with high level aluminum sulfate-treated pens at any time; although control pens were 95, 78, and 38% positive at Weeks 1, 4, and 6, respectively. *Salmonella* colonization frequency and populations in the ceca were not significantly decreased by any of the treatments investigated. Although effective pathogen control will most likely require a combination of interventions, acidifying treatment of litter in poultry production may serve as a means to help control *Campylobacter* and to reduce horizontal transmission of pathogens in broiler flocks.

(*Key words*: acidified litter, Salmonella, Campylobacter, colonization, broiler)

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INTRODUCTION

There are numerous challenges presented when attempting to reduce human exposure to foodborne pathogens, especially when those pathogens are associated with foods of animal origin. Reduction of pathogens on farms is necessary if cleaner products are expected after processing; however, few effective intervention methods are presently available for use during poultry production.

Several poultry litter treatments intended primarily for use in reducing ammonia levels, promoting animal health, or inhibiting mold growth do exist in the poultry industry, including aluminum sulfate (alum) and sodium bisulfate. Because many of these products are acidic compounds, it is reasonable to suspect that proper application could significantly lower pH and water activity of poultry litter, conditions that directly affect the survivability of microorganisms present in the litter. Reducing pathogen populations in the litter could, in turn, reduce horizontal transmission of pathogens among the flock and result in a lower frequency of intestinal colonization and fewer bacteria on the broiler carcasses entering the processing facility. Reducing pathogen populations in the litter is also important when the litter is removed and used as fertilizer. Potential contamination of irrigation water through runoff and subsequent contamination of minimally processed foods such as fresh fruits and vegetables is an increasing food safety concern.

Researchers have known for over 30 yr that ammonia levels build up in poultry rearing facilities, and this buildup adversely affects body weight, feed conversions, and livability in poultry. Charles and Payne (1966) observed that broilers exposed to 100 ppm NH_3 had lower respiration, weight gain, and feed consumption than unexposed birds. Ammonia is also harmful to farm laborers (Donham, 1996). Attempts to reduce ammonia volatilization and pathogen populations from poultry litter have

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Abbreviation Key: BGS + nal = brilliant green sulfa agar containing 200 mg/L nalidixic acid; BPW = buffered peptone water; WCR = whole carcass rinse.

been reported for the last five decades (Bullis et al., 1950; Botts et al., 1952). Acidic poultry litter treatments reduce volatile ammonia by reducing pH, which shifts the NH_4/NH_3 equilibria toward NH_4. Reduction in ammonia is beneficial not only to workers, but to poultry as well, by contributing to decreased susceptibility to certain diseases, increased feed efficiency, and higher growth rate.

Aluminum sulfate has been shown to effectively reduce ammonia and ammonia-related stress (Moore et al., 1996) and helps reduce energy costs as less ventilation is required when ammonia levels are reduced (Worley et al., 2000). Aluminum sulfate has been demonstrated to reduce phosphorous run-off from fields to which aluminum sulfate-treated poultry litter has been applied (Moore and Miller, 1994; Shreve et al., 1995; Moore et al. 1999). Recent studies have demonstrated that treatment of litter with the recommended level of aluminum sulfate (100 g aluminum sulfate/kg litter) presents little to no risk of toxicity to young broiler chicks by typical litter consumption (Moore et al., 1995; Huff et al., 1996); furthermore, some treatment levels may effectively suppress darkling beetle populations (Worley et al., 1999).

Sodium bisulfate is a dry granular acid that has also been shown to effectively reduce ammonia volatilization from poultry litter and improve poultry performance. Terzich et al. (1998a) demonstrated that broiler chickens reared on sodium bisulfate-treated litter have less respiratory distress and gain more body weight than those grown on untreated litter. Recent studies have demonstrated that broiler chicks raised on sodium-bisulfate-treated litter had significantly less mortalities ($P = 0.001$) associated with acites when compared to groups raised on untreated litter (Terzich et al., 1998b).

Significant reductions in litter pH have been demonstrated by the addition of aluminum sulfate or sodium bisulfate. Some enteric pathogens, including *Salmonella* spp., have the ability to regulate cytoplasmic pH close to neutral conditions when exposed to mild decreases in pH; however, this procedure is strenuous (especially when extracellular pH levels are much lower than internal pH) and commonly leads to cell death (Hill et al., 1995). Significant reductions in litter pH and exposure to pH levels below 4.5 would be expected to be bacteriostatic or bacteriocidal for *Salmonella* and *Campylobacter*. The purpose of this study was to determine if the introduction of aluminum sulfate or sodium bisulfate would serve as an intervention method for reducing pathogen populations associated with broiler chickens during production.

MATERIALS AND METHODS

Design

Two commercially available products, aluminum sulfate (alum; AlClear+)[2] and sodium bisulfate (Poultry Litter Treatment; PLT)[3] were tested in three replicate trials, using duplicate treatment groups to determine their effects on *Salmonella* and *Campylobacter* populations associated with broilers raised on treated litter. Fresh pine shavings served as litter in five isolation floor pens about [4.6 m^2 (50 ft^2)] equipped with nipple drinkers and a filtered, conditioned air supply. Broiler chicks (375) were obtained on day of hatch from a commercial Georgia hatchery and were individually inoculated by oral gavage with a three-strain mixture of wild-type campylobacters (poultry isolates) and a three-strain mixture of nalidixic-acid-resistant *Salmonella* (*S. heidelberg*, *S. montevideo*, *S. typhimurium*). Each chick received 0.1 mL containing approximately log 7 cfu/mL campylobacters and salmonellae. The chicks were released into the pens (about 65 per pen) at approximate commercial densities. The chicks were raised under simulated commercial conditions for approximately 6 wk to shed pathogens and to contaminate the litter naturally. These broilers were then removed from the pens, and the litter was treated in each pen with low or high levels of the two litter treatment products.

[2]General Chemical Co., East Point, GA.
[3]Jones-Hamilton Co., Salisbury, MD.

Treatments

The low level aluminum sulfate treated pen received 3.63 kg (8 lb) of aluminum sulfate/4.6m^2 (50 ft^2). The high level aluminum sulfate treated pen received 7.26 kg (16 lb) of aluminum sulfate /4.6m^2 (50 ft^2). The aluminum sulfate was gently raked into the litter, which was then sprayed with 1 L of water to provide adequate moisture for activation of the product. The pen treated with the low level of sodium bisulfate received approximately 1.13 kg (2.5 lb) of sodium bisulfate/4.6m^2 (50 ft^2), whereas pen treated with the high level of sodium bisulfate received approximately 1.81 kg (4 lb) sodium bisulfate/4.6m^2 (50 ft^2).

At this point, each pen was divided into two equal halves using stainless steel dividers to create duplicate experimental groups for each trial. The sodium bisulfate treated pens were also sprayed with 1 L of water to ensure activation of the product. The untreated control pen received no litter treatment but the surface was sprayed with 1 L water as in the treated pens. Water application in all pens (1 L/pen) was repeated every second day throughout the trial to maintain moisture levels near commercial conditions. Litter treatments were reapplied at Week 5 as suggested by the manufacturers for this experimental situation.

Placement and Analysis of Samples

Newly hatched chicks (about 35, uninoculated) were released into each half pen (to serve as duplicate pens) within 2 h of treatment application. Chickens from each duplicate pen (n = 5 to 10) were analyzed at Weeks 1, 4, and 6 for *Salmonella* and *Campylobacter* populations associated with the skin and feathers on the outside of the birds and internal colonization of the ceca. Litter pH levels were also monitored by placing about 50 g litter in 100 mL of deionized water and determining the pH with a pH meter.[4] At the appropriate sampling times, chickens to be analyzed were humanely sacrificed by cervical dislocation and the entire, unprocessed carcasses were individually placed in large plastic bags[5] and rinsed for 2 min in 400 mL of buffered peptone water (BPW) by using a carcass shaking machine to standardize the rinse procedure (Dickens, 1985). The whole carcass rinse (WCR) solution was poured into sterile containers and held on ice for analysis.

The carcasses were then aseptically dissected and the ceca were removed and placed in small plastic bags. The ceca and contents were diluted (1:3) in BPW and mixed using a laboratory blender.[6] The ceca and WCR samples were plated on brilliant green sulfa agar containing 200 mg/L nalidixic acid (BGS + nal) for enumeration of salmonellae. Plates (BGS + nal) were incubated for 24 h at 37 C. The ceca and WCR samples in BPW were also incubated for enrichment. Any enriched samples found to be negative for salmonellae by direct plating were streaked for isolation on BGS + nal. These plates were inspected for presence of salmonellae after 24 h at 37 C. The diluted ceca and WCR samples were also plated on Campy-Cefex agar (Stern et al., 1992) for enumeration of campylobacters. The Cefex plates were incubated for 48 h at 42 C under microaerobic conditions prior to enumeration of *Campylobacter* colonies. Typical *Campylobacter* colonies were confirmed by microscopic examination and latex agglutination.[7]

Statistical Analyses

Results from duplicate treatment groups (in each of three replicate trials) were combined for analysis. A one-way ANOVA and multiple-range test was performed to determine differences in pathogen populations between treatment group means for all samples. Differences in colonization frequencies were determined using the chi-squared or Fisher exact test[8] where appropriate.

RESULTS AND DISCUSSION

Both levels of the aluminum sulfate and sodium bisulfate litter treatments tested significantly ($P < 0.05$) reduced *Campylobacter* colonization frequency in the ceca (Table 1). Populations of campylobacters isolated from the ceca were also significantly ($P < 0.05$) reduced by all litter treatments as compared to the untreated controls. The high level of aluminum sulfate treatment was responsible for the most significant ($P < 0.05$) reduction in *Campylobacter* populations in the ceca (a nearly 5 log reduction at Week 6) and similarly reduced the incidence of *Campylobacter* cecal colonization from about 90% in the controls to only 10% in birds at a marketable age of 6 wk. The lower level aluminum sulfate treatment also significantly reduced cecal campylobacter colonization frequency by 65% at Week 6 and effected a 3.4 log reduction in cecal campylobacter populations. The treatment with a high level of sodium bisulfate significantly ($P < 0.05$) reduced cecal campylobacter colonization incidence by 30% at Week 6 and diminished campylobacter populations by 1.5 log. The low level of sodium bisulfate treatment was also effective and reduced cecal colonization frequency by about 27% and populations by 1.3 log.

Campylobacter incidence and populations on the exteriors of the broilers (WCR samples) diminished over time from Weeks 1 to 6 in the untreated controls, which were 95, 78, and 38% positive at Weeks 1, 4, and 6, respectively, so that significant reductions observed from all treatments at Weeks 1 and 4 were no longer statistically significant by Week 6. One exception to this observation was noted, however; no *Campylobacter* were recovered at any time from the exterior carcass of broiler chickens in the high level of aluminum sulftate-treated pens.

Salmonella colonization was not as readily affected by the acidifying litter treatments as was *Campylobacter*. *Salmonella* incidence and populations were not significantly decreased by any of the treatments investigated, and some actual increases were observed (Table 2). The high level of sodium bisulfate treatment did significantly ($P < 0.05$) reduce salmonella populations on the carcass exterior as compared to the untreated controls at Week 6, but this 0.4 log reduction may not be microbiologically significant. In general, the salmonellae were more prevalent on the exterior surfaces of the broilers than in cecal contents, whereas campylobacters were more prevalent in the ceca and less likely to survive on the carcass exterior perhaps due to the microaerobic growth requirements of campylobacters and the greater environmental survivability of salmonellae.

Campylobacter and *Salmonella* populations present at Week 6 probably represent the most important data points as these levels reflect the pathogen loads the birds would likely carry to the processing plant. At Week 6, all of the litter treatments reduced the populations of campylobacter associated with the ceca (Table 1). Although aluminum sulfate treatment appeared more effective than sodium bisulfate treatment under these study conditions, differences could be attributed to the quantities of the products used. By weight, nearly four times more aluminum sulfate was applied than sodium bisulfate. The treatment rates used were those suggested by the suppliers to simulate potential commercial economically feasible treatment levels (low) and experimentally more intensive treatment amounts (high).

It was the pH reduction caused by application of these acidic compounds that could be a possible mechanism for reducing pathogen populations in the litter. The pH data demonstrate that both litter treatments reduce pH in the litter soon after application and activation of the products (Figure 1). Although the high level sodium bi-

[4]Model 440, Corning, Corning, NY.
[5]Cryovac, Duncan, SC.
[6]Seward Medical, London, UK.
[7]INDX, Integrated Diagnostics, Inc., Baltimore, MD.
[8]SigmaStat, Jandel Software, San Rafael, CA.

TABLE 1. Frequencies and populations of *Camploybacter* recovered from the ceca and carcass exterior of broiler chickens raised on treated litter[1] at Weeks 1, 4, and 6

	Control	Low aluminum sulfate	High aluminum sulfate	Low sodium bisulfate	High sodium bisulfate
	Ceca				
Frequency, mean % positive					
Week 1	97.5[a]	0[c]	0[c]	50.0[b]	22.0[b]
Week 4	100.0[a]	20.0[c]	3.3[c]	66.7[b]	53.3[b]
Week 6	90.0[a]	25.0[c]	10.0[c]	63.3[b]	60.0[b]
Population, mean log cfu/mL					
Week 1	6.04[a]	0[c]	0[c]	3.30[b]	2.24[b]
Week 4	5.72[a]	1.19[c]	0.14[c]	5.00[b]	3.70[b]
Week 6	5.03[a]	1.65[c]	0.12[d]	3.73[b]	3.51[b]
	Whole carcass rinse				
Frequency, mean % positive					
Week 1	95.0[a]	0[c]	0[c]	23.3[b]	16.7[b]
Week 4	77.5[a]	13.3[c]	0[c]	36.7[bc]	30.0[bc]
Week 6	38.0[a]	25.0[a]	0[b]	30.0[a]	23.3[a]
Population, mean log cfu/mL					
Week 1	3.48[a]	0[c]	0[c]	0.76[b]	0.42[bc]
Week 4	2.20[a]	0.63[c]	0[c]	1.44[abc]	1.25[bc]
Week 6	0.90[a]	0.51[a]	0[b]	0.70[a]	0.43[a]

[a-c]Means or percentages within a row, with no common superscript differ significantly ($P < 0.05$).

[1]Litter was treated with 3.63 kg (8 lb) aluminum sulfate/4.6 m^2 (50 ft^2) (Low aluminum sulfate), or 7.26 kg (16 lb) aluminum sulfate/4.6 m^2 (High aluminum sulfate), or 1.13 kg (2.5 lb) sodium bisulfate/4.6 m^2 (Low sodium bisulfate), or 1.81 kg (4 lb) sodium bisulfate/4.6 m^2 (High sodium bisulfate). Treatments were reapplied at Week 5. Controls received no litter treatment. Sample number (n) ranged from 30 to 50, depending on mortalities through each trial.

sulfate treatment initially reduced the pH by the greatest amount, the aluminum sulfate treatments maintained a lower pH for a longer period of time, perhaps contributing to their greater efficacy.

Demonstration of significant pathogen-reducing capabilities for products now on the market and currently in use for other purposes would help to rapidly fill a need for additional food safety intervention methods suitable for application during poultry production. Such treatments could provide the industry with economical means to help meet food safety objectives. Although effective pathogen control will most likely require a combination of interventions, treatment of litter in poultry production may serve as a means to help control *Campylobacter* and

TABLE 2. Frequencies and populations of *Salmonella* recovered from the ceca and carcass exterior of broiler chickens raised on treated litter[1] at Weeks 1, 4, and 6

	Control	Low aluminum sulfate	High aluminum sulfate	Low sodium bilsulfate	High sodium bisulfate
	Ceca				
Frequency, mean % positive					
Week 1	36.0[b]	57.1[b]	37.5[b]	83.3[a]	80.0[a]
Week 4	30.0[b]	37.1[b]	15.0[bc]	53.3[b]	63.3[a]
Week 6	20.0[c]	26.3[bc]	5.0[c]	50.0[a]	43.3[ab]
Population, mean log cfu/mL					
Week 1	0.88[b]	1.35[a,b]	0.82[b]	1.85[a]	1.51[ab]
Week 4	0.48	0.63	0.33	0.52	0.80
Week 6	0.29[a,b]	0.48[a]	0.08[b]	0.55[a]	0.23[ab]
	Whole carcass rinse				
Frequency, mean % positive					
Week 1	68.0[b]	71.4[b]	50.0[b]	100.0[a]	100.0[a]
Week 4	50.0[b]	51.4[b]	20.0[c]	93.3[a]	70.0[b]
Week 6	44.0[ab]	50.0[ab]	27.5[b]	66.7[a]	43.3[ab]
Population, mean log cfu/mL					
Week 1	1.09[ab]	1.28[a]	0.83[b]	1.50[a]	1.75[a]
Week 4	0.78[b]	0.74[b]	0.30[c]	1.38[a]	0.75[b]
Week 6	0.67[a]	0.76[a]	0.42[ab]	0.75[a]	0.23[b]

[a-c]Means or percentages within a row, with no common superscript differ significantly ($P < 0.05$).

[1]Litter was treated with 3.63 kg (8 lb) aluminum sulfate/4.6 m^2 (50 ft^2) (Low aluminum sulfate), or 7.26 kg (16 lb) aluminum sulfate/4.6 m^2 (High aluminum sulfate), or 1.13 kg (2.5 lb) sodium bisulfate/4.6 m^2 (Low sodium bisulfate), or 1.81 kg (4 lb) sodium bisulfate/4.6 m^2 (High sodium bisulfate). Treatments were reapplied at Week 5. Controls received no litter treatment. Sample number (n) ranged from 30 to 50, depending on mortalities throughout each trial.

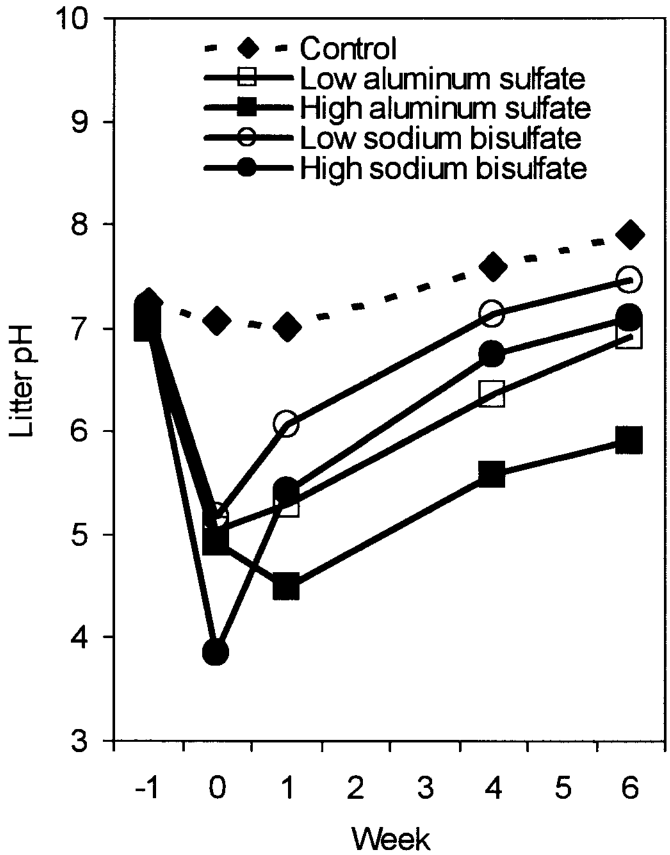

FIGURE 1. Effect of acidified litter treatments on litter pH. Litter was treated with 3.63 kg (8 lb) aluminum sulfate/4.6 m^2 (50 ft^2) (Low aluminum sulfate), or 7.26 kg (16 lb) aluminum sulfate/4.6 m^2 (High aluminum sulfate), or 1.13 kg (2.5 lb) sodium bisulfate/4.6 m^2 (Low sodium bisulfate), or 1.81 kg (4 lb) sodium bisulfate/4.6 m^2 (High sodium bisulfate). Treatments were reapplied at Week 5. Controls received no litter treatment.

to reduce horizontal transmission of pathogens in broiler flocks. Field trails in commercial broiler houses are currently in progress to determine the influence of these acidified litter treatments on pathogen populations during large-scale broiler production.

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