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Use of organic acids in water supplementation and its effects on *Salmonella* prevalence in broilers

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Abstract

This study investigated the effects of supplementing drinking water with organic acids on the prevalence of *Salmonella enteritidis* (SE) in broiler chickens. The research was conducted at the Charles C. Miller Poultry Research and Education Center, Auburn University, Alabama. A total of 900 male broiler chickens were divided into three treatment groups: acetic acid, citric acid, and a control group with plain water. The organic acids were administered at a concentration of 2 mL per liter of drinking water from day one until the end of the grow-out period. The results showed that supplementing water with organic acids did not significantly influence *Salmonella* colonization in the ceca or reduce the incidence of *Salmonella* in poultry carcasses. This suggests that while organic acids may have some benefits, they are not as effective as standalone pre-harvest interventions for controlling *Salmonella* contamination in poultry. The study emphasized the critical role of Best Management Practices (BMPs) in controlling *Salmonella* and highlighted the importance of educating poultry producers and processors about BMPs. Additionally, the study evaluated the impact of the SE inoculation on day 7, administered via oral gavage, and the subsequent monitoring through various sampling methods, including chick papers, boot swabs, ceca enrichment, and carcass rinse samples. Despite the consistent administration of organic acids, no significant reduction in *Salmonella* colonization was observed. The findings underscore the need for combining organic acids with other mitigation strategies, such as litter composting, to enhance overall effectiveness in controlling *Salmonella* and other pathogens.

Keywords: Broiler, organic acids, *Salmonella*

Resumen

Este estudio investigó los efectos de la suplementación del agua con ácidos orgánicos sobre la prevalencia de *Salmonella enteritidis* (SE) en pollos de engorde. El estudio se desarrolló en el Centro de Investigación y Educación Avícola Charles C. Miller de la Universidad de Auburn, Alabama. Un total de 900 pollos machos se dividieron en tres grupos de tratamiento: ácido acético, ácido cítrico y un grupo de control con agua corriente. Los ácidos orgánicos se administraron en concentración de 2 ml/L de agua desde el primer día hasta el final del período de crecimiento. Los resultados mostraron que la suplementación del agua con ácidos orgánicos no influyó significativamente en la colonización de *Salmonella* en el ciego ni redujo la prevalencia de Salmonella en las canales. Esto sugiere que, si bien los ácidos orgánicos pueden tener beneficios, no son tan efectivos como intervenciones precosecha independientes para controlar la contaminación por *Salmonella*. El estudio enfatizó el papel crítico de las Buenas Prácticas de Manejo en el control de *Salmonella* y destacó la importancia de educar a los productores y procesadores avícolas. Además, el estudio evaluó el impacto de la inoculación de SE en el día 7, administrada por vía oral, y el monitoreo posterior a través de varios métodos de muestreo: papeles, cobertores de botas, enriquecimiento de ceca y enjuague de carcasas. Los hallazgos recalcan la necesidad de combinar los ácidos orgánicos con otras estrategias de mitigación, como el compostaje de la cama, para mejorar la efectividad general en el control de Salmonella y otros patógenos.

Palabras clave: Ácidos orgánicos, pollo de engorde, *Salmonella*

Introduction

Salmonella remains the leading pathogen of food safety concern worldwide and poultry meat has been identified as one of the primary transmission vectors (Centers for Disease Control and Prevention [CDC] et al., 2024). According to the CDC, *Salmonella* infections have remained consistent over the past two decades. The CDC estimates that 1.35 million people in the United States (US) get sick from *Salmonella* each year, with approximately 26,500 hospitalizations and 420 deaths annually . Additionally, the CDC et al. (2024) estimate that over 23% of foodborne *Salmonella* illnesses are linked to poultry meat consumption. The U.S. Department of Agriculture (2024) estimated that in 2018, the annual cost of foodborne illness due to non-typhoidal *Salmonella* was more than $4.1 billion. Therefore, research, is required to develop efficient and cost-effective pre-harvest programs to control and reduce the spread of *Salmonella* in chickens in order to reduce human illness due to this pathogen.

Poultry represents a significant reservoir for *Salmonella*, raising public health concerns about the transmission of this pathogen from poultry products to humans (Antunes et al., 2016). Various potential sources of *Salmonella* contamination exist within integrated poultry operations, encompassing chicks, litter, water, feed, rodents, wild birds, pets, humans, transportation, and processing procedures (J. Stan Bailey, 1988). Preventing *Salmonella* colonization in chickens poses considerable challenges, particularly as serovars such as *Salmonella enteritidis* form commensal relationships with birds (Antunes et al., 2016; J. Stan Bailey, 1988) .

Traditionally, antibiotic usage has been the primary method for *Salmonella* control in the animal food industry, including poultry (Ruvalcaba-Gómez et al., 2022). However, increasing attention has been directed towards contaminated poultry products due to the rising incidence of antibiotic-resistant bacteria associated with human illnesses (Hur et al., 2012; Varma et al., 2005). Reports suggest that prophylactic antibiotic use or growth promoters contribute to the emergence of antibiotic-resistant bacteria (Huyghebaert et al., 2011; van Immerseel et al., 2009). Consequently, regulatory bodies like the United States Food and Drug Administration (FDA) have prohibited the use of in-feed antibiotics as growth promoters since 2017, prompting the exploration of alternatives to control bacterial pathogens such as *Salmonella* spp. (Ruvalcaba-Gómez et al., 2022; van Immerseel et al., 2009). These alternatives focus on holistic approaches throughout the production chain, beginning with pre-harvest interventions and management practices to minimize *Salmonella* colonization in poultry (Foof Safety and Inspection Service [FSIS] & United States Department of Agriculture [USDA], 2021). General hygienic and biosecurity measures during the primary production phase are important components of poultry producers' management plans. Various reports have demonstrated the success of these measures in controlling *Salmonella* at the pre-harvest level (Barrow et al., 2012; European Food Safety Authority [EFSA] & European Centre for Disease (ECDC), 2016; Hugas & Beloeil, 2014).

Salmonella exhibits resilience and adaptability, capable of persisting in litter or dust for extended periods. This persistence facilitates transmission between flocks (Davies & Wray, 1996; Mani-López et al., 2012). Experimental evidence suggests that *Salmonella* can be eliminated from litter through composting procedures, such as on-site windrow composting (Macklin et al., 2008). In-house composting presents a viable solution for producers due to its cost-effectiveness compared to chemical treatments. On the other hand, there are optimum conditions to consider when in-house litter composting, for example, for a compost pile to function properly, its temperature must exceed 50 °C and be sustained at that level for at least one day to eliminate bacteria. Generally, the pile is turned every few days. This mixing process introduces oxygen to the bacteria, enabling them to further decompose the organic material in the compost and generate heat (Macklin et al., 2006). Additionally, for optimal composting rates, primary ingredients, amendments, and recycled compost are combined to achieve ideal initial ranges for carbon/nitrogen ratio (C/N) (20-40), moisture (45-65%), and porosity (30-50%). This mixture is then placed into a pile or windrow to initiate the composting process. Depending on the system and composting phase, the mix is turned daily, twice a week, weekly or monthly. In certain systems, air is forced through the compost to regulate temperature and maintain adequate oxygen levels (Keener et al., 2014). However, further validation of the composting conditions under commercial farm conditions is necessary (Williams et al., 2012).

Given the multifaceted factors influencing *Salmonella* colonization in birds, including environmental conditions, reservoirs, shedding patterns, bacterial characteristics, and animal-related factors, a comprehensive, integrated approach is imperative to reduce or eliminate *Salmonella* colonization in poultry during the grow-out phase. The "multi-hurdle" approach, which involves sequential pathogen interventions, shows promise by combining pre-harvest products in feed or water with appropriate management and biosecurity programs to further restrict *Salmonella* colonization in poultry (FSIS & USDA, 2021). Organic acids have garnered attention for their antimicrobial properties and nutritional benefits in poultry. Studies have demonstrated their efficacy in reducing *Salmonella* counts, particularly the use of acetic acid in drinking water (Hamed & Hassan, 2013)

In light of the significant public health concerns posed by *Salmonella* contamination in poultry products, this study seeks to evaluate and implement effective pre-harvest mitigation strategies to reduce or eliminate *Salmonella* contamination levels in poultry products intended for human consumption. Specifically, this study aims to determine the impact of water supplementation using organic acids to reduce or prevent *Salmonella* infection pressure in both the environment and within the birds themselves. Additionally, it aims to assess the *Salmonella* status of commercial farms in Alabama and evaluate current management practices related to *Salmonella* occurrence. Furthermore, this study endeavors to raise awareness about Best Management Practices (BMPs) for controlling *Salmonella* among poultry producers and processors in Alabama. Through these objectives, it aims to contribute to enhanced food safety and public health outcomes related to *Salmonella* contamination in poultry products.

Materials and Methods

Study Site and Geo-climatic Conditions

The experiment was conducted at the Charles C. Miller Poultry Research and Education Center, Auburn University, Auburn, Alabama. The site has an average annual temperature of 18 °C, a precipitation of 1216 mm per year, and an elevation of 214 masl.

Animals

For this study, 900 male broiler chickens (Ross 708 × YPM) were allocated across 36 different pens and subjected to three water treatments: acetic acid, citric acid, and plain water as the control. Each treatment was replicated 12 times, ensuring robust and reliable results.

Productive Performance

During each experimental phase (starter, grower, and finisher), the productive performance indicators of the broiler chickens were measured. Birds and feed were measured at the time of placement and again at 14, 28, and 39 days of age to calculate body weight (BW), feed intake (FI), and feed conversion ratio (FCR). The birds were checked twice daily to ensure their comfort, proper room temperature, and the availability of feed and water, as well as to remove any mortalities. The weight of the mortality was used to adjust FCR. Both initial and final weights for each grow-out phase were measured individually using an industrial scale. Additional parameters assessed included water consumption, cecal content analysis for *Salmonella* and microbiota, and carcass rinse analysis.

Effects of Organic Acids in *Salmonella enteritidis* Colonization

The organic acids tested were used at a concentration of 2 mL per liter of drinking water from day one until the end of the grow-out period. The purpose of this consistent administration was to evaluate the impact of acidifiers on the health and growth performance of the chicks throughout their growing period. By maintaining this concentration of acidifiers, any potential benefits or drawbacks could be accurately assessed over the entire duration of the grow-out phase.

Additionally, the prevalence of *Salmonella* was assessed through the collection of samples from chick papers prior to placement as well as boot swabs before, during, and after the grow-out period to monitor the colonization of *Salmonella enteritidis*, which was inoculated via gavage on day 7. Furthermore, ceca enrichment and carcass rinse samples were cultured in XLT4+ enrichment media to determine *Salmonella enteritidis* colonization post-slaughter and evaluate overall prevalence.

Salmonella entiritidis (SE) Inoculation

All chicks were inoculated with a 10^7-colony forming unit (CFU) dose of a nalidixic acid resistant strain of SE on day 7 via oral gavage. To ensure the selective growth of the inoculated *Salmonella*-marked strain, XLT4+ plates containing 100 µg/mL of nalidixic acid and 15 µg/mL of novobiocin were used. This selective medium effectively prevents the growth of non-resistant *Salmonella*, thereby favoring the proliferation of the inoculated strain to determine prevalence.

Poultry Carcass Rinse Samples

Carcasses were rinsed using buffered peptone water (BPW) during processing at the Fortenberry processing plant at Auburn University to evaluate the effect of the organic acids on the prevalence of *Salmonella* in carcasses. To assess potential *Salmonella* contamination in carcasses from the flocks, 180 carcass rinse samples, five birds per pen, were collected to calculate *Salmonella* prevalence. All rinses were conducted in accordance with FSIS Directive 10,250.1 and were analyzed for prevalence using the Molecular Detection System (MDS).

Additionally, nalidixic acid was incorporated into the XLT4+ enrichment media to inhibit non-*Salmonella enteritidis* strains, the nalidixic-marked SE was recovered and enumerated using standard plating microbiological techniques, aiding in the determination of prevalence specific to the inoculated strain. For each type of sample collected, *Salmonella* presence was identified using both classical culture methods, following USDA's Microbiology Laboratory Guidebook (MLG) 4.10, and molecular detection techniques. Molecular detection employed MDS (3M, 2022), a method combining isothermal DNA amplification and bioluminescence detection technologies. This approach utilized multiple primers to target distinct genome regions and Bst DNA polymerase for rapid and continuous amplification, ensuring high specificity (3M, 2022). Validation included appropriate controls to minimize false positives and negatives. Samples that tested positive via culture for *Salmonella* were also expected to yield positive results via MDS for the organism.

Investigate Pathogen Reduction by Windrow Composting

At the end of the trial, all the litter from each experimental treatment was composted. During the composting period, 16 subsamples from both internal (eight samples) and external (eight samples) locations throughout the litter were collected. Standard methods for detection and enumeration of *Salmonella* were utilized for microbial analyses. This approach allowed for a comprehensive assessment of *Salmonella* presence and distribution within the composting litter.

This method of sampling and analysis was not unique to this study; it was also employed in two subsequent studies. The consistent use of these procedures ensured reliable and comparable data across multiple investigations. Finally, after the composting period and before chick placement, when the litter was dispersed throughout the house, eight litter samples were collected to further monitor and analyze the microbial status of the litter. This final sampling step ensured that any potential changes in the *Salmonella* population during litter dispersion were documented, contributing to a thorough understanding of the microbial dynamics in the experimental settings.

Treatments

It is of interest to evaluate the effect of supplementing two types of organic acids, acetic and citric acid and plain water as the control, in drinking water when an oral inoculation of SE is given to the chicks. Based on previous results observed when using acetic acid (Hamed & Hassan, 2013) or citric acid (Dunca, 2009) as acidifiers in water.

The acidifiers tested were used at a concentration of 2 mL per liter of drinking water starting at chick placement until the end of the grow-outs. The grow-out provided data about the effect of both organic acids under a *S. enteritidis* challenge without an intervention in the litter. Each experimental group was composed of 12 pens of 25 broilers (36 pens; 900 birds total); each pen representing an experimental unit. All the birds in the study were fed with a common feed throughout the different grow-out phases. This standardized feeding regimen ensured that any observed differences in the health and microbial status of the birds could be attributed to the experimental treatments rather than variations in diet. By providing a consistent feed, the study aimed to eliminate potential confounding factors related to nutrition, thereby enhancing the reliability of the results and allowing for a clearer interpretation of the impact of the experimental variables, as shown in Appendix 1, 2, and 3.

Statystical Analysis

Growth performance and water consumption were analyzed using an analysis of variance (ANOVA) to determine the statistical significance of the differences between treatment groups for growth performance metrics, such as weight gain, feed consumption, feed conversion ratio (FCR) and water consumption.

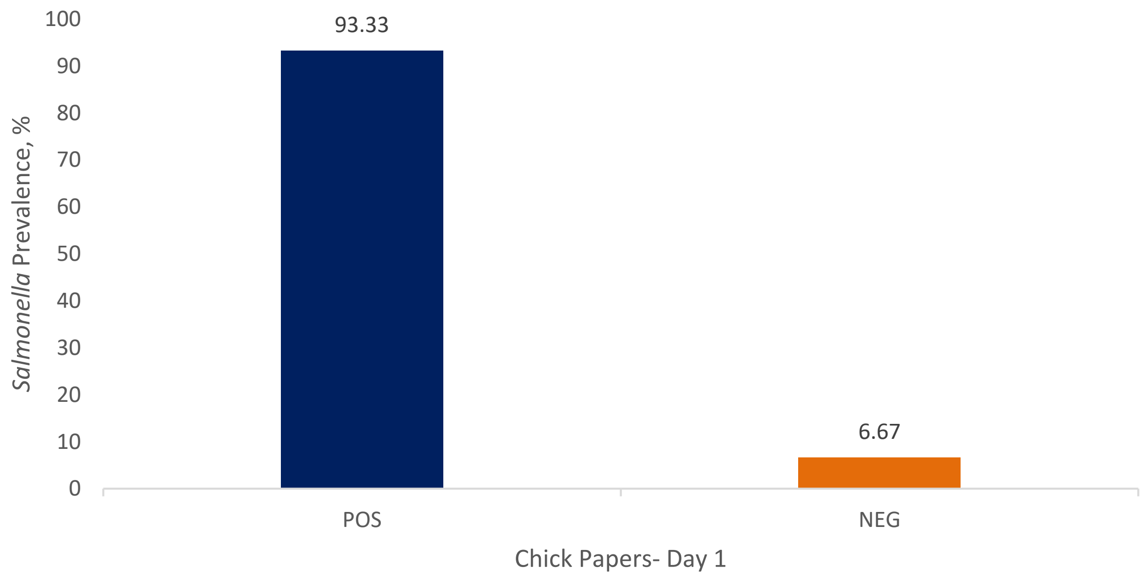
Results and Discussion

Chick Papers

Figure 1 illustrates the prevalence of *Salmonella* in 15 samples of chick papers when chicks were received and placed in the house, with a significant majority testing positive. Specifically, 93.33% of the chick papers were found to be positive for *Salmonella*, indicating a widespread presence of the bacteria among the samples. Conversely, only 6.67% of the chick papers tested negative, suggesting a relatively small proportion were free from contamination. This high rate of positive results underscores the potential risk of *Salmonella* exposure of broiler chicks in commercial hatcheries, highlighting the need for strict hygiene and monitoring practices to mitigate the spread of this pathogen. There is a trend of increasing contamination in hatcheries as the workflow progresses, starting from egg receiving areas and continuing through incubators and hatchers (Oastler et al., 2022). Additionally, an increase in *Salmonella*-positive samples from setters onward was also found by (Davies & Wray, 1996) who reported that incubated broiler eggs exhibit a rise in bacterial load between incubation and hatching. In light of this, eggs contaminated by *Salmonella* are likely to contaminate other chicks in the same hatcher when they hatch (Cason et al., 1993). This contamination can be spread to chick handling areas and into commercial farms.

Figure 1

Prevalence of Salmonella in Chick Papers- Day 1

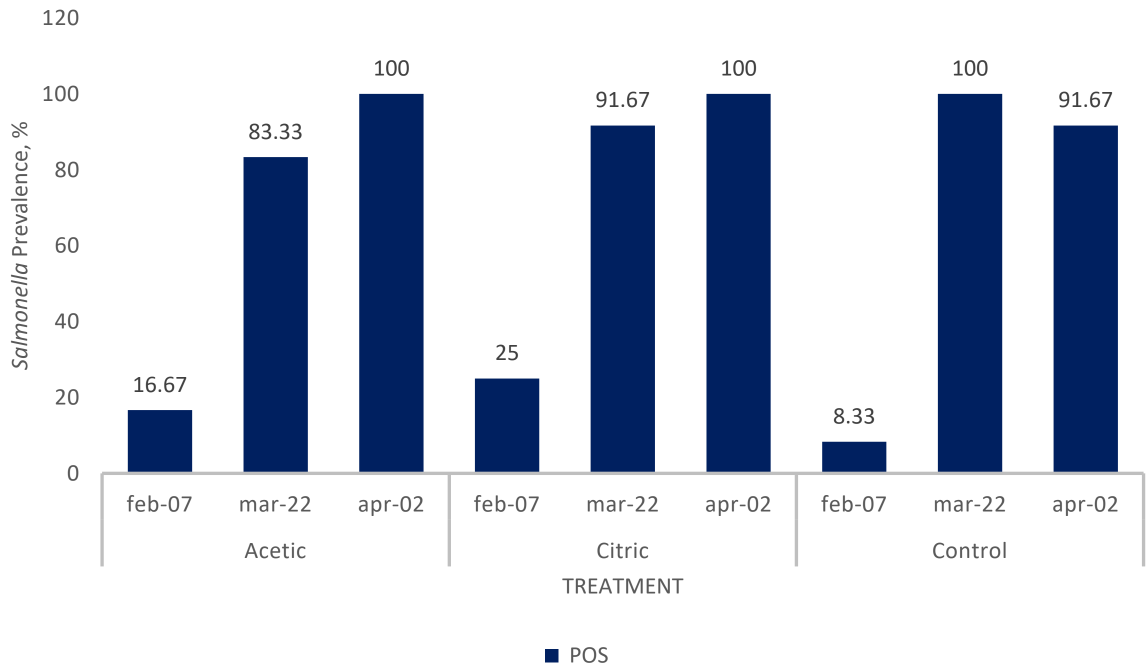


Boot Swabs

Figure 2 shows the prevalence *Salmonella* positive samples for acetic, citric, and control treatments across three dates of sample analysis: Feb-07, Mar-22, and Apr-02. For acetic and citric treatments, positive prevalence starts low in February, increases in March, and reaches 100% by April. The control treatment also starts low in February and hits 100% by March, maintaining this level in April. Overall, all treatments showed a marked increase in prevalence over time, with all reaching full prevalence by April. This indicates that *Salmonella* prevalence remained consistent from the placement of the contaminated chicks and later when they were inoculated with *S. enteritidis* in day 7. The goal of the experiment was to contaminate all the pens to evaluate the effects of the organic acids on the prevalence of *Salmonella* in broiler carcasses. This matches studies indicating that the frequency of *Salmonella* recovery by sample time shows that *Salmonella* was detected in most sample types throughout the entire grow-out period (J. S. Bailey et al., 2001). Finally, this finding aligns with studies by Davies and Wray (1996), which indicate that *Salmonella* is a resilient and adaptive bacterium capable of persisting in litter or dust for months to years. This persistence facilitates transmission between flocks when litter is reused without proper treatment.

Figure 2

Prevalence of Salmonella in Boot Swabs



Growth Performance

The inclusion of organic acids in water influenced feed intake from 1 to 39 days of age. However, no differences in feed intake were observed among the treatments during the periods from 1 to 14 days, 14 to 28 days, and 1 to 28 days. In general, broilers drinking water without organic acids had higher feed intake. Metabolic and digestive adjustments, influenced by changes in gut pH and microbial populations due to the inclusion of acetic and citric acids in feed or water, might become more pronounced over time. Feed intake significantly impacts body weight gain and feed conversion in meat-type poultry. On the other hand, addressing poor feed intake is challenging due to the numerous influencing factors, necessitating a thorough review of feed and management practices. Typically, management and flock health issues cause feed intake problems more than dietary factors. Dietary influences on feed intake tend to affect all flocks similarly within a complex, whereas environmental or immunological stresses cause significant variations between flocks. Implementing management protocols to reduce these stressors can enhance feed intake (Ferket & Gernat, 2006). However other studies show that feed intake in broilers is influenced by the size of feed particles, with the effects differing based on the birds' age and the type of grain in their diet (Abdollahi et al., 2018). In conclusion, higher feed intake cannot be directly attributed to the consumption of organic acids. Instead, the optimal conditions provided to the birds, such as reduced heat stress and lower bird density (1 cubic foot per bird compared to 0.7-0.8 cubic feet in commercial farms), played a significant role.

Table 1

Feed Intake (FI; kg)

Treatment	Days of Age			
	1 to 14	14 to 28	28 to 39	1 to 39
Control	0.55	1.79	2.22	4.56
Acetic	0.55	1.77	2.18	4.53
Citric	0.56	1.77	2.16	4.49
P-value	0.69	0.33	0.21	0.046
Root SEM	18.39	42.25	56.77	85.91

Table 2 illustrates that there are no significant differences among the treatments for both water intake and the water: feed intake ratio. The inclusion of organic acids in the drinking water did not influence water intake or water: feed intake ratio. The root SEM values, which indicate the variability within the groups, also did not show any significant differences attributable to the treatments.

Table 2

Water Consumption

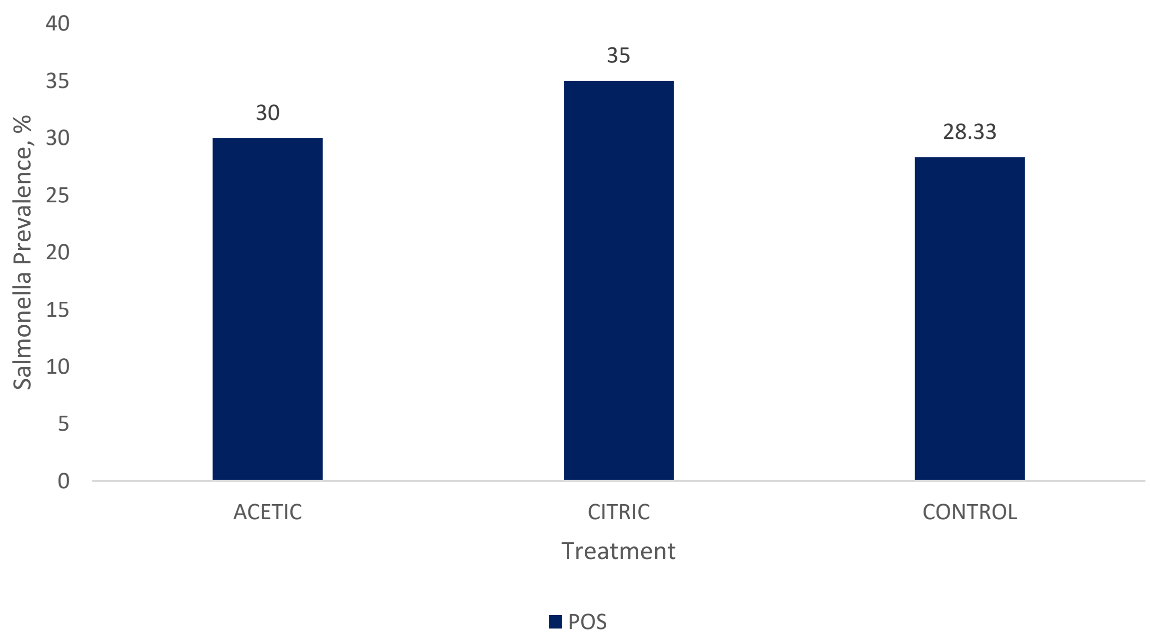
Treatment	Water Intake (WI) (kg)	Water: Feed Intake Ratio (WFIR)
Control	8.63	1.88
Acetic	8.68	1.89
Citric	8.39	1.86
P-Value	0.13	0.67
Root SEM	0.37	0.09

XLT4+ Ceca Enrichment

The ceca have been identified as the primary site of *Salmonella* colonization in poultry (Fanelli et al., 1971). Therefore, cecal and intestinal contents are considered the primary sources of *Salmonella* contamination in poultry litter, on the external surfaces and feathers of broilers, and on processed carcasses when the intestinal tract ruptures during evisceration in processing facilities. Figure 3 shows the effect of the organic acids in drinking water on the prevalence of *S. enteritidis* in the ceca. The citric treatment shows the highest prevalence at 35%, suggesting it is the least effective in controlling *Salmonella* prevalence. The acetic treatment follows with a prevalence of 30%, indicating moderate *Salmonella* prevalence. The control group, with a prevalence of 28.33%, has the lowest *Salmonella* prevalence in the ceca among the three groups. Previous studies have shown that fermentative bacteria, including *Salmonella*, tend to lower their intracellular pH in response to acidic extracellular conditions. This adaptation requires a metabolic strategy able to tolerate a lower pH levels, facilitating their ability to thrive in acidic environments (van Immerseel et al., 2009).

Figure 3

Prevalence of Salmonella enteritidis in XLT4+ Ceca Enrichment



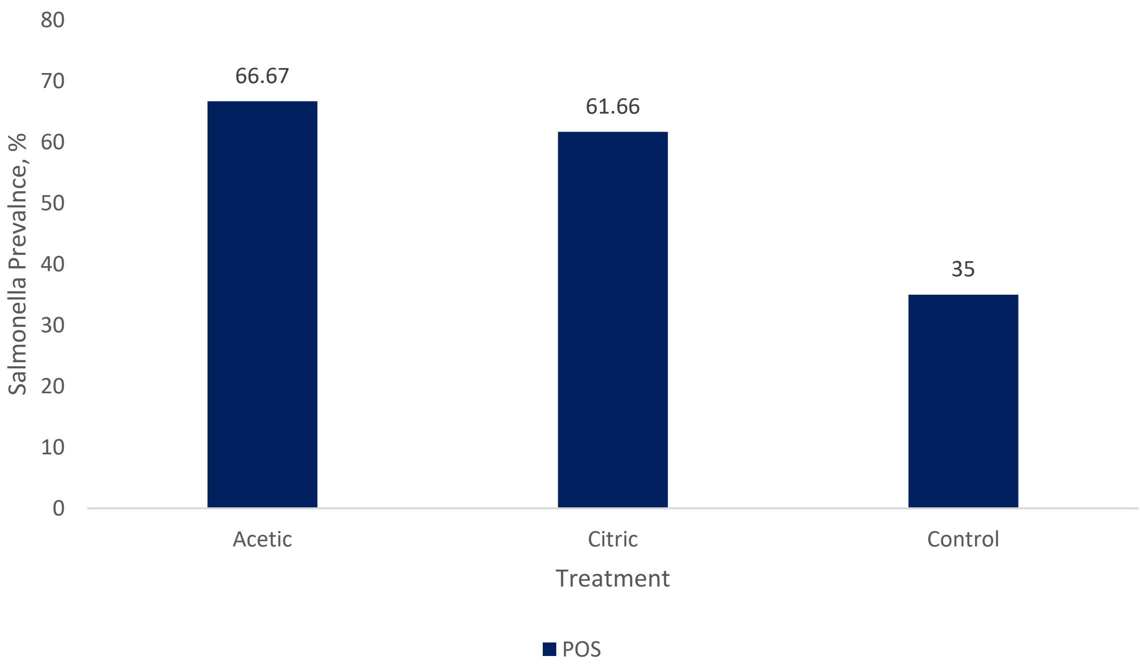
XLT4+ Carcass Enrichment

A total of 180 carcasses of broilers were obtained after processing through the picker to determine *S. enteritidis* before chilling. Figure 4 compares the prevalence of *S. enteritidis* in poultry carcasses among treatments. The study's results indicate varying levels of effectiveness in the treatments applied to control *Salmonella* prevalence. The control group, which did not receive any treatment, had a prevalence of 35%. In contrast, the acetic acid treatment group showed the highest prevalence at 66.67%, making it the least effective treatment. The citric acid treatment group exhibited a prevalence of 61.67%, indicating it was also relatively ineffective but slightly more effective than the acetic treatment. These results highlight that higher prevalence rates of *Salmonella* correspond to lower effectiveness of the water-supplied treatments. Specifically, the acetic treatment with a higher prevalence underscores its limited impact in controlling *Salmonella* after processing. *Salmonella* has been linked to poultry and meat due to contamination of carcasses by fecal material, dirt from feathers and hides, and dirty processing equipment during slaughter and packinghouse operations (Mani-López et al., 2012). On the other hand, carcass rinses were conducted after picking and no evisceration was performed since the study was mostly focused on determining exterior contamination. Studies have shown that usage of peracetic acid during the chilling helps to decrease *Salmonella* colonization. However, previous reports have differed in their interpretation of whether the chilling, chlorination or usage of peracetic acid significantly reduce pathogen levels on poultry carcasses (Salina Parveen et al., 2007). Finally, the use of pre-slaughter strategies using organic acids showed no response to the control of *S. enteritidis.*

The prevalence of *S. enteritidis* is particularly important to assess after carcass rinses because accurately quantifying this pathogen poses significant challenges. When *Salmonella* is isolated for culturing on media, the pathogen continues to proliferate, complicating precise quantification efforts. Therefore, the USDA relies on prevalence measurements rather than exact counts to classify processing plants. This method allows for a more practical and standardized approach to monitoring and controlling *Salmonella* contamination, as the consistent growth of the pathogen in culture media would otherwise skew quantitative data. By focusing on prevalence, the USDA can effectively evaluate the effectiveness of sanitation practices and interventions, ensuring that processing plants maintain compliance with food safety standards. Ultimately, establishments that have exceeded the maximum allowable percent positive during the most recently completed 52-week moving window (FSIS & USDA, 2021).

Figure 4

Prevalence of Salmonella enteritidis in XLT4+ Carcass Enrichment



Conclusions

The experiment demonstrated that supplementing water with organic acids did not influence *Salmonella* colonization in the ceca or reduce the incidence of *Salmonella* in poultry carcasses. This suggests that while organic acids may have some benefits, they are not as effective as a standalone pre-harvest intervention for controlling *Salmonella* contamination in poultry.

Despite the lack of significant reduction in *Salmonella* colonization through organic acid supplementation, the project emphasized the critical role of BMPs in controlling *Salmonella* and how extensive outreach efforts to educate poultry producers and processors about BMPs is essential for preventing and reducing *Salmonella* colonization and maintaining growth performance standards.

The findings underscore the importance of pre-harvest biosecurity and hygienic strategies in enhancing the effectiveness of post-slaughter interventions. These strategies are crucial for the poultry industry, both in the US and globally, to reduce the prevalence and spread of *Salmonella* in poultry products, thereby contributing to public health benefits.

Recommendations

Considering the possibility that the identical *Salmonella*-marked strain may not be recovered after treatments, future studies should include comprehensive sampling and analysis protocols to confirm the absence of the marked strain. This would provide robust evidence of the efficacy of composting as a pathogen control method.

To further test the efficacy of chemical and composting treatments in reducing *Salmonella* spp. levels in litter, litter treatments will be evaluated after challenging one flock with a *Salmonella* e*nteritidis* (SE) marker strain (experiment 1) and raising two flocks on the same litter (experiments 2 and 3).

A higher dosification of acid may be applied to evaluate more success in reducing prevalence in broilers.

Necropsies should be conducted to clearly observe the effects of organic acids in the ceca, for example, on day 7, 28, and 39, to calculate the reduction of the *Salmonella enteritidis* strain inoculated on day 7.

Combining the use of organic acids with other mitigation strategies, such as litter composting, can enhance overall effectiveness in controlling Salmonella and other pathogens.

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Appendices

Appendix A

Starter Feed Diet (1-14 days of age)

Ingredient	%	Kg
Corn	60.546	631.65
Soybean meal	31.898	332.78
Poultry by product meal	4.134	43.13
Poultry fat	0.500	5.22
Dl-methionine	0.369	3.85
L-Lysine HCl	0.221	2.30
L-Threonine	0.156	1.63
L-Valine	0.068	0.71
L-Isoleucine	0.018	0.19
Dicalcium phosphate	0.990	10.33
Limestone	0.392	4.09
Salt	0.304	3.17
Sodium bicarbonate	0.031	0.32
Trace mineral premix	0.100	1.04
Vitamin premix	0.100	1.04
Choline chloride (60%)	0.101Kg	1.05
Quantum Blue10g (1,500 FTU; 0.15% Ca and NPP)	0.015	0.16
Econase (0.008%; 44 kcal/kg)	0.008	0.08
Sand	0.050	0.52
Subtotal	100.016	1043.27

Appendix B

Grower Feed Diet (14-28 days of age)

Ingredient	%	Kg
Corn	64.373	525.58
Soybean meal	27.423	223.90
Poultry by product meal	4.500	36.74
Poultry fat	1.212	9.90
Dl-methionine	0.334	2.73
L-Lysine HCl	0.195	1.59
L-Threonine	0.129	1.05
L-Valine	0.042	0.34
L-Isoleucine	0.017	0.14
Dicalcium phosphate	0.756	6.17
Limestone	0.323	2.64
Salt	0.302	2.47
Sodium bicarbonate	0.035	0.29
Trace mineral premix	0.100	0.82
Vitamin premix	0.075	0.61
Choline chloride (60%)	0.112	0.92
QuantumBlue10g (1,500 FTU; 0.15% Ca and NPP)	0.015	0.12
Econase (0.008%; 44 kcal/kg)	0.008	0.06
Sand	0.050	0.41
Subtotal	100.001	**816.47**

Appendix C

Finisher Feed Diet (28-39 days of age)

Ingredient	%	Kg
Corn	69.962	634.67
Soybean meal	22.424	203.44
Poultry by product meal	4.500	40.82
Poultry fat	0.804	7.30
Dl-methionine	0.295	2.68
L-Lysine HCl	0.180	1.63
L-Threonine	0.102	0.91
L-Valine	0.031	0.27
L-Isoleucine	0.019	0.18
Dicalcium phosphate	0.689	6.26
Limestone	0.303	2.77
Salt	0.297	2.68
Sodium bicarbonate	0.049	0.45
Trace mineral premix	0.100	0.91
Vitamin premix	0.050	0.45
Choline chloride (60%)	0.124	1.13
QuantumBlue10g (1,500 FTU; 0.15% Ca and NPP)	0.015	0.14
Econase (0.008%; 44 kcal/kg)	0.008	0.09
Sand	0.050	0.45
Subtotal	100.002	907.18