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Reduction of *Salmonella* and Ammonia Emissions in Broiler Litter Using Sulfuric Acid and Aluminum Sulfate

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Abstract: In recent years an emphasis has been placed on reducing food borne pathogens on the farm. Of particular interest to the poultry industry is *Salmonella enterica*. In the United States, a common practice in broiler management is the reuse of litter for consecutive flocks; this practice can lead to a *Salmonella* positive flock causing the colonization of sequential flocks. Reuse of litter can also lead to high levels of ammonia volatilization which can impact broiler health and performance. In this paper, two experiments are presented using chemical litter amendments to reduce both ammonia and *Salmonella*. In the first experiment, liquid sulfuric acid was applied to litter at one of three application rates; 9.07, 18.14 and 27.21 L/92.9 m^2. In experiment two, commercially available aluminum sulfate was applied to litter at three different application rates; 22.7, 45.5 and 68.0 kg/92.9 m^2. In addition, a cocktail of five poultry associated *S. enterica* serovars was applied to the litter. Salmonella, ammonia, moisture and pH levels were measured immediately before treatments and every 24 hours up to 96 hours. For treated litter, a reduction was seen in ammonia levels 24 hours after application regardless of application rate, the reduction continued for the duration of the experiment ($P<0.001$). No *Salmonella* was detected in litter treated with sulfuric acid. *Salmonella* was recovered in litter treated with aluminum sulfate. The data presented here indicates that sulfuric acid and aluminum sulfate are suitable candidates for reducing ammonia emissions and sulfuric acid as a candidate for *Salmonella* reduction.

Key words: Sulfuric acid, aluminum sulfate, *Salmonella*, ammonia, litter management

INTRODUCTION

Poultry meat is the leading source of food borne illness in the United States accounting for 1.5 million total cases, nearly 12,000 hospitalizations and 180 deaths per year (Batz *et al*., 2011). Of these cases, it is estimated that 35.1% are caused by *Salmonella* spp. Recently it has been shown that *Salmonella* has been recovered from 83% of Australian broiler chicken farms that reuse litter (Chinivasagam *et al*., 2010). In a study of the effects of soil characteristics on incidence of *Salmonella* in litter, nearly a third (29%) of litter samples tested were *Salmonella* positive (Volkova *et al*., 2009).

One problem that has arisen with the intensive rearing conditions associated with broiler chicken production is the high level of ammonia that can be volatilized from litter. This ammonia is produced by the ureolytic action of bacteria found in the litter (Rothrock *et al*., 2010). Eight hour exposure to ammonia levels higher than 25 ppm has been banned by the United States Occupational Safety and Health Association. Ammonia in high enough concentrations (100 ppm or greater) can cause lesions to the mucosal linings of the nasal passages, esophagus and lungs which will in turn lead to a significant reduction in bird live performance (Fulton, 2008). Ammonia emissions from litter can be reduced to acceptable levels by the application of acidifying litter amendments, more specifically acidifiers. Acidifying litter amendments work by reducing litter pH and trapping ammonia as ammonium, these amendments can also reduce the number of ureolytic bacteria present (Terzich *et al*., 1998).

It has also been shown that acidifying litter amendments reduce *Salmonella* concentrations in litter if a low enough pH can be achieved. Some of these amendments have shown reduction of *Salmonella* at a litter pH of 4 or less (Pope and Cherry, 2000 and Payne *et al*., 2002; Payne *et al*., 2007). These researchers applied either aluminum sulfate, sodium bisulfate or granulated sulfuric acid to achieve the low pH 's needed to first trap ammonia and secondly reduce *Salmonella*. These chemicals work by trapping ammonia as an ammonium salt. At higher litter pH levels, 5 or higher, it has been observed that no reduction in *Salmonella* was observed (Line, 2002; Payne *et al*., 2007; Williams *et al*., 2012).

Salmonella in contaminated food must first pass through the acidic environment of the stomach before it can enter the small intestine and cause illness. The production of acid shock proteins in response to acidic environments enables *Salmonella* to adapt and survive (Foster, 1993). It has also been found that when *Salmonella* is adapted to an acidic environment its

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virulence can increase, researchers noted that virulent strains can survive better in acidic environments such as the stomach (Foster and Hall, 1990). The goal of these experiments was to determine the effectiveness of sulfuric acid and aluminum sulfate at reducing ammonia emissions and *Salmonella* concentrations in broiler litter. It was hypothesized that due to the acidic nature of both treatments, they would be highly effective at reducing litter pH, trapping ammonia and eliminating or reducing *Salmonella* concentrations.

MATERIALS AND METHODS

Recovery and culture of *Salmonella*: Five *Salmonella* species were used in this experiment; *Salmonella* Enteritidis, *Salmonella* Montevideo, *Salmonella* Heidelberg, *Salmonella* Typhimurium and *Salmonella* Kentucky. The *Salmonella* isolates were previously cultured from either commercial broiler houses or processing plants and identified by 16S rRNA sequencing. Cultures were stored in glycerol solution at -80°C, when needed cultures were streaked onto Tryptic Soy Agar containing 5% sheep red blood cells (BBL™, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) and incubated aerobically at 37° C for 24 hours. After incubation, a single isolated colony was picked and streaked onto Brain Heart Infusion Agar (Difco™,Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) slants and incubated as above. These slants were stored at 4°C for future use. Brain Heart Infusion Broth (Difco™)was inoculated from the above mentioned Brain Heart Infusion slants. This broth culture was incubated at 37°C for 24 hours with shaking (150 rotations per minute). A cocktail of the five *Salmonella* species was made by combining 6 ml of each species, making a 30 ml cocktail of 109 cfu/ml that was applied to the litter. *Salmonella* concentrations were enumerated by spread plating 0.1 ml of each serovar onto Plate Count Agar (Difco™).

Litter preparation: Broiler litter, composed of pine shavings and poultry manure, was obtained, weighed and stored in a heated room (27°C) at the Auburn University Poultry Science Research Unit. Five days before the start of the experiments, litter moisture was determined by taking three samples from the litter. Ten grams of litter from each sample was dried at 110°C for 48 hours and then reweighed, to obtain percent moisture. From these results, water was added to adjust litter moisture to 25-30% which is the level typically observed on a commercial broiler farm (Martin and McCann, 1998). As the water was added, litter was turned to facilitate even dispersal. After addition of the water, litter was heated (27° C) for 72 hours to enhance ammonia production. On the day of each experiment, three kg of litter was placed into one of twelve identical plastic tubs each measuring 0.53 x 0.39 m. After placement in plastic tubs, litter was allowed to equilibrate for one hour before any samples were taken or treatments added. Initial samples were taken after equilibration and the *Salmonella* cocktail, as described previously, was applied to each replicate. The cocktail was applied, by even dispersion on the surface of the litter and then thoroughly mixed into the litter by hand.

Treatment application: In each experiment, immediately following the application of *Salmonella* cocktail, chemical treatments were applied. Treatments were applied by evenly dispersing the chemical on the surface of litter and then thoroughly mixed into the litter using glass rod. Each treatment was applied to three replicate litter beds.

For the sulfuric acid experiment treatments utilized were; either sterile, deionized water (negative control), sulfuric acid at a rate of 9.07, 18.14, or 27.21 liters (L)/92.9 m^2. Sulfuric acid was mixed with sufficient amount of sterile, deionized water to obtain final treatment volumes totaling, 75.6, 151.2 or 226.8 L/92.9 m^2. The dilution of sulfuric acid prior to treatment would prevent a dangerous, violent reaction with the litter.

In the second experiment, commercially available aluminum sulfate (Al+Clear® Poultry Grade Alum) was applied to litter in one of four possible application rates: 0 (negative control), 22.7, 45.4 or 68 kg/92.9 m^2.

Sampling and analysis techniques: Litter samples were taken at 0, 24 and 96 hours post treatment. Litter was collected from each of the four corners and the center of each replicate using a gloved hand and combined in a whirl-pak bag; approximately 50 g of litter was removed from each replicate. Litter collected was thoroughly mixed in a whirl-pak bag before analysis. Remaining litter was evenly spread to maintain a flat surface area. Litter pH measurements were taken with a Fisher Scientific Accumet pH meter 50 (Denver Instrument Company, Bohemia, NY, USA). This was performed by adding 45 ml of distilled water to 5 g of litter and allowed to equilibrate for 1 hour. After which time, the litters' pH was measured. Litter moisture was obtained by taking 10 g of litter and drying for 48 hours at 100°C. Using the weight loss between initial and dried litter the percent moisture was determine.

Ammonia measurements were taken immediately before sulfuric acid addition (time 0), then, 24, 48, 72 and 96 hours post treatment application. A Dräger Chip Measuring System (CMS) was used, with a plastic rectangular measurement tub (17×25×12 cm) attached to pump (Dräger Safety, Inc., Lubeck, Germany). The measurement tub was placed directly on the litter filled tub and air was pumped through the ammonia detection machine for 60 seconds before measurements were taken. After each measurement, fresh air was forced into the pump, by fanning the attached measurement tub, with the pump running, up and down four to five times.

A ten gram sub sample of litter obtained from the 0, 24 and 96 hour samples was utilized for bacteria recovery. This sub sample was placed in sterile filter bags with 90 ml of sterile Phosphate Buffered Saline (Difco™) and homogenized in an AES Laboratoire Stomacher for 60 s. Serial dilutions were made by taking 1 ml of this initial dilution and adding it to 9 ml of sterile Phosphate Buffered Saline, each dilution was vortexed for five seconds before sequential dilutions were made. For each dilution, 0.1 ml was spread plated, in duplicate, onto each of the following media types.

Media utilized were; Plate Count Agar (Difco™)for total aerobic bacteria; Anaerobic Agar (Difco™)for total anaerobic bacteria; MacConkey's Agar (Difco™)for *E. coli*. *Salmonella* recovery was performed by two methods, direct plating and enrichment. Direct plating was performed utilizing the methods described above for the other media types using Xylose-Lysine-Tergitol 4 agar (Difco™). *Salmonella* enrichments were performed by incubating 1 g of each litter sample in 9 mL of Tetrathionate Broth, Hajna (Difco™) at 37°C for 48 hours and then streaked onto XLT4. This step was performed to ensure that any sample with *Salmonella* concentrations below enumeration limits were not falsely identified as negative.

Aerobic bacteria, *Salmonella* and *E. coli* were incubated aerobically overnight at 37°C. While anaerobic bacteria were incubated in an atmosphere consisting of 90% N2, 5% CO_2 and 5% H_2 overnight at 37° C.

Statistics: Data analysis was performed with SAS 9.2, using the general linear model procedure at 0.05 level of significance (SAS Institute 2009). Cfu/g litter was transformed from visual counts to log10 and litter moisture was arcsine transformed. Means with significant differences were separated using Tukey 's Honestly Significant Difference test. Data were analyzed for effects due to application rate and time.

RESULTS

Sulfuric acid: Sulfuric acid treated litter had lower ammonia levels than the control at, 24, 48, 72 and 96 hours (P<0.001, Table 1). By 48 hours, the 27.21 L/92.9 m^2 application rate had lower ammonia levels than the 9.07 application rate, 1.3 and 3.8 pm respectively (P<0.001). At the 72 and 96 hour sampling time there was no difference in ammonia levels between any of the sulfuric acid treatments (P<0.001).

Bacterial data for total aerobe, anaerobe and *Salmonella* levels are presented in Table 2. Total aerobic bacteria counts were lower in litter that received 27.21 L/92.9 m^2 sulfuric acid, at 24 (P<0.001) and 96 hours (P = 0.004). Twenty four hours after sulfuric acid treatment, total anaerobic bacteria were lowest in the 27.21 L/92.9 m^2 treatment (3.1 log10 cfu/g litter) while the control was higher with 5.4 log10 cfu/g litter. Sulfuric acid when applied at 9.07 and 18.14 L/92.9 m^2, had the highest anaerobic bacteria levels, with both having an average of 7.1 log10 cfu/g litter. By 96 hours litter treated with the 27.21 L/92.9 m^2 application rate had lower anaerobic concentrations, 4.2 log10 cfu/g litter, compared to the control and other treatments (P<0.001). No *Salmonella* was recovered before the addition of the *Salmonella* cocktail (0 hour, Table 2). At 24 and 96 hours, no *Salmonella* was recovered from sulfuric acid treated

Table 1: Average ammonia levels (ppm) for each treatment at 0, 24, 48, 72 and 96 hours after treatment with sulfuric acid

	Time (hours) post treatment				
L/92.9 m^2	0	24	48	72	96
9.07	270	3.8^{z}	3.8^{y}	2.8^{z}	2.7^{z}
18.14	266	2.3^{z}	2.7^{yz}	2.6^{z}	2.4^{z}
27.21	225	1.4^{z}	1.3^{z}	0.9^{z}	1.1^{z}
0 (Control)	210	63.7^{y}	36.7^{x}	27.9^{y}	20.8^{y}
SEM	14.9	5.4	0.5	1.4	2.3
P-value	0.053	<0.001	<0.001	<0.001	0.007

No. in the same column with different superscripts are different (P<0.05); SEM = Standard error of the mean

Table 2: Total aerobic, anaerobic and *Salmonella* concentrations at 0, 24 and 96 hours after treatment with sulfuric acid (log10 cfu/g litter)

Sulfuric acid	Aerobic			Anaerobic			*Salmonella*		
L/92.9 m^2	0	24	96	0	24	96	0	24	96
9.07	7.2	7.9^{y}	8.3^{y}	4.1^{z}	7.1^{x}	6.9^{y}	0	0^{z}	0^{z}
18.14	7.3	8.0^{y}	8.4^{y}	4.4^{yz}	7.1^{x}	7.3^{y}	0	0^{z}	0^{z}
27.21	7.3	4.3^{z}	6.0^{z}	4.5^{y}	3.1^{z}	4.2^{z}	0	0^{z}	0^{z}
0 (Control)	7.2	8.1^{y}	8.1^{y}	4.5^{y}	5.4^{y}	6.5^{y}	0	2.4^{y}	2.7^{y}
SEM	0.038	0.239	0.367	0.067	0.189	0.281	0	0.113	0.451
P-Value	0.087	<0.001	0.004	0.012	<0.001	<0.001	NS	<0.001	0.006

No. in the same column with different superscripts are different (P<0.05). SEM = Standard error of the mean

Table 3: Litter pH levels at 0, 24 and 96 hours after sulfuric acid application

	Time (hours) post treatment		
L/92.9 m^2	0	24	96
9.07	8.90	6.33^{y}	7.17^{x}
18.14	8.89	4.78^{y}	5.73^{y}
27.21	8.89	2.07^{z}	2.31^{z}
Control	8.87	8.80^{x}	8.81^{w}
SEM	0.0007	0.268	0.194
P-value	0.06	<0.001	<0.001

No. in the same column with different superscripts are different *(*P<0.05). SEM = Standard error of the mean

Table 4: Litter moisture (percent) for litter treated with sulfuric acid

	Time (hours) post treatment		
L/92.9 m^2	0	24	96
9.07	37.90	41.16^{y}	30.05^{y}
18.14	40.28	41.30^{y}	34.65^{x}
27.21	39.78	44.19^{y}	39.27^{w}
0 (Control)	36.39	33.34^{z}	25.55^{z}
SEM	1.04	1.16	0.82
P-value	0.096	0.001	<0.001

$^{w\text{-}z}$No. in the same column with the different superscripts are different at P ≤ 0.05. SEM = Standard error of the mean

Table 5: Ammonia measurements (ppm) for litter treated with 22.7, 45.4, 68 or 0 kg/92.9 m^2 of aluminum sulfate

	Time (Hours) Post Treatment				
kg/92.9 m^2	0	24	48	72	96
22.7	195.3	16.7^{z}	15.4^{z}	15.4^{z}	12.3^{z}
45.4	283.7	10.9^{z}	9.0^{z}	7.4^{z}	6.6^{z}
68	250.3	7.5^{z}	6.4^{z}	6.0^{z}	3.3^{z}
0 (Control)	225.3	100.3^{y}	88.3^{y}	67.0^{y}	46.3^{y}
SEM	32.8	6.9	5.5	5.1	3.3
P-value	0.337	<0.001	<0.001	<0.001	<0.001

$^{x\text{-}z}$No. in the same column with difference superscripts are different at P ≤ 0.05. SEM = Standard error of the mean

litter regardless of sulfuric acid application rate, while control litter had 2.4 and 2.7 log10 cfu/g litter, respectively (P<0.001).

The 24 hours control litter pH remained basic at 8.8, while treated litter pH decreased to 6.3, 4.8 and 2.1, for 9.07, 18.14 and 21.27 L/92.9 m^2 sulfuric acid treatment levels, respectively (Table 3). By 96 hours, control litter pH remained at 8.8, while treated litter pHs were 7.2, 5.7 and 2.3 for the increasing sulfuric acid application rates (9.07, 18.14 and 21.27 L/92.9 m^2).

Observed moisture percent for litter treated with sulfuric acid are shown in Table 4. Litter moisture correlated with the application rate; litter receiving higher application rates containing more liquid had higher percentage of moisture.

Aluminum sulfate: Twenty-four hours after treatment, litter receiving aluminum sulfate had lower ammonia emissions than the control litter which received only *Salmonella* (P<0.001), this trend would continue until the conclusion of the experiment at 96 hours (Table 5). At 24 hours, ammonia levels in treated litter were: 16.7, 10.9 and 7.5 ppm. With increasing amounts of aluminum sulfate there was a decrease in ammonia. Ammonia measurement for the untreated litter was 100.3 ppm. Samples collected at the 48 hour period showed that ammonia emissions in the control litter decreased to 88.3 ppm; however litter treated with aluminum sulfate still had lower ammonia emissions, 15.4, 9.0 and 6.4 ppm, again with ammonia decreasing with increasing amount of aluminum sulfate (P<0.001). Seventy two hours after treatment, ammonia emissions for litter treated with aluminum sulfate remained steady at 15.4, 7.4 and 6.0 ppm. At this time, the control decreased to 67 ppm but this was still significantly higher than the treated litter (P<0.001). Ammonia measurements at 96 hours reflected those of the previous sampling times, with aluminum sulfate treated litter having significantly lower ammonia measurements than the untreated litter; 12.3, 6.6, 3.3 ppm at application levels 22.7, 45.4 or 68 kg/92.9 m^2, respectively and control at 46.3 ppm (P<0.001). For the 24, 48, 72 and 96 hour sampling times there was no difference in ammonia levels between the three application rates.

At 24 hours, total aerobic bacteria were 9.3, 9.5 and 9.2 for treated litter and 9.6 log10 cfu/g for the untreated control (Table 6). A decrease was observed at 96 hours in the 68 kg/92.9 204 m^2 treated litter. The mean aerobic

Table 6: Total aerobic, anaerobic and *Salmonella* concentrations (log10 cfu/g litter) at 0, 24 and 96 hours after treatment with aluminum sulfate

Aerobic				Anaerobic			*Salmonella*		
kg/92.9 m^2	0	24	96	0	24	96	0	24	96
22.7	10.5^{x}	9.3	9.9^{y}	4.9^{z}	5.7	5.6	0	4.2	3.9
45.4	10.2^{xy}	9.5	9.6^{y}	5.9^{yz}	6.4	5.1	0	4.3	1.7
68	10.1^{yz}	9.2	8.2^{z}	6.2^{y}	5.4	5.2	0	3.9	1.7
0 (Control)	9.9^{z}	9.6	9.2^{yz}	5.8^{yz}	5.5	4.9	0	4.6	2.5
SEM	0.067	0.199	0.240	0.278	0.371	0.162	0	0.604	0.995
P-value	0.002	0.613	0.004	0.045	0.28	0.099	NS	0.894	0.407

$^{x-z}$No. in the same column with different superscripts are different at $P \leq 0.05$. SEM = Standard error of the mean

Table 7: Litter pH levels at 0, 24 and 96 hours after aluminum sulfate application

Time (hours) post treatment			
kg/92.9 m^2	0	24	96
22.7	7.50^{yz}	7.46^{xy}	7.42^{y}
45.4	7.43^{z}	5.85^{yz}	5.99^{z}
68	7.54^{yz}	4.31^{z}	5.83^{z}
0	7.82^{y}	8.80^{x}	8.38^{y}
SEM	0.072	0.421	0.293
P-value	0.023	<0.001	<0.001

$^{x-z}$No. with different superscripts are different at $P \leq 0.05$. SEM = standard error of the mean

Table 8: Litter moisture (percent) for litter treated with aluminum sulfate

Time (hours) post treatment			
kg/92.9 m^2	0	24	96
22.7	25.26^{y}	20.13	16.36
45.4	25.58^{yz}	19.96	15.61
68	23.89^{yz}	20.86	15.74
0 (Control)	23.20^{z}	21.71	15.46
SEM	0.24	0.91	0.01
P-value	0.0018	0.5436	0.5331

$^{y-z}$No. in the same column with different superscripts are significantly different at $P \leq 0.05$. SEM = Standard error of the mean

bacteria concentration for the 68 kg/92.9 m^2 treated litter was 8.2 log10 cfu/g, this was significantly less than both the 22.7 and 45.4 kg/92.9 m^2 treated litter (9.9 and 9.6 log10 cfu/g). However, this was not less than the control which had 9.2 log10 cfu/g (P = 0.005). Twenty four hours after treatment with aluminum sulfate, anaerobic bacteria concentrations for litter treated with either 22.7, 45.4 or 68 kg/92.9 m^2 of aluminum sulfate were; 5.7, 6.4 and 5.4 log10 cfu/g, respectively. At this time the untreated litter had 5.5 log10 cfu/g (P = 0.28). Anaerobic bacteria concentrations at 96 hours reflected those at 24 hours, with no observable differences between treated litter (5.6, 5.1 and 5.2 log10 cfu/g) and untreated litter (4.9 log10 cfu/g).

Salmonella results for aluminum sulfate were not as promising as the sulfuric acid results. Before addition of the 30 ml *Salmonella* cocktail no *Salmonella* was recovered from the litter(Table 6). At 24 hours 4.6 log10 cfu/g of *Salmonella* was recovered from the untreated litter; while litter treated with aluminum sulfate at 22.7, 45.4 or 68 kg/92.9 m^2 application rates had *Salmonella* concentrations of 4.2, 4.3 and 3.9 log10 cfu/g, respectively (P = 0.894). *Salmonella* numbers at 96 hours in litter treated with 45.4 or 68 kg/92.9m^2 of aluminum sulfate were 1.7 log10 cfu/g. Litter treated with the lowest application rate, 22.7 kg/92.9 m^2 had the highest concentration of *Salmonell*a, with 3.9 log10 cfu/g. Untreated litter was intermediate with 2.5 log10 cfu/g. None of these were statistically different from each other (P = 0.162).

Litter pH measurements (Table 7) before treatment with aluminum sulfate were all between 7.4 and 7.8 which is slightly more neutral than normal litter pH. Twenty four hours after treatment, a decrease was seen in 45.4 and 68 kg/92.9 m^2 application rates, 5.9 and 4.3, respectively. The pH for litter receiving aluminum sulfate at 22.7 kg/92.9 m^2 remained unchanged from time 0 at 7.5; while the pH for untreated litter increased to 8.8 (P = 0.023). At 96 hours, pH values were almost unchanged from the 24 hour sampling with pHs of; 7.4, 5.9, 5.8 and 8.4 for the treatments 22.7, 45.4, 68 and 0 kg/92.9 m^2 respectively (P<0.001). The observed pH values for both the 45.4 and 68 kg/92.9 m^2 application rates were significantly lower than the pH values for the 22.7 and 0 kg/92.9 m^2 treated litter at 24 and 48 hours.

Percent litter moisture measurements are given in Table 8. No differences were observed after application of aluminum sulfate. The decreasing moisture in the

untreated litter was the probable cause for the decreasing ammonia emissions observed in the untreated control litter.

DISCUSSION

While several types of poultry litter amendments exist for ammonia reduction the most used and effective are acidifiers. These amendments work by reducing both litter pH; it does this by trapping ammonia as ammonium. This study investigated the efficacy of sulfuric acid and aluminum sulfate at reducing ammonia levels and *Salmonella* levels in broiler litter. Previous research has shown that reduction in bacterial concentrations is greatest when a litter pH of 4 or less is achieved (Pope and Cherry 2000; Payne *et al*., 2002; Payne *et al*., 2007; Williams *et al*., 2012).

In agreement with previous research, this experiment showed that as litter pH decreased below 4, a significant difference was observed in the amount of bacteria present. The highest application rate of sulfuric acid (21.27 L/92.9 m^2) produced a litter pH of 2.1 and 2.3 at 24 and 96 hours, respectively. Coinciding with pH reduction an almost 4 log reduction was observed for total aerobic and anaerobic bacteria at 24 hours and an approximate 2 log reduction at 96 hours. In regards to *Salmonell*a, litter treated with sulfuric acid had no recoverable *Salmonella* at 24 and 96 hours.

Sulfuric acid worked extremely well at reducing litter ammonia levels regardless of application rate, with ammonia levels of <4 ppm at 24 hours reduced from over 200 ppm before treatment. These ammonia levels remained relatively unchanged for the duration of the experiment with only a slight fluctuation between sampling times. It is interesting to note that no difference was observed in ammonia levels between application rates even though differences were observed in litter pH. Sulfuric acid does pose a certain risk as it is very corrosive, must be handled with appropriate caution and training or certification.

Aluminum sulfate produced similar ammonia reduction results as sulfuric acid and as with previous research the lower the litter pH the more effective an amendment is at trapping ammonia as ammonium. Treatment with aluminum sulfate reduced ammonia to acceptable levels that would not impact broiler live performance (Fulton, 2008). Despite differences in litter pH, additional amounts of aluminum sulfate did result in observable lower amounts of ammonia. This would be helpful to the farmer as using less of the product would save money. Aluminum sulfate did not impact bacterial numbers as dramatically as sulfuric acid did. Only total aerobic bacteria were affected by the addition of 68 kg/92.9 m^2, the highest application rate used and this was not observed until 96 hours after treatment. Aluminum sulfate had no effect on total anaerobic bacteria or *Salmonell*a.

Overall, both aluminum sulfate and sulfuric acid were effective at reducing litter ammonia emissions. Sulfuric acid produced promising results for bacteria reduction, as it almost completely eliminated *Salmonell*a and had significant reduction of total aerobic and anaerobic bacteria. The last finding of interest is that higher amounts of either chemical did not result in lower ammonia emissions or lower bacteria numbers up to 96 hours after treatment.

REFERENCES

Batz, M.B., S. Hoffmann and J.G. Morris Jr., 2011. Ranking the risks: The 10 pathogen- food combinations with the greatest burden on public health. Emerging Pathogens Institute, University of Florida.

Chinivasagam, H.N., M. Redding, G. Runger and P.J. Blackall, 2010. Presence and incidence of food-borne pathogens in Australian chicken litter. Br. Poult. Sci., 51: 311-318.

Foster, J.W. and H.K. Hall, 1990. Adaptive acidification tolerance response of *Salmonella* typhimurium. J. Bacteriol., 172: 771-778.

Foster, J.W., 1993. The acid tolerance response of *Salmonella* typhimurium involves transient synthesis of key acid shock proteins. J. Bacteriol., 175: 1981-1987.

Fulton, R.M., 2008. Other toxins and poisons. Pages 1245 in Diseases of Poultry 12 th Edition. Y.M. Said ed. Blackwell Publishing Professional. Ames, Iowa.

Line, J.E., 2002. Campylobacter and *Salmonella* populations associated with chickens raised on acidified litter. Poult. Sci., 81: 1473-1477.

Martin, S.A. and M.A. McCann, 1998. Microbiological aurvey of Georgia poultry litter. J. Appl. Poult. Res., 7: 90-98.

Payne, J.B., E.C. Kroger and S.E. Watkins, 2002. Evaluation of litter treatments on *Salmonella* recovery from poultry litter. J. Appl. Poult. Res., 11: 239-243.

Payne, J. B., J. A. Osborne, P. K. Jenkins and B. W. Sheldon. 2007. Modeling the growth and death kinetics of *Salmonella* in poultry litter as a function of pH and water activity. Poult. Sci., 86: 191-201.

Pope, M.J. and T.E. Cherry, 2000. An evaluation of the presence of pathogen on broilers raised on Poultry Litter Treatment®-treated litter. Poult. Sci., 79: 1351-1355.

Rothrock, Jr., M.J., K.L. Cook and J.G. Warren, 2010. Microbial mineralization of organic nitrogen forms in poultry litters. J. Environ. Quality, 39: 1848-2010.

SAS Institute, 2009. SAS User 's Guide.Statistics.Versi on 9.2 Edition. SAS Inst. Inc, Cary, NC.

Terzich, M., C. Quarles, M.A. Goodwin and J. Brown, 1998. Effect of poultry Litter Treatment® (SODIUM BISULFATE®) on death due to ascites in broilers. Avian Dis., 42: 385-387.

Volkova, V.V., R.H. Bailey and R.W. Willis, 2009. *Salmonella* in broiler litter and properties of soil at farm location. PLoS ONE. 4: 6403.

Williams, Z., J.P. Blake and K.S. Macklin, 2012. The effect of sodium bisulfate on *Salmonella* viability in broiler litter. Poult. Sci., 91: 2083-2088.