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Research Article
Litter Treatment with Aluminum Sulfate (Alum) Produced an Inconsistent Reduction in Horizontal Transmission of *Campylobacter* in Chickens

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Abstract

Background and Objective: Campylobacteriosis is a significant health problem worldwide and poultry are considered as one of the main vehicles of transmission. This study was conducted to determine whether alum reduces *Campylobacter* colonization in broilers by reducing horizontal transmission between birds or by reducing *Campylobacter* counts in birds already colonized (Therapeutic efficacy). **Materials and Methods:** Two replicate experiments were conducted and in each experiment, day of hatch broiler chicks (n = 295) were divided into 7 treatment groups including controls. Each treatment was reared in either no (0 kg), low (0.78 kg m^{-2}) or high (1.58 kg m^{-2}) concentrations of aluminum sulfate (alum; Al^{+} Clear). During days 7, 14, 28 and 42, ten birds from each treatment were analyzed for *Campylobacter* counts in the ceca. To evaluate whether alum inhibits horizontal transmission between birds, *Campylobacter* negative birds were reared with seeder birds that served as carriers. **Results:** Alum reduced ($p<0.05$) horizontal transmission of *Campylobacter* at 14 and 28 days in experiment 1 and only with the highest concentration of alum at 42 days in experiment 2. To evaluate the therapeutic efficacy of alum, all birds were inoculated with *Campylobacter* (5.2×10^{6} CFU mL^{-1}) prior to placement in pens. Infected birds reared on low or high alum had lower ($p<0.05$) *Campylobacter* counts at 14 and 28 days in only 1 of 2 experiments. At 42 days, there were no differences in cecal *Campylobacter* counts between alum treated and untreated controls in experiment 1 and for only the highest concentration in experiment 2. **Conclusion:** It appears treating litter with alum is not a consistent way to reduce enteric *Campylobacter* counts.

Key words: Alum, poultry litter, horizontal transmission, *Campylobacter jejuni*, foodborne illness

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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INTRODUCTION

Campylobacter has been recognized as the leading cause of bacterial foodborne illness in the world and poultry products are reported to be the prevailing vehicles of campylobacteriosis transmission[1-3]. The colonization of *Campylobacter* in the bird's gut and the subsequent contamination of the meat during mechanized processing is the contributing factor in the prevalence of this pathogen on poultry carcasses[4]. Effective pre-harvest intervention strategies are therefore needed to reduce enteric colonization and the incidence of campylobacteriosis in humans[5-8].

To this end, Line[9] supplemented poultry litter with aluminum sulfate (alum) [$Al_2(SO_4)_3.14H_2O$] and reported a decrease in cecal *Campylobacter* frequency and counts and corresponding reduction in whole carcass contamination. Alum may be added to poultry litter to precipitate soluble phosphorous and consequently minimize phosphorous runoff[10-12]. Alum is also used to reduce ammonia concentrations and thus, promotes poultry health and well being[13,14]. It appears that litter application of alum may also help control the prevalence of *Campylobacter*, however, it is unknown how alum exerts its effects on *Campylobacter* colonization in the intestinal tract of birds.

The ability of alum to lessen *Campylobacter* contaminations in chickens may be related to alum's effect on litter pH. Litter is acidified upon alum application[9,15] and a reduced pH can be lethal to *Campylobacter*[16-19]. This reduction in litter pH may lessen horizontal transmission of *Campylobacter* within flock. Although horizontal transmission was not directly determined by Line[9], other mechanisms may play a role for the reduction in this foodborne pathogen following alum administration. Because of litter consumption by chickens and corresponding alum ingestion, this compound may also act as a therapeutic agent against *Campylobacter* to limit colonization in the gut of chickens. To investigate the manner in which alum exerts its effects, this study was conducted to determine whether alum effects *Campylobacter* colonization in the ceca of broilers by reducing horizontal transmission between birds or by reducing *Campylobacter* counts in birds already colonized (Therapeutic efficacy). This study may provide an understanding of the mechanisms of action of alum and how it may be used to control the colonization of *Campylobacter* in chickens.

MATERIALS AND METHODS

Animal care and alum administration: At the beginning of each of two replicate experiments, fresh pine shaving were used and served as litter in seven isolation pens of about 3.06 m^2 (33 ft^2) equipped with feeders and bell-shaped water drinkers. Of the 7 and 4 pens were top dressed with commercially available aluminum sulfate (Al^+ Clear, General Chemical, Parsippany, N.J.), treated with either low (0.78 kg m^{-2}) or high (1.58 kg m^{-2}) concentrations of alum as per the procedure used by Line[9]. The litter was then sprayed with 1 L of water to provide adequate moisture for activation of the product. Although untreated control pens received no litter treatment, water (1L) was sprayed to maintain moisture as the rest of the pens. Water application in all pens (1 L pen^{-1}) was repeated every week throughout the experiment to maintain moisture concentrations near commercial conditions. The birds were reared for 6 weeks, feed and water were available *ad libitum* throughout the study. Alum was re-applied at 5 weeks as practiced from the previous alum study[9].

Experimental treatments: Two separate replicate experiments were conducted in this study. A total of 590 chicks were used in this study (n = 295 replicate $trials^{-1}$). Birds were divided into 7 treatment groups. These were: (1) Negative controls (NC, birds not inoculated with *C. jejuni*, no alum), (2) Horizontal transmission controls (HC, negative control birds reared together with *C. jejuni* infected seeders, no alum), (3) Horizontal transmission/low alum (HL, birds not inoculated with *C. jejuni* but reared with *C. jejuni* infected seeders on a low concentrations of alum), (4) Horizontal transmission/high alum (HH, birds not inoculated with *C. jejuni* but reared with *C. jejuni* infected seeders on high concentrations of alum, (5) Therapeutic efficacy controls (TC, all birds inoculated with *C. jejuni*, no alum), (6) Therapeutic efficacy/low alum (TL, all birds inoculated with *C. jejuni* and raised on low concentrations of alum) and (7) Therapeutic efficacy/high alum (TH, all birds inoculated with *C. jejuni* and raised on high concentrations of alum).

Of the 295 total newly hatched chicks used in each trial, 160 were released into pens (NC, HC, HL, HH, 40 birds pen^{-1}), whereas another 135 birds were orally gavaged with 0.2 mL of *C. jejuni* (approx. 5×10^6 CFU mL^{-1}) and released in TC (n = 55), TL (n = 40), TH (n = 40) treatment pens. After 7 days, a total of 15 birds from TC were transferred to HC, HL and HH pens to serve as *Campylobacter* seeders (n = 5 $group^{-1}$) in these treatment groups. Seeders used to evaluate horizontal transmission were fitted with leg bands to distinguish them from the rest of the birds in the pen and were not included in the data analysis. Seven strains of *Campylobacter jejuni* previously isolated from chickens were used to colonize the birds in this study following the procedure described previously[20].

Data collection: Birds from each treatment group (n = 10) were analyzed at days 7, 14, 28 and 42 (n = 10 day^{-1}) for *C. jejuni* counts in the ceca as previously described by Cole *et al.*[20]. Briefly, chickens were euthanized by CO_2 and the ceca of each bird were aseptically dissected and diluted in Butterfield's Phosphate Diluent (BPD, 68 g L^{-1} KH_2PO_4, pH 7.2) and vortexed. The homogenous mixture was serially diluted (1:10) with BPD and inoculated on Campy Line Agar (CLA) plates[20]. Labeled CLA plates were incubated for 48 h at 42°C under microaerophilic conditions. Counts were recorded and direct bacterial counts were converted to CFU g^{-1} of the cecal content. *Campylobacter* colonies were confirmed by latex agglutination test (PANBIO, Inc. Columbia, MD) and further identified as *C. jejuni* isolates using API® Campy (Biomėrieux® Durham, NC). In addition, about 20 g of litter sample were obtained weekly from each treatment group, which were used to monitor litter pH levels. Litter pH levels were analyzed by diluting (1:2) the litter samples with de-ionized water and pH was determined by a pH meter.

Statistical analysis: The numbers of *Campylobacter* colonies were logarithmically transformed (log_{10} CFU g^{-1}) prior to analysis to achieve homogeneity of variance[21]. Data were subjected to ANOVA using the GLM procedure of SAS[22]. Treatment means were partitioned by LSMEANS analysis. A probability of $p<0.05$ was required for statistical significance.

RESULTS

Cecal *Campylobacter* counts for the alum treatments are presented in Table 1 and 2. In birds evaluated for horizontal transmission, *Campylobacter* was not detected in non-inoculated groups (HC, HL, HH) at 7 days of age in both replicate experiments. However, after transfer (7 days) of *Campylobacter* inoculated seeder chicks in groups HC, HL and HH, birds in these groups became *Campylobacter* positive by 14 days of age. In experiment 1, HH demonstrated reduced *Campylobacter* counts compared to HL and HC at 14 days ($p<0.05$). On day 28, both HL and HH had lower ($p<0.05$) *Campylobacter* counts compared to HC. By 42 days, no difference in *Campylobacter* counts were observed between treatments. In experiment 2, no differences were observed in *Campylobacter* counts at 14 and 28 days for horizontal treatments. However, at 42 days, a reduction ($p<0.05$) in *Campylobacter* counts was observed in HH compared to HC. None of the negative controls (NC, not inoculated, no seeder birds) had *Campylobacter* counts during the entire study. Birds inoculated with *Campylobacter* were evaluated for therapeutic efficacy of alum. All chicks inoculated at day of hatch with *Campylobacter* were colonized by 7 days (groups TC, TL, TH) in both replicate experiments. In experiment 1, both TL and TH reduced ($p<0.05$) *Campylobacter* counts compared to TC at 14 and

Table 1: Mean counts of *Campylobacter jejuni* recovered from ceca of broilers reared on litter with or without alum at 7, 14, 28 and 42 days (Experiment 1)[1]

	Mean cecal *Campylobacter* counts (log_{10} CFU g^{-1} cecal content)			
	7	14	28	42
Treatments	(days)			
Horizontal control/no alum	ND	5.8×10^{5c}	7.7×10^{8a}	1.2×10^{7a}
Horizontal/low alum	ND	3.1×10^{5c}	5.0×10^{8b}	1.2×10^{6a}
Horizontal/high alum	ND	6.9×10^{4d}	2.2×10^{7c}	1.1×10^{6a}
Therapeutic control/no alum	1.0×10^{8a}	5.9×10^{8a}	5.5×10^{8a}	2.4×10^{7a}
Therapeutic/low alum	1.0×10^{8a}	2.6×10^{8b}	4.0×10^{8b}	2.1×10^{6a}
Therapeutic/high alum	1.0×10^{8a}	1.1×10^{8b}	1.1×10^{8b}	1.3×10^{6a}

[a,b,c,d]Different letter superscripts in the same column indicates significant difference ($p<0.05$), [1]All data were log_{10} transformed for statistical analysis. For clarity of presentation, arithmetic means are presented, ND: Not detected

Table 2: Mean counts of *Campylobacter jejuni* recovered from ceca of broilers reared on litter with or without alum at 7, 14, 28 and 42 days (Experiment 2)[1]

	Mean cecal *Campylobacter* counts (log_{10} CFU g^{-1} cecal content)			
	7	14	28	42
Treatments	(days)			
Horizontal control/no alum	ND	1.6×10^{6b}	2.5×10^{8ab}	2.6×10^{8a}
Horizontal/low alum	ND	1.5×10^{6b}	2.1×10^{8ab}	5.0×10^{6ab}
Horizontal/high alum	ND	8.4×10^{5b}	3.3×10^{7b}	3.8×10^{6b}
Therapeutic control/no alum	1.0×10^{a}	5.9×10^{8a}	2.7×10^{8a}	9.1×10^{7a}
Therapeutic/low alum	1.2×10^{8a}	7.9×10^{8a}	2.7×10^{8a}	6.1×10^{6ab}
Therapeutic/high alum	9.3×10^{7a}	3.3×10^{8a}	2.5×10^{8a}	5.0×106^{ab}

[a,b]Different letter superscripts in the same column indicates significant difference ($p<0.05$), [1]All data were log_{10} transformed for statistical analysis. For clarity of presentation, arithmetic means are presented, ND: Not detected

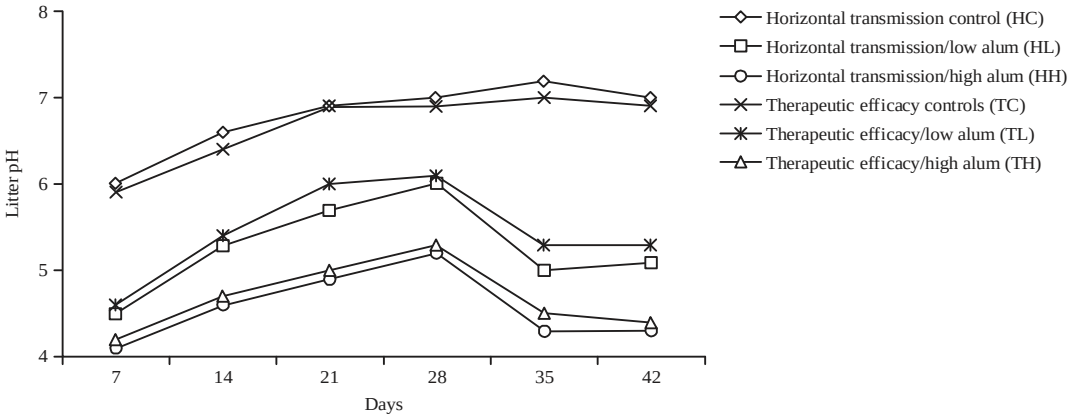

Fig. 1: Effect of alum on litter pH, litter was treated with low (2.4-3.06 kg m^{-2}) or high (4.8-3.06 kg m^{-2}) concentrations of alum. Controls received no litter treatment. Alum was re-applied at 5th week

28 days. By 42 days, no reduction in *Campylobacter* counts were observed. In experiment 2, the therapeutic efficacy of alum to reduce *Campylobacter* counts in inoculated birds were not evident. In both replicate experiments, birds in the therapeutic groups (TC, TL, TH) had higher cecal *Campylobacter* counts at 14 days of age when compared with the horizontal group (HC, HL, HH). This is possibly due to the fact that the therapeutic groups were inoculated with *Campylobacter* directly as opposed to horizontal group being indirectly exposed to *Campylobacter* infected seeders.

Top dressing the litter with alum reduced ($p<0.05$) litter pH when evaluated weekly during the 6 weeks study (Fig. 1). The highest alum concentrations yielded the greatest reduction ($p<0.05$) in litter pH. There were no differences in litter pH for pens treated with alum and inoculated with *Campylobacter* compared to those pens treated with alum but not inoculated (Fig. 1).

DISCUSSION

In a previous study, alum applied on chicken litter reduced *Campylobacter* colonization frequency and populations in the ceca[9]. Line[9] suggested that the decrease in pH of the litter caused by alum treatment may have reduced *Campylobacter* populations in the litter, thus reducing *Campylobacter* transmission in poultry flocks. As reported from other studies, *Campylobacter* cells cannot survive under acidic conditions, e.g., below 4.0[18,23]. The appropriate pH range for growth of *Campylobacter* is reported to be around 5.5-7.5[23].

In this study, two potential mechanisms (reduced horizontal transmission or therapeutic efficacy) on how alum might reduce *Campylobacter* counts in chickens were evaluated. In both replicate experiments, litter treatments with alum reduced cecal *Campylobacter* counts in both the birds tested for horizontal or therapeutic efficacy. The decrease in *Campylobacter* counts in the horizontal group in both replicate trials may be due to the acidifying effect of alum in litter (Fig. 1). Litter application of alum decreased litter pH compared to the non-acidified litters, however, the reductions in *Campylobacter* counts in treated litters are minimal and inconsistent in both replicate experiments. This suggests that although alum lowers the pH of the litter, the acidifying effect of alum to lyse *Campylobacter* cells is either limited or conversely did not have enough contact with *Campylobacter* contained within fecal dropping sitting on top of the alum treated litter. The difference in the outcome between this study and Line's study[9] may be explained by the experimental design employed by that researcher. In this study, researchers utilized used chicken litter contaminated with *Campylobacter* followed by alum treatment prior to bird placement[9]. The alum litter treatment may have reduced or eliminated *Campylobacter* counts, reducing the potential for *Campylobacter* colonization in placed birds. In the present study, to evaluate whether alum inhibits horizontal transmission between birds, *Campylobacter* inoculated chicks (seeders) were used as the source of *Campylobacter* infection. The experimental design used in this study might be more representative of a commercial broiler setting because uninfected birds would be exposed to the constant shedding from *Campylobacter* contaminated birds. The results obtained in the present study are consistent with a follow-up study conducted by Line[9] and Bailey[15] who reported that alum application in litter in a commercial broiler farm reduced the onset of *Campylobacter* colonization in broilers but did not reduce final *Campylobacter* counts in 6 weeks old market age birds.

Because of the coprophagous nature of chickens, it is proposed that alum may have therapeutic efficacy to reduce *Campylobacter* counts in the ceca. Interestingly, the therapeutic efficacy of alum was observed during 14 and 28 days of the first experiment. It is possible that birds may have ingested alum particles through litter pecking, acidifying their gut and consequently reduced *Campylobacter* counts in the ceca. Alternatively, the application of alum may have caused litter aggregation making the *Campylobacter* contaminated litter unavailable for ingestion by the birds.

In this study, the effect of alum on cecal *Campylobacter* counts declined over time. Unfortunately, re-application (35 days) of alum elicited little effect on reducing *Campylobacter* counts towards the end of this study. Nonetheless, this study demonstrated that alum has some potential to reduce *Campylobacter* loads at specific times during the rearing period. Further research is necessary to optimize the efficacy of alum as a tool to reduce *Campylobacter* colonization during broiler production.

SIGNIFICANCE STATEMENT

- Previous study has shown that litter amendments, mainly acidification of litter, using alum can reduce ammonia levels and improve the health and performance of birds
- This study explored the potential application of alum to reduce *Campylobacter* colonization or horizontal transmission of *Campylobacter* in poultry
- Alum demonstrated some potential to reduce both *Campylobacter* colonization and horizontal transmission to some extent, however, the effect of alum on cecal *Campylobacter* counts declined over time
- Further study is aimed at combing litter amendments with other pre-harvest invention strategies for reducing *Campylobacter* colonization in broiler chickens

REFERENCES

1. WHO., 2011. *Campylobacter*. Fact Sheet, World Health Organization, Geneva, Switzerland. http://www.who.int/mediacentre/factsheets/fs255/en/
2. CDC., 2015. 2015 food safety report. Centers for Disease Control and Prevention. https://www.cdc.gov/foodnet/ pdfs/ foodnet-mmwr-progress-508-final.pdf
3. Wagenaar, J.A., D.G. Newell, R.S. Kalupahana and L. Mughini-Gras, 2015. *Campylobacter* Animal Reservoirs, Human Infections and Options for Control. In: Zoonoses-Infections Affecting Humans and Animals, Sing, A. (Ed.). Springer, Netherlands, pp: 159-177.
4. Robyn, J., G. Rasschaert, F. Pasmans and M. Heyndrickx, 2015. Thermotolerant *Campylobacter* during broiler rearing: Risk factors and intervention. Comp. Rev. Food Sci. Food Saf., 14: 81-105.
5. Vandeplas, S., C. Marcq, R.D. Dauphin, Y. Beckers, P. Thonart and A. Thewis, 2008. Contamination of poultry flocks by the human pathogen *Campylobacter* spp. and strategies to reduce its prevalence at the farm level. Biotechnol. Agron. Soc. Environ., 12: 317-334.
6. Newell, D.G., K.T. Elvers, D. Dopfer, I. Hansson and P. Jones *et al*., 2011. Biosecurity-based interventions and strategies to reduce *Campylobacter* spp. on poultry farms. Applied Environ. Microbiol., 77: 8605-8614.
7. WHO., 2013. The global view of campylobacteriosis: Report of an expert consultation, Utrecht, Netherlands, 9-11 July 2012. World Health Organization, Geneva. http://apps.who.int/iris/handle/10665/80751
8. Epps, S.V.R., R.B. Harvey, M.E. Hume, T.D. Phillips, R.C. Anderson and D.J. Nisbet, 2013. Foodborne *Campylobacter*, Infections, metabolism, pathogenesis and reservoirs. Int. J. Environ. Res. Public Health, 10: 6292-6304.
9. Line, J.E., 2002. *Campylobacter* and *Salmonella* populations associated with chickens raised on acidified litter. Poult. Sci., 81: 1473-1477.
10. Moore, P.A. and D.R. Edwards, 2007. Long-term effects of poultry litter, alum-treated litter and ammonium nitrate on phosphorus availability in soils. J. Environ. Qual., 36: 163-174.
11. Seiter, J.M., K.E. Staata-Borda, M. Ginder-Vogel and D.L. Sparks, 2008. XANES spectroscopic analysis of phosphorus speciation in alum-amended poultry litter. J. Eniron. Qual., 37: 477-485.
12. Guo, M. and W. Song, 2009. Nutrient value of alum-treated poultry litter for land application. Poult. Sci., 88: 1782-1792.
13. Choi, I.H. and P.A. Moore Junior, 2008. Effects of liquid aluminum chloride additions to poultry litter on broiler performance, ammonia emissions, soluble phosphorus, total volatile fatty acids and nitrogen contents of litter. Poult. Sci., 87: 1955-1963.
14. Zhang, H.F., H.C. Jiao, Z.G. Song and H. Lin, 2011. Effect of alum-amended litter and stocking density on ammonia release and footpad and hock dermatitis of broilers. Agric. Sci. China, 10: 777-785.
15. Line, J.E. and J.S. Bailey, 2006. Effect of on-farm litter acidification treatments on *Campylobacter* and *salmonella* populations in commercial broiler houses in Northeast Georgia. Poult. Sci., 85: 1529-1534.
16. Choi, I.H., J.N. Kim and Y.M. Kwon, 2008. Effects of chemical treatments on pH and bacterial population in poultry litter: A laboratory experiment. Br. Poult. Sci., 49: 497-501.
17. Rothrock, M.J.Jr., K.L. Cook, J.G. Warren and K. Sistani, 2008. The effect of alum addition on microbial communities in poultry litter. Poult. Sci., 87: 1493-1503.

18. Chen, Z. and X. Jiang, 2014. Microbiological safety of chicken litter or chicken litter-based organic fertilizers: A review. Agriculture, 4: 1-29.
19. Sahin, O., I.I. Kassem, Z. Shen, J. Lin, G. Rajashekara and Q. Zhang, 2015. *Campylobacter* in poultry: Ecology and potential interventions. Avian Dis., 59: 185-200.
20. Cole, K., M.B. Farnell, A.M. Donoghue, N.J. Stern and E.A. Svetoch *et al*., 2006. Bacteriocins reduce *Campylobacter* colonization and alter gut morphology in Turkey poults. Poult. Sci., 85: 1570-1575.
21. Perveen, F. and Z. Hussain, 2012. Use of statistical techniques in analysis of biological data. Basic Res. J. Agric. Sci. Rev., 1: 1-10.
22. SAS., 2011. Base SAS® 9.3 Procedures Guide. SAS Institute Inc., Cary, NC., USA.
23. Shaheen, B.W., M.E. Miller and O.A. Oyarzabal, 2007. *In vitro* survival at low pH and acid adaptation response of *Campylobacter jejuni* and *Campylobacter coli*. J. Food Saf., 27: 326-343.