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Impact of acidified clay and sodium bisulfate on litter ammonia, pH, total aerobic plate counts and broiler footpad dermatitis

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SUMMARY

Acidified clay and sodium bisulfate are common litter amendments utilized to control ammonia and lower pH in poultry litter. Three separate pen trials were conducted to compare the efficacy of both amendments for controlling litter ammonia, pH, total aerobic plate counts (APC), and improving footpad lesions in commercial broilers. A commercial broiler research farm was chosen as the test site. Day-old broiler chicks were placed in 40 pens (60 per pen for a total of 2400 birds) on used pine shavings. Pens were blocked within the house. Prior to placement pens were de-caked and top dressed as necessary. Treatments consisted of (1) control; (2) acidified clay and (3) sodium bisulfate. Composite litter surface samples were collected from each pen and analyzed for pH and APC on days 0-5. Litter surface ammonia concentrations were measured on days 0-5. Ammonia flux measurements were recorded on days 3 and 6. Broiler footpad lesion scores were assessed on day 38. Ammonia, pH, total APC and ammonia flux values were significantly lower in treated litter compared to values in the control. Ammonia concentrations in the acidified clay group were significantly lower than values in the sodium bisulfate group. On day 1, pH and total APC values in the acidified clay treated litter were lower than values in the sodium bisulfate treated litter. Average footpad lesion scores within a pen were significantly higher in the control group as compared to the acidified clay and sodium bisulfate groups. Additionally, average footpad lesion scores were significantly higher in the sodium bisulfate group as compared to the acidified clay group. Both litter amendments proved effective for controlling ammonia, pH, total APC and improving footpad dermatitis, supporting greater broiler rearing efficiency, productivity, and marketable broiler paws.

DESCRIPTION OF PROBLEM

Poultry litter consists of bedding, manure, feathers, spilled feedstuff and soil. Ammonia, a colorless gas, is produced via the microbial breakdown of uric acid in the litter. Ammonia can be detrimental to bird health and performance (Miles et al., 2004) and, along with increased litter moisture, can cause footpad dermatitis negatively affecting bird welfare (Shepherd and Fairchild, 2010). Controlling footpad dermatitis is economically significant to the commercial poultry industry as broiler paw exports to China in 2020 generated a revenue of $461 million US dollars (USDA Foreign Ag Service, 2021). Chemical litter amendments are commonly used during downtime in commercial poultry houses to control ammonia and lower litter pH during the first 7-14 days post placement. Amendments have also been used to reduce incidence and severity of footpad dermatitis (McWard and Taylor, 2000; Nagaraj et al., 2007). Acidified bentonite clay (Poultry Guard®, C&S Chemicals, Roswell, GA) and sodium bisulfate (PLT®, Jones-Hamilton Co., Walbridge, OH) are two common dry chemical litter amendments applied to the surface of litter just prior to bird placement.

Research has shown that both acidified clay and sodium bisulfate can lower litter pH (Payne et al., 2002) and lower ammonia concentrations (McWard and Taylor, 2000). However, recent research comparing the efficacy of these products is limited. A meta-analysis (de Toledo et al., 2020; Table 1) reported that these two products were only directly

Primary Audience: Live Production Managers, Researchers, Veterinarians

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Table 1
Daily litter surface pH values.

	pH		
Day	Control[1]	Acidified clay[2]	Sodium bisulfate[3]
0	9.38[a]	1.10[b]	1.27[b]
1	9.15[a]	1.33[c]	1.87[b]
2	9.32[a]	1.31[b]	1.60[b]
3	9.34[a]	2.45[b]	2.70[b]
4	9.47[a]	3.48[b]	3.54[b]
5	9.42[a]	3.95[b]	3.69[b]
SEM	0.36	0.32	0.32
P-value	<0.0001		

[1] No amendment added.
[2] Acidified clay at 57 kg/93 m^2 (125 lbs/1000 ft^2).
[3] Sodium bisulfate at 57 kg/93 m^2 (125 lbs/1000 ft^2).
[a–c] Within each row, values with different superscripts differ significantly ($P <$ 0.05).

compared once between 1998 and 2019 in a trial involving live birds (lab studies were excluded) and that trial occurred more than 20 years ago (McWard and Taylor, 2000). Additionally, acidified clay recently reentered the market in 2021. The objective of this study was to determine the efficacy of acidified clay and sodium bisulfate for reducing litter surface ammonia concentrations, ammonia flux, pH, total aerobic plate counts (APC) and improving broiler footpad dermatitis. Direct comparison of the two materials allows for effectiveness to be determined under the same environmental conditions with live bird inputs. This is especially important since litter properties can significantly vary among different studies as well as exhibit spatial variability within the same house (Miles et al., 2017). Further, the current research assessed aerial ammonia (early in the flocks) and paw lesions (at the end of the flocks), two aspects of bird well-being, in addition to measuring litter properties related to ammonia generation. Few litter amendment studies report litter surface ammonia concentrations. This is crucial early in the growout when minimum ventilation creates a stagnant atmosphere (Miles et al., 2008).

MATERIALS AND METHODS

All procedures relating to the use of live birds were approved by Pilgrim's rearing standards for commercial broiler production. All flocks related to this project were reared in commercially ventilated housing specifically dedicated to research trials. Water and feed were provided *ad libitum* for every flock and mortality checks were made and recorded up to three times daily. No deceased birds were replaced throughout the trial in order to maintain flock consistency. Three separate pen trials were conducted on a broiler research farm located in Pittsburg, TX, from August 2021-September 2022. For each trial, 40 pens were blocked into 8 sections (4 per each side) within the house to provide equal representation of treatments laterally and across the barn. Each block consisted of 2 pens per treatment and 1 control pen. Each pen contained used pine shavings at a 10 cm depth. The age of the litter was 6 years since the last complete clean-out. Prior to placement pens were de-caked and top dressed as necessary. Litter amendments were spread evenly by hand on the litter surface in each treated pen. Sixteen pens were treated with acidified clay and 16 pens were treated with sodium bisulfate at 57 kg/93 m^2 (125 lb/1000 ft^2) on day of placement (day 0). Application rate was based on manufacturer's guidelines as a common use rate. Eight control pens received no litter treatment. Day-old straight run broiler chicks were placed in 40 pens (60 per pen for a total of 2400 birds). Each pen contained 1 feeder and 5 nipple drinkers in 4.5 m^2 (48 ft^2) of floor space.

Composite surface litter samples were collected from each pen on days −1, 0, 1, 2, 3, 4 and 5. Each composite sample consisted of approximately 50 g of litter collected at a 1.27 cm depth from 15 locations within each pen. Day −1 represented pre-treatment sampling while all other days represented post-treatment sampling. Litter samples were placed into a resealable plastic bag for pH analysis or a sterile bag (Whirl-Pak®, Nasco Sampling, Pleasant Prairie, WI) for bacterial analysis. Litter was homogenized and litter pH was analyzed by stirring 25 g of litter with 75 ml of distilled water and then measured using a portable pH meter (Oakton Instruments, Vernon Hills, IL). For microbial analysis, samples were transported on ice to the laboratory. Upon arrival at the lab, samples were mixed, serially diluted in sterile 0.1 % peptone water and then cultured to determine plate counts for total APC by transferring 1 ml onto Petrifilm™ (3M™ Center, Saint Paul, MN). Samples were incubated at 30°C for 48 h as recommended by the manufacturer. Following incubation, the Petrifilm™ samples were examined for growth and colonies were counted.

Litter surface ammonia concentrations were measured from each pen using an ammonia sampling pump (AP-20, Kitagawa America. Milford, PA) on days 0, 1, 2, 4 and 5. The ammonia tube and sampling pump were placed on the litter surface near the center of each pen until a reading was taken. To measure ammonia flux, cylindrical chambers (14.3-cm radius, 35-cm height) were inverted over the litter in each pen on days 3 and 6. Care was used to form a seal by pushing litter along the bottom edge of each chamber. An ammonia diffusion tube (Drager, Houston, TX) was broken and hung from inside each chamber through a hole in the top. The hole was re-sealed immediately. After 1 hour, tubes were removed from each chamber and an ammonia flux [ppm $m^{-2}h^{-1}$] reading was recorded. The units of flux values are mass of ammonia gas per area per time. Similar inverted chamber methods have been used by Moore et al. (2011) and Miles et al. (2006) over the past two decades. The adaptation of ammonia diffusion tubes in the current study eliminated the need for the expensive optical analyzers and power requirements used in previous studies. In addition, the simplicity of the ammonia diffusion tube/chamber method given here can be utilized by a broader audience like growers and field technicians.

Five random birds from each pen were selected and scored for paw lesions at the end of the flock (day 38). The following scoring system was utilized (McWard and Taylor, 2000):

0 = Normal (no burn, scab or lesion)
1 = Lesion the size of a No. 2 pencil eraser (5 mm) or smaller
2 = Lesion larger than a No. 2 pencil eraser but less than the size of a U.S. Quarter (24 mm)
3 = Lesion larger than a U.S. Quarter

Total APC data were transformed to a base-10 logarithm prior to analysis. All analyses were conducted using SAS (Version 9.4, SAS Institute Inc., Cary, NC). Pen was evaluated as the experimental unit. Significance was defined as alpha = 0.05. Ammonia, pH, ammonia flux and total APC were statistically analyzed by repeated measures ANOVA (the MIXED procedure). Where pre-treatment values were available, these values were included as a covariate. The model included study day, treatment group and the study day by treatment interaction as fixed effects. Trial was included in the analysis as a random effect. Where fixed effects were significant, an all pair-wise comparison between groups was made using two-sided tests and an unadjusted alpha = 0.05. The assumption of normal distribution was assessed by inspection of the residuals. Paw scores were averaged within a pen, then evaluated using ANOVA. Like the other parameters, the model for paw scores included treatment group as a fixed effect and trial as a random effect. If the main effect of treatment was significant, an all pair-wise comparison between groups was made using two-sided tests and an unadjusted alpha = 0.05.

RESULTS AND DISCUSSION

For litter surface pH, the interaction between treatment and day was significant, thus within day comparisons were evaluated. Average litter surface pH values pre-treatment (day −1) were 9.62, 9.27, and 9.30 for control, acidified clay, and sodium bisulfate treatments, respectively. For comparison, untreated litter in commercial flocks on reused pine shavings has been previously reported in a range of 8.23 to 8.68 (Miles et al., 2017). On each day post-treatment, litter surface pH values in acidified clay and sodium bisulfate treatments were significantly lower than values in the control (Table 1). Additionally, on day 1, pH values in the acidified clay group were significantly lower than values in the sodium bisulfate group which agrees with previous research (Payne et al., 2002). The acidified clay litter pH was numerically lower than the sodium bisulfate litter on days 0, 2-4, but not on day 5.

Other findings illustrate that common litter pathogens are destroyed when pH is reduced below 4 (Pope and Cherry, 2000; Payne et al., 2002, 2007). In contrast, Joerger et al. (2020) observed coliform counts were higher in sodium bisulfate treated litter compared to unamended litter at pH below 4. It was speculated that ammonia in the untreated litter had an inhibitory effect. Similarly, Williams et al. (2012) reported *Salmonella* survival below a pH 4 in litter amended with sodium bisulfate. Both treatments in the present study maintained litter surface pH below 4 through day 5.

Results from the litter surface ammonia concentration analysis are summarized in Table 2. None of the interactions involving treatment were significant thus values were averaged across days. The main effect of treatment was highly significant ($P < 0.0001$). Ammonia concentrations in acidified clay (0.54 ppm) and sodium bisulfate (1.08 ppm) treatments were significantly lower than values in the control treatment (8.05 ppm). Additionally, values in the acidified clay group were significantly lower than values in the sodium bisulfate group.

Ammonia flux results are summarized in Table 3. None of the interactions involving treatment were significant thus values were averaged across days. Again, the main effect of treatment was highly significant ($P < 0.0001$). Ammonia flux values in both acidified clay (0.0 ppm $m^{-2}h^{-1}$) and sodium bisulfate (0.58 ppm $m^{-2}h^{-1}$) groups were significantly lower than values in the control bird pens (47.1 ppm $m^{-2}h^{-1}$). A recent meta-analysis of the impacts of litter treatment on litter quality concluded that acidifying treatments reduce ammonia as well as litter pH and moisture, pathogenic microbiota, and mortality rate while improving broiler weight gain (de Toledo et al., 2020).

Results from the total APC analysis are summarized in Table 4. The interaction between treatment and day was significant, thus within day comparisons were evaluated. Average total APC values pre-treatment (day −1) were 7.65, 7.47, and 7.50 $\log^{10}$ cfu mL^{-1} for control, acidified clay, and sodium bisulfate pens, respectively. On each day, total APC values in acidified clay and sodium bisulfate treatments were significantly lower than values in the control. Additionally, on day 1, values in the acidified clay group were significantly lower than values in the sodium bisulfate group. This is similar to the litter surface pH on day 1 where the acidified clay was less than the sodium bisulfate. On all other days, the acidified clay APC values were numerically less than those of the sodium bisulfate, which is again like the trend in litter surface pH (Table 1). Previous research has shown that sodium bisulfate can reduce total APC on broiler house floors at 24 h and 72 h at application rates of 45 kg/93 m^2 and 68 kg/93 m^2 (Payne et al., 2019). In contrast, Joerger et al. (2020) reported an increase in total APC in shavings/manure samples supplemented with 10 % sodium bisulfate; however, this was most likely due to adjusting the pH to 6.5 during the extraction procedure. Mohammadi-Aragh et al. (2022) found analogous total APC in control and sodium bisulfate treatments. Both acidified clay and sodium bisulfate have also been shown to significantly reduce experimentally inoculated litter *Salmonella* populations (45 kg/93 m^2) 24 h post treatment (Payne et al., 2002).

The results of the analysis of the paw lesion scores are summarized in Table 5 and Fig. 1. The main effect of treatment was significant. Average paw scores within a pen were significantly higher in the control group as compared to the acidified clay and sodium bisulfate groups. Additionally, average paw scores were significantly lower in the acidified clay group as compared to the sodium bisulfate group. Paw scores graded as 0 were 0, 24.4, and 14.4 % for control, acidified clay, and sodium bisulfate treatments, respectively. The results agree with previous research illustrating that acidified clay had improved footpad lesion scores compared to control, dry alum and sodium bisulfate litter amendments (McWard and Taylor, 2000). The moisture absorbing characteristics of dried acidified bentonite clay along with the ammonia and pH reduction capabilities most likely contributed to improved paw lesion scores.

CONCLUSIONS AND APPLICATIONS

1. Acidified clay and sodium bisulfate litter amendments applied at

Table 2
Litter surface ammonia concentrations averaged across days.

	Ammonia [ppm]		
	Control[1]	Acidified clay[2]	Sodium bisulfate[3]
	8.05[a]	0.54[c]	1.08[b]
SEM	0.65	0.59	0.59
P-value	<0.0001		

[1] No amendment added.
[2] Acidified clay at 57 kg/93 m^2 (125 lbs/1000 ft^2).
[3] Sodium bisulfate at 57 kg/93 m^2 (125 lbs/1000 ft^2).
[a–c] Values with different superscripts differ significantly ($P < 0.05$).

Table 3
Litter ammonia flux values averaged across days.

	Ammonia flux [ppm m^{-2} h^{-1}]		
	Control[1]	Acidified clay[2]	Sodium bisulfate[3]
	47.1[a]	0.0[b]	0.58[b]
SEM	2.1	1.6	1.6
P-value	<0.0001		

[1] No amendment added.
[2] Acidified clay at 57 kg/93 m^2 (125 lbs/1000 ft^2).
[3] Sodium bisulfate at 57 kg/93 m^2 (125 lbs/1000 ft^2).
[a–b] Values with different superscripts differ significantly ($P < 0.05$).

Table 4
Daily total aerobic plate count (APC) values of broiler litter surface.

	APC $\log^{10}$ cfu mL^{-1}		
Day	Control[1]	Acidified clay[2]	Sodium bisulfate[3]
0	6.95[a]	4.23[b]	4.54[b]
1	5.25[a]	3.53[c]	4.09[b]
2	5.91[a]	4.20[b]	4.52[b]
3	6.60[a]	5.50[b]	5.61[b]
4	6.23[a]	5.37[b]	5.49[b]
5	6.30[a]	5.48[b]	5.58[b]
SEM	0.23	0.17	0.17
P-value	<0.0001		

[1] No amendment added.
[2] Acidified clay at 57 kg/93 m^2 (125 lbs/1000 ft^2).
[3] Sodium bisulfate at 57 kg/93 m^2 (125 lbs/1000 ft^2).
[a–c] Within each row, values with different superscripts differ significantly ($P < 0.05$).

Table 5
Broiler paw lesion scores at the end of three flocks (day 38).

	Paw lesion scores		
	Control[1]	Acidified clay[2]	Sodium bisulfate[3]
	2.16[a]	1.39[c]	01.69[b]
SEM	0.33	0.31	0.31
P-value	0.0002		

[1] No amendment added.
[2] Acidified clay at 57 kg/93 m^2 (125 lbs/1000 ft^2).
[3] Sodium bisulfate at 57 kg/93 m^2 (125 lbs/1000 ft^2).
[a–c] Values with different superscripts differ significantly ($P < 0.05$).

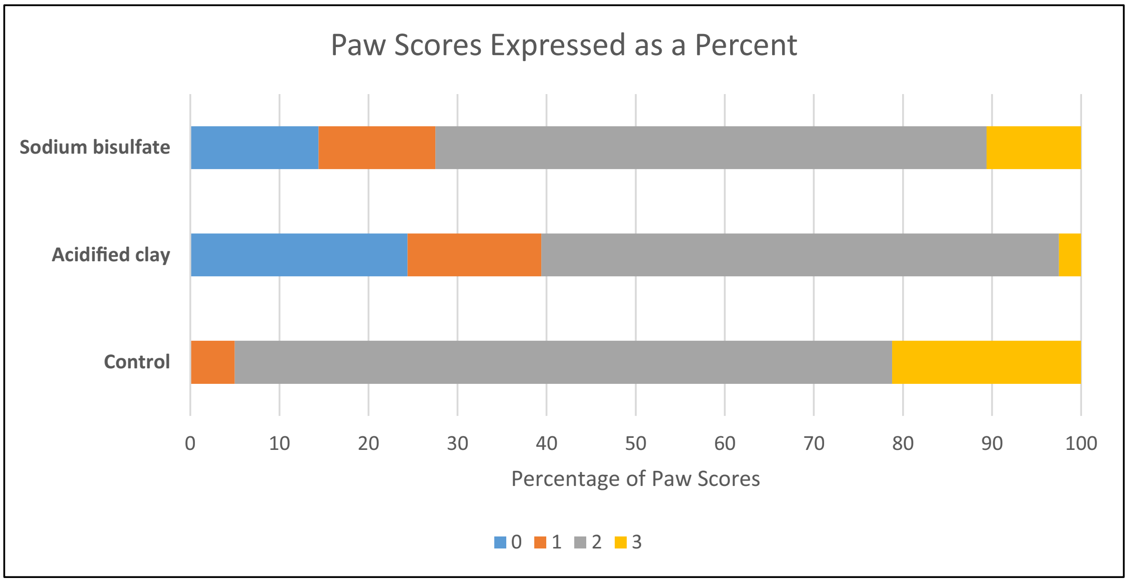

Fig. 1. Broiler paw lesion scores at the end of three flocks (day 38) expressed as a percent.

57 kg/93 m^2 (125 lbs/1000 ft^2) significantly lowered litter surface pH below 4 for up to 5 days post bird placement.

2. On day 1, litter treated with acidified clay showed significantly lower pH and total APC compared to sodium bisulfate treated litter.

3. Both chemical amendments significantly reduced ammonia, ammonia flux, and total APC compared to the control. Ammonia concentrations in the acidified clay group were significantly lower than values in the sodium bisulfate group.

4. Paw lesion scores were significantly lowered by acidified clay and sodium bisulfate treatments compared to the control pens. Scores were significantly lower in the acidified clay treated pens compared to the sodium bisulfate treated pens.

CRediT authorship contribution statement

J.B. Payne: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **D.M. Miles:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **K.L. Moncada:** Data curation, Investigation, Project administration, Supervision, Writing – review & editing. **K. Gardner:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – review & editing.

DECLARATION OF COMPETING INTEREST

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

J.B. Payne reports a relationship with C&S Chemicals that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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