Research Note: Effect of organic acid mixture on growth performance and *Salmonella* Typhimurium colonization in broiler chickens

Pratima Adhikari,* Sudhir Yadav,† Douglas E. Cosby,‡ Nelson A. Cox,‡ Joshua A. Jendza,§ and Woo Kyun Kim†,[1]

*Department of Poultry Science, Mississippi State University, Starkville, MS, USA; †Department of Poultry Science, University of Georgia, Athens, GA, USA; ‡USDA, ARS, The U.S. National Poultry Research Center, Athens, GA, 30605, USA; and §BASF Corporation, Florham Park, NJ, United States

ABSTRACT Feed additives can be alternatives to antibiotics for routinely encountered pathogens in the poultry production. The objective of this study was to understand effects of organic acid mixture on growth parameters and *Salmonella* Typhimurium (**ST**) colonization in broilers. Organic acid mixture is a feed-grade buffered formic acid and sodium formate mixture (Amasil NA). A total of 800 1-day-old Cobb500 males were fed one of the five dietary treatments: a negative control diet without ST challenge (**NC**), positive control diet with ST challenge (**PC**), 0.3% organic acid mixture with ST, 0.6% organic acid mixture with ST, and 0.9% organic acid mixture with ST. Treatments were assigned to 20 pens with 40 chicks/pen and 4 replicates of each treatment. Chickens were challenged with 10^7 CFU/mL of nalidixic acid–resistant ST (**ST^{NAR}**) 4-D posthatch. In the grower phase, feed conversion rate was significantly reduced in the 9% organic acid mixture compared with the PC. The body weight and body weight gain (**BWG**) were not affected either in the starter or grower phases. However, in the finisher phase, the non-challenged NC had higher BWG than the PC ($P < 0.05$), whereas there were no differences in BWG among the NC and organic acid mixture fed groups. In addition, there was a significant effect of organic acid mixture on the colonization of cecal ST^{NAR}. At 9 dpi, cecal ST^{NAR} was 3.28 $\log_{10}$ in the PC that was reduced to 2.65 $\log_{10}$ at 0.3%, 1.40 $\log_{10}$ at 0.6%, and 0.84 $\log_{10}$ in 0.9% organic acid mixture. At 24 dpi, cecal ST^{NAR} recovery was 0.81, 0.99, 0.53, and 0.33 $\log_{10}$ in the PC and 0.3, 0.6, and 0.9% organic acid mixture, respectively. Similarly, at 38 dpi, cecal ST^{NAR} was 0.26, 0.11, 0.33, and 0 $\log_{10}$ in the PC, 0.3, 0.6, and 0.9%, respectively. These results show that organic acid mixture can be one dietary strategy to control ST infection and maintain efficient growth performance.

Key words: organic acid, antibiotic alternative, broilers performance, *Salmonella* Typhimurium

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INTRODUCTION

The profit to the poultry industry largely depends on the feed quality because feed comprises around 70% of the total cost of production. Feed ingredients are one of the more probable potential sources of *Salmonella* contamination in production (EFSA, 2008). Feed additives have long been part of feed and play substantial roles in success of poultry production (Ragaa et al., 2016). Some of the commonly used feed additives are organic acids, probiotics, prebiotics, medicinal plants extract, and exogenous enzymes. They are used as antimicrobials, antioxidants, emulsifiers, binders, pH control agents, and enzymes in the poultry diet (Ragaa et al., 2016). Specifically, organic acid has been incorporated in feed or water for the benefit with prevention of intestinal tract disease, immunity, digestibility of nutrients, and effect on growth performance (Yadav and Jha, 2019). Previously, antimicrobial growth promotors (**AGP**) were used to maintain bird health and improvement, but because of its increasing resistance, AGP have been banned or their use discouraged (Huyghebaert et al., 2011). Organic acids are approved and are safe to use in animal as feed additives (Baaboua et al., 2018). Organic acids are used to reduce or eliminate *Salmonella* in feed ingredients either before heat treatment or supplemented in the feed (Koyuncu et al., 2013). Ample studies have been done using organic acids either in feed or water alone or in a combination for their

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[1]Corresponding author: wkkim@uga.edu

beneficial effects to reduce the amount of *Salmonella* Typhimurium (**ST**). An *in vitro* study by Menconi et al. (2013) found better results to reduce ST in the crop and cecal tonsils (**CT**) using a blend of acetic, citric, and propionic acids at a final concentration of either 0.031 or 0.062%. Similarly, supplementing organic acid blends with oregano extract in water at 0.08% and in feed at 0.2% significantly reduced the shedding of *Salmonella* Enteritidis at 22 and 42 D of life, although this combination did not reduce the prevalence in crop at slaughter age (Machado Jr. et al., 2014).

Salmonellosis is one of the most common and widely observed food-borne diseases. *Salmonella* can cause reduction in feed efficiency along with increased mortality. *Salmonella* has been found to be highly resistant at the rate of ≥89.28% to vancomycin, tetracycline, streptomycin, or nalidixic acid (Siriken et al., 2015). Previous studies have reported that formic and propionic acids reduce *Salmonella* and *E. coli* in the chicken gastrointestinal tract (AL- Tarazi and Alshawabkeh, 2003; Moharrery and Mahzonieh, 2005). No known study seems to be published on this organic acid mixture and its role against ST. Organic acid mixture used in this study is a commercially available feed grade formic acid (**FA**) and sodium formate produced by BASF, which is used as feed acidifier hypothesized to decrease in the number of bacteria (Menconi et al., 2013). Thus, the objective of our study was to evaluate the effects of this organic acid mixture on growth performance (BW, BW gain, FI, and FCR), and *Salmonella* colonization in broilers challenged with nalidixic acid (**NAL**)–resistant ST (**ST^{NAR}**).

MATERIALS AND METHODS

All feed preparation and broiler housing took place at the University of Georgia Poultry Research Center. The experiment protocol was approved by the University of Georgia Animal Care and Use Committee.

Management

A total of 800 1-day-old chicks were used for this study. The chicks were allocated into 5 treatments with 40 chicks per pen and 4 replicates of each treatment. Five treatment groups were no *Salmonella* challenge (T1 = Negative control: **NC**); *Salmonella* challenge (T2 = Positive control: **PC**); *Salmonella* challenge + 3 kg organic acid mixture/ton of feed (T3 = 0.3% organic acid mixture); *Salmonella* challenge + 6 kg organic acid mixture/ton of feed (T4 = 0.6% organic acid mixture); and *Salmonella* challenge + 9 kg organic acid mixture/ton of feed (T5 = 0.9% organic acid mixture). The organic acid mixture used in this study was mixture of FA and sodium formate commercially available in the name of Amasil NA. The active substance of product includes 61% FA and 20.5% sodium formate. For example, in a feed containing 0.3% organic acid mixture, there are 0.183% of FA and 0.0615% of sodium formate. It was available in liquid form and was buffered FA fully miscible in water. Chicks were fed treatment diets from day 1. Basal diet formulation for starter, grower, and finisher phase is shown in Table 1. At 14, 28, and 42 D, BW, BW gain, FI, FCR, and mortality percentage were measured.

Microbiology

All microbiology analysis was performed at the USDA-ARS U.S. National Poultry Research Center, Athens, GA.

***Salmonella* Challenge** At 4 D, chicks were orally gavaged with ST^{NAR}. The individual chicks were challenged with approximately 10^7 CFU/mL ST^{NAR} organisms. The dose of ST^{NAR} organisms used in this study was based on Bjerrum et al. (2003). The ST^{NAR} culture used in our challenge protocol was obtained from USDA Athens, GA. A night before, ST^{NAR} organisms were streaked onto Brilliant Green Agar with 200 ppm nalidixic acid (BGS-NAL) plates and were grown overnight at 37°C (Adhikari et al., 2018 and 2019). Ten birds/pen were randomly selected for enumeration of the cecal *Salmonella* at 9-, 24- and 38-d postinfection (dpi).

Table 1. Ingredients used and nutrient composition of different phase basal diets.

Ingredients, %	Starter	Grower	Finisher
Corn	61.98	65.83	70.19
SBM	31.56	29.05	23.87
Soybean oil	2.74	2.03	2.87
Limestone	0.67	0.64	0.61
Defluor. Phos.	2.01	1.58	1.40
Lysine	0.113	0.03	0.22
Methionine	0.22	0.16	0.16
NaCl	0.3	0.3	0.3
Vitamin mix[1]	0.25	0.25	0.25
Mineral mix[1]	0.08	0.08	0.08
Coccidiostat	0.05	0.05	0.05
Total	100	100	100
Calculated Content, %			
MEn, Kcal/kg	3,100	3,100	3,200
CP	21.0	20.0	18.05
Ca	1.00	0.84	0.76
Total P	0.73	0.65	0.60
Avail. Phos.	0.5	0.42	0.38
Lysine	1.18	1.05	1.05
Methionine	0.54	0.47	0.44
Cysteine	0.34	0.33	0.30
Threonine	0.77	0.73	0.65
Tryptophan	0.27	0.25	0.22
Methionine + Cysteine	0.88	0.80	0.74
Arginine	1.33	1.26	1.10
Valine	0.95	0.91	0.81
Isoleucine	0.85	0.81	0.71
CF	2.59	2.58	2.47
Na	0.24	0.22	0.21
Cl	0.24	0.23	0.26
Choline (mg/kg)	1.47	1.42	1.31

[1]Providing the following (per kg of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D_3 (cholecalciferol), 3,000 IU; vitamin E (all-*rac*-tocopherol-acetate), 30 mg; vitamin B_1, 2 mg; vitamin B_2, 8 mg; vitamin B_6, 4 mg; vitamin B_{12} (cyanocobalamin), 0.025 mg; vitamin K_3 (bisulphatemenadione complex), 3 mg; choline (choline chloride), 250 mg; nicotinic acid, 60 mg; pantothenic acid (D-calcium pantothenate), 15 mg; folic acid, 1.5 mg; betaine anhydrous, 80 mg; D-biotin, 0.15 mg; zinc (ZnO), 80 mg; manganese (MnO), 70 mg iron ($FeCO_3$), 60 mg; copper ($CuSO_4 \cdot 5H_2O$), 8 mg; iodine (KI), 2 mg; selenium (Na_2SeO_3), 0.2 mg.

Blanch-Field Method/Swab Plate Method Cecal contents were examined for the presence of the marker *Salmonella* using the modified 3-Swab method of Blanchfield et al. (1984). Briefly, the ceca were aseptically removed and placed into individual stomacher bags. The ceca were gently macerated with a rubber mallet, weighed, and 3 times the weight of buffered peptone water (**BPW**) with 3 times the weight was added. The bags were stomached for 60 s. Two cotton-tipped applicators were inserted into the cecal/BPW solution to coat the swab. One swab was streaked onto a BGS-NAL plate (A plate). The second swab was transferred into a tube containing 9.9 mL of BPW and vortexed for approximately 15 s. A third swab was inserted into the dilution tube and streaked onto a BGS-NAL plate (B plate). The contents of the tube were returned to the stomacher bag for overnight pre-enrichment as required. All plates and samples and plates were incubated overnight at 35°C ± 2°C. After overnight incubation, estimated counts were obtained, and negative samples were re-streak from overnight pre-enrichment of the sample bags onto BGS-NAL (C plate). These plates were incubated overnight under the same conditions as above. After overnight incubation, the plates were observed for growth of typical *Salmonella* like colonies and recorded as a positive or negative. A colonization correction factor was used to transform the data for analysis (3.7 for the A plate; 1.7 for the B plate; and 1.5 for the C plate).

Statistical Analysis

Calculated CFU/g was first converted to $\log_{10}$ CFU/g and analyzed. For both growth performance and *Salmonella* count, data were analyzed by ANOVA using Mixed procedure of SAS (SAS 9.2; SAS Institute Inc., Cary, NC). Significant differences among treatments were assessed by Duncan's test. Statistical significance was considered at $P < 0.05$. Results were expressed as mean and SEM. For mortality, data were transformed to $\sqrt{n+1}$ before analysis. Data were processed using one-way ANOVA, GLM procedure of SAS. Significant differences among treatments were assessed by Duncan's test. Statistical significance was considered at $P < 0.05$.

RESULTS

Growth Performance

For growth performance, BW, BWG, FI, and FCR were recorded for the starter (0 to14 D), grower (15 to 28 D), and finisher (29 to 42 D) phases (Table 2). There was an effect of organic acid mixture on growth performance of broiler chickens challenged with ST^{NAR}. There were no differences in growth performance parameters during the starter period and overall period ($P > 0.05$). The FCR of birds fed 0.9% organic acid mixture was similar to that of the NC group, whereas other treatment groups had significantly higher FCR compared with the NC or 0.9% organic acid mixture group ($P < 0.01$) during the grower phase (15 to 28 D). BWG was highest in NC and lowest in PC; however, different levels of organic acid mixture included diets fed birds had intermediate BWG. There were no significant differences in mortality among the treatments (Table 3).

Table 2. Growth performance of broilers challenged with ST and supplemented with different levels of organic acid mixture.

	Treatment[1]						
Parameters	NC	PC	PC + 0.3%	PC+ 0.6%	PC + 0.9%	SEM	*P*-value
0–14 D							
FI[2]	430	430	496	433	421	3.14	0.319
BW[3]	378	374	383	379	367	3.80	0.763
BWG[4]	338	333	343	338	327	3.77	0.308
FCR[5]	1.27	1.30	1.43	1.28	1.28	0.03	0.120
15–28 D							
FI	1,325	1,325	1,311	1,339	1,300	11.11	0.331
BW	1,238	1,203	1,197	1,213	1,192	10.86	0.726
BWG	858	836	813	848	832	9.14	0.866
FCR	1.54[b]	1.58[a]	1.61[a]	1.58[a]	1.56[b]	0.01	0.007
29–42 D							
FI	1,759	1,637	1,660	1,714	1,720	28.75	0.175
BW	2,436	2,259	2,277	2,304	2,299	51.12	0.860
BWG	1,225[a]	1,071[b]	1,111[a,b]	1,102[a,b]	1,119[a,b]	50.18	0.043
FCR	1.50	1.56	1.55	1.59	1.57	0.06	0.431
0–42 D							
FI	3,515	3,392	3,391	3,486	3,441	40.21	0.861
BW	2,436	2,259	2,277	2,304	2,299	51.12	0.860
BWG	2,395	2,218	2,237	2,264	2,259	51.11	0.860
FCR	1.48	1.54	1.51	1.55	1.53	0.03	0.964

[a,b]Means followed by different letters in the same row are different Duncan's test ($P < 0.05$).
[1]NC, No Challenge; C, ST^{NAR} challenge; C + 0.3%, ST^{NAR} challenge +0.3% organic acid mixture; C + 0.6%, ST^{NAR} challenge +0.6% organic acid mixture; T5, ST^{NAR} challenge +0.9% organic acid mixture.
[2]Feed intake.
[3]Body weight.
[4]Body weight gain.
[5]Feed conversion rate.

Table 3. Mortality (%) during starter, grower, finisher, and overall experiment time.

Parameters	Treatment							
	NC	PC	PC + 0.3%	PC+ 0.6%	PC + 0.9%	CV (%)	SEM	*P*-value
0–14 D								
Mortality (%)	4.38	1.88	3.75	0.63	1.25	31.13	0.08	0.429
14–28 D								
Mortality (%)	3.27	1.91	1.95	1.26	0.00	24.87	0.06	0.315
28–42 D								
Mortality (%)	0.68	0.65	0.66	0.64	1.27	16.45	0.04	0.954
0–42 D								
Mortality (%)	8.13	4.38	6.25	2.50	2.50	30.23	0.09	0.346

Abbreviations: C, ST^{NAR} challenge; C + 0.3%, ST^{NAR} challenge +0.3% organic acid mixture; C + 0.6%, ST^{NAR} challenge +0.6% organic acid mixture; NC, No Challenge; T5, ST^{NAR} challenge +0.9% organic acid mixture.

Salmonella Recovery

There was significant reduction of ST colonization when organic acid mixture was added in feed as shown in Table 4. *Salmonella* recovery was recorded on 9-, 24-, and 38-dpi. On day 9 dpi, the percentage of positive birds with ST^{NAR} decreased from 92.5% in PC to 75%, 52.5 and 45% in PC + 0.3%, PC + 0.6%, and PC + 0.9%, respectively. On day 24 dpi, the higher inclusions of organic acid mixture (0.6 and 0.9%) had lower positive cases (32.5 and 22.5%) compared with PC + 0.3% (52.5%). On day 38 dpi, highest organic acid mixture group had zero positive birds, indicating that these birds had completely recovered *Salmonella*.

For ST^{NAR} count in ceca, on 9 dpi, PC + 0.6% (1.41 Log_{10} CFU) and PC + 0.9% (0.85 Log_{10} CFU) treatments significantly reduced ST^{NAR} colonization compared with PC (3.28 Log_{10} CFU) or PC+ 0.3% (2.65 Log_{10} CFU). On 24 dpi, organic acid mixture at higher levels (0.6 and 0.9%) significantly reduced ST^{NAR} compared with the lower level ones (0 and 0.3%). On 38 dpi, the group fed 0.9% organic acid mixture had significantly lower ST^{NAR} compared with the other treatments ($P < 0.05$), whereas the one fed 0.3% organic acid mixture significantly reduced the counts compared with PC and 0.6% groups. Thus, supplementation at the highest level showed better result in all tested dpi.

DISCUSSION

Organic acid use in poultry has been studied for a long time with inconsistent results with respect to the growth benefit or reduction of unwanted bacteria. Our study was focused on the effect of a FA-based compound on the reduction or elimination of ST and the growth performance of broiler chickens. Some differences in growth performance between treatments with different doses of organic acid mixture, and *Salmonella* challenged or non-challenged group were observed. In a study by Hernandez et al. (2006), no differences were found in weight gain, FI, and/or FCR, when FA was fed at 5 or 10 g/kg to the broiler chickens, whereas our study indicated differences in BWG and FCR. Our study aligns with a study by Ragaa et al. (2016), where feeding a diet supplemented with FA at 5 g/kg diet found significant increase in BWG and decreased FCR ($P < 0.05$). The positive response on growth performance by organic acid in early life could be because of decrease in pH of the gut and increase the proteolytic enzyme activity and nutrient digestibility along with bacteriostatic and bactericidal action to the pathogenic bacteria (Papatisiros et al., 2013). Similarly, a decrease in FCR was found by Garcia et al. (2007), when chicks were fed a diet supplemented with 10,000 ppm of FA ($P < 0.05$). These differences could be because of the use of different form, doses, source of FA, and bird health (challenged or healthy birds in controlled environment).

Table 4. Effect of organic acid mixture supplementation on cecal nalidixic acid resistant *Salmonella* Typhimurium (ST^{NAR}) colonization in broiler chickens at different days postinfection (dpi).[1]

dpi	Items	Treatment[2]			
		PC	PC + 0.3%	PC + 0.6%	PC + 0.9%
9	Positive birds per total[3]	37/40 (92.5%)	30/40 (75%)	21/40 (52.5%)	18/40 (45%)
	Log_{10} CFU/g	3.282^{a}	2.652^{a}	1.407^{b}	0.847^{b}
24	Positive birds per total	11/40 (27.5%)	21/40 (52.5%)	13/40 (32.5%)	9/40 (22.5%)
	Log_{10} CFU/g	0.817^{a}	0.992^{a}	0.537^{b}	0.337^{b}
38	Positive birds per total	8/40 (20%)	3/40 (7.5%)	8/40 (20%)	0/40 (0%)
	Log_{10} CFU/g	0.265^{a}	0.112^{b}	0.337^{a}	0^{c}

Means followed by different letters in the same row are different by Duncan's test ($P < 0.05$).

[1]Chickens were challenged with approximately 10^7 CFU/mL of ST^{NAR}.

[2]PC, ST^{NAR} challenge; PC + 0.3%, ST^{NAR} challenge +0.3% organic acid mixture; PC + 0.6%, ST^{NAR} challenge +0.6% organic acid mixture; PC + 0.9%, ST^{NAR} challenge +0.9% organic acid mixture.

[3]Data represent the number positive per numbered sampled (N = 40).

A study by Van Immerseel et al. (2006) stated that possible mechanism for antimicrobial activity of organic acid is attributed to its regulation of invasive phenotype of *Salmonella*.

Many studies were done during the 1980s to examine the effect of supplementing acids such as FA and propionic acid on the *Salmonella* colonization in chickens, and results were variable. Hinton (1986), in a short-term trial, found that FA was very effective at decreasing *Salmonella,* where 50% of control group were positive for *Salmonella*. In another study, when FA was fed from day of hatch, the treatment groups had 3, 0, and 0% of *Salmonella*-positive fecal samples, and control groups were 25, 27, and 60% positive (Hinton and Linton, 1988). They also found no difference between control and treatment group when FA was supplemented after 2 wk of age. A study conducted by Koyuncu et al. (2013) found that organic acid mixture of FA and sodium formate was more efficacious (0.5-1 $\log_{10}$ reduction) in *Salmonella* reduction than a blend of FA and propionic acid when added in mash feed. In a recent study, Jendza et al. (2018) found similar results to our study in relation to *Salmonella* and concluded that acidification of the feed reduces the number of *Salmonella* in both unconditioned mash feed and pellets when *Salmonella* was reinoculated postpelleting.

In conclusion, the findings from the current study show that the use of organic acid mixture as an antibiotic alternative in the feed of broiler chickens can significantly decrease ST as well as improve the growth performance of the birds. We could also suggest that 0.9% organic acid mixture is an appropriate treatment level to maintain feed hygiene and infection/contamination by *Salmonella*.

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