

Research Note

Assessing the Efficacy of Sodium Bisulfate and Organic Acid Treatments for Control of *Salmonella* Typhimurium in Rendered Chicken Fat Applied to Pet Foods

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ABSTRACT

This study was conducted to evaluate the effects of sodium bisulfate (SBS), lactic acid (LA), phosphoric acid (PA), and combinations of organic acids with SBS on *Salmonella* in rendered chicken fat and in water. The MICs of the antimicrobials individually and in combination were determined. Efficacies of the antimicrobials against *Salmonella* were tested in both media. The MICs of SBS, LA, and PA were 0.5, 0.5, and 0.25%, respectively. At the given concentrations in the water phase, 0.5% SBS was more effective ($P < 0.05$; 2.7-log reduction) than LA and PA at 0 h. SBS and LA were more effective ($P < 0.05$) than PA with >4-log reductions at 2 h and complete kill at 6 h. After 24 h, each of the chemicals completely eliminated the *Salmonella*. However, because of low recovery in the fat phase, *Salmonella* was not detected after 12 h and all three chemicals effectively reduced ($P < 0.05$) *Salmonella* at 6 h compared with the control. When combinations were used in the water phase, SBS plus butyric acid decreased ($P < 0.05$) *Salmonella* by >5.5 log CFU/mL after 12 h. The SBS+LA combinations were effective ($P < 0.05$) after 2 h. The combinations of SBS+PA resulted in ~3.5-log reductions in *Salmonella* ($P < 0.05$) after 6 h. In the fat phase, except for the SBS+PA combination, *Salmonella* reduction was not different from that for the positive control. When SBS was combined with organic acids, *Salmonella* inhibition was achieved at a lower SBS concentration, indicating a possibly synergistic effect of these chemicals. These results suggest that inclusion of SBS or LA at 0.5% individually or a combination of SBS with organic acids could reduce *Salmonella* in rendered chicken fat contaminated by residual water encountered during storage and transport.

Key words: Organic acids; Rendered chicken fat; *Salmonella*; Sodium bisulfate

Salmonella infections in humans have occurred after handling of contaminated pet foods and pet treats (1, 6, 13, 16). Li et al. (25) reported that 12.4% of pet foods and treats were *Salmonella* positive from 2002 to 2006, and this percentage was reduced to 6.1% from 2007 to 2009. Two major multistate outbreaks of human salmonellosis caused by *Salmonella* Schwarzengrund and *Salmonella* Infantis in 2008 and 2012, respectively, were traced back to contaminated dry pet foods.

In a survey sponsored by the Fats and Proteins Research Foundation, raw materials (animal waste tissues), crax (material from cooking or expelling process), and final rendered products were evaluated for five human pathogenic bacteria, including *Salmonella* (37). *Salmonella* was found in 84.5% of the raw ingredients sampled, 0% of crax samples, and 26.1% of final rendered products (37). These results are similar to those of other research in which the presence of *Salmonella* was evaluated in final rendered products, including protein meals, meat and bone meals, feather meal, meat meal, and poultry meal (10, 15, 21, 23,

29, 34). However, evaluation of microbial contamination of rendered fats, specifically poultry fat, beef tallow, or other animal fat products commonly used by the pet food industry, was not included in these surveys. Although the rendering process is effective for pathogen reduction, it is a point-in-time mitigation technique with no residual activity. Recontamination after processing has been proposed as the primary factor for the presence of *Salmonella* in final rendered products (10, 21, 37). Cochrane (8) found that the addition of chemical additives to rendered animal proteins, including feather meal, avian blood meal, porcine meat and bone meal, and poultry by-product meal, was an effective strategy for control of *Salmonella*. However, this research did not include rendered animal fats.

An outbreak of human salmonellosis in the United States from 2006 to 2008 was attributed to *Salmonella* Schwarzengrund and traced back to dried dog food. During further investigation, the enrobing and flavoring room in the manufacturing facility was positive for the outbreak strain. In this room, material is sprayed onto the surface of the finished product to enhance palatability (4). Although not identified, the material sprayed on the finished product likely included some kind of rendered animal fat. Rendered

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animal fats are commonly incorporated into animal feed and pet foods to increase the energy density and concentrations of essential fatty acids and to enhance palatability.

Sodium bisulfate (SBS) is an acidulant ($pK_a = 1.99$) and a known hygroscopic chemical that kills pathogens such as *Salmonella* and *Campylobacter* (26) through its desiccant effect. In 1997, SBS was approved for use in animal feed as a general-purpose additive (22), and in 1998 the U.S. Food and Drug Administration (38) designated SBS as generally recognized as safe for use in human foods. SBS is commonly used in animal diets for acidification of feline urine and for preservation of soft-moist treats and liquid digests. Lactic acid (LA) is an organic acid commonly used in the food industry as a preservative. Phosphoric acid (PA) is a weak inorganic acid commonly used as a food additive for flavoring and as an antimicrobial agent. Short-chain fatty acids such as acetic acid, propionic acid (ProA), and butyric acid (BA) are food additives known to extend the shelf life and provide antimicrobial activity in pre- and postharvest food production systems (33).

Rendered chicken fats are transported and stored in bulk in trucks and storage tanks in the rendering facility and pet food plants. The coating of dry pet foods with these fats occurs after the established kill steps in dry pet food production. Hence, postprocessing contamination by pathogens such as *Salmonella* from these additives is always possible in the food supply stream. SBS and organic acids are food-grade antimicrobials that can control *Salmonella* in a variety of food products. However, their application via fat for *Salmonella* mitigation has not been evaluated. We hypothesized that the residual water from storage or transport systems in bulk fat is a source of *Salmonella* contamination of pet food. The use of SBS individually and in combination with established antimicrobials in chicken fat was evaluated for its efficacy against *Salmonella*.

MATERIALS AND METHODS

***Salmonella* serotypes and sources of antimicrobials and chicken fat.** *Salmonella* Typhimurium (ATCC 14028) was maintained in tryptic soy broth (TSB):glycerol (7:3) at -80°C . Before use, the frozen cultures were streaked on tryptic soy agar (TSA) plates and incubated at 37°C for 24 h. A single colony of this *Salmonella* strain was inoculated into 10 mL of TSB and incubated at 37°C for 18 to 24 h. SBS was provided by the Jones-Hamilton Co. (Walbridge, OH). The DL-LA (85%), PA (85%), ProA ($\geq 99.5\%$), and BA ($\geq 99\%$) were procured from a commercial supplier (Fisher Scientific, Hampton, NH). Rendered chicken fat was provided by an established poultry rendering company (Simmons Foods, Siloam Springs, AR).

MIC assay. The MICs of individual SBS, LA, and PA solutions and combinations of SBS with the organic acids LA, ProA, and BA were determined by the broth microdilution assay according to the method of the Clinical and Laboratory Standards Institute (7) in TSB. A 100- μL aliquot of TSB bacterial culture containing ~ 6 log CFU/mL was added to each well of a plate containing 100 μL of decreasing concentrations of antimicrobials for a final volume of 200 μL per well. The positive control consisted of *Salmonella* inoculum only (no antimicrobials), and the negative control consisted of TSB alone. The MIC was defined as the lowest concentration of an antimicrobial agent that inhibited

TABLE 1. MICs and pH of various antimicrobial agents

Agent ^a	MIC (%)	pH (mean \pm SD)
SBS	0.50	3.8 \pm 0.026
LA	0.50	3.8 \pm 0.125
PA	0.25	3.8 \pm 0.173
ProA	0.25	4.9 \pm 0.153
BA	0.25	5.2 \pm 0.153
SBS+BA	0.10 + 0.05	2.3 \pm 0.036
	0.05 + 0.075	2.6 \pm 0.153
SBS+LA	0.10 + 0.10	2.3 \pm 0.011
	0.05 + 0.15	2.4 \pm 0.006
SBS+ProA	0.10 + 0.05	2.3 \pm 0.010
	0.05 + 0.075	2.5 \pm 0.265

^a SBS, sodium bisulfate; LA, lactic acid; PA, phosphoric acid; ProA, propionic acid; BA, butyric acid.

the visible growth of *Salmonella* after 24 h of incubation at 37°C . The pH values of the chemicals and their combinations are presented in Table 1.

***Salmonella* mitigation in chicken fat.** Aliquots of autoclave-sterilized rendered fat were transferred to 15-mL sterile plastic centrifuge tubes. Separate tubes for each chemical treatment and time interval were set up and treated with each of the antimicrobials at a concentration determined from the MIC assay and then incubated overnight at 45°C . The final concentrations of SBS, LA, and PA in chicken fat were maintained at 0.5% (w/v), 0.5% (v/v), and 0.05% (v/v), respectively. The series of treatments involving a combination of organic acids with SBS are listed in Table 1. The concentrations of the combinations of SBS plus an organic acid were based on the results of the MIC assay of the chemical combinations. The positive control was *Salmonella* without antimicrobials, and the negative control was medium without *Salmonella*.

The moisture percentage in chicken fat was maintained at $\sim 3\%$. *Salmonella* culture (~ 7 log CFU/mL) was added to the overnight chemically treated chicken fat. After vortex mixing, the tubes were incubated at 45°C . Microbiological analyses were conducted separately for the fat-phase and water-phase treatments at predetermined time intervals (0, 2, 6, 12, and 24 h). From each subsample, the liquid fat and aqueous phases were gently removed by pipetting, diluted in 0.1% peptone water, and plated onto nutrient TSA. For the fat phase, samples were drawn by vigorously vortexing the tubes and removing 100 μL while the suspension was still emulsified. The plates were incubated at 37°C for 24 h, and then colonies were counted.

Data were analyzed utilizing the GLIMMIX procedure of SAS (SAS Institute, Cary, NC) separately for the two studies. The first experiment was a 5×5 factorial design utilizing three chemicals (SBS, LA, and PA) and the positive and negative controls and five sampling intervals. The second experiment was an 8×5 factorial design utilizing six chemical combinations and the positive and negative controls and five sampling intervals. Three replicates were made for each treatment.

RESULTS

MICs. The MICs of all antimicrobials against *Salmonella* Typhimurium (ATCC 14028) are presented in Table 1. To determine the MICs of the combination of SBS with each of the organic acids, starting with half the MICs of each of the chemicals, two MIC combinations were

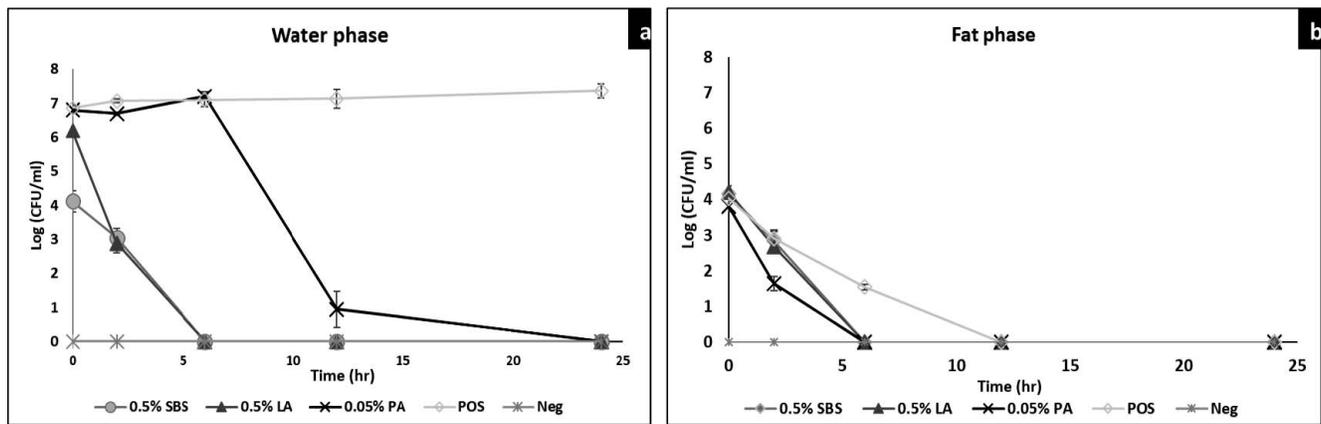


FIGURE 1. Effects of various antimicrobials against *Salmonella Typhimurium* (ATCC 14028) evaluated in the water phase (a) and fat phase (b) of a chicken fat system at 45°C. Treatments from each phase were plated on TSA at different times. SBS, sodium bisulfate; LA, lactic acid; PA, phosphoric acid; POS, positive control (*Salmonella* only, no antimicrobials added); Neg, negative control (no *Salmonella*, no antimicrobials).

determined. Individual MICs required in all the combinations were <50% of the MICs of each of the individual chemicals, which indicates potential synergistic effects of SBS with the organic acids.

Salmonella control in chicken fat. In the water phase at 0 h, the 2.7-log reduction with 0.5% SBS was greater than ($P < 0.05$) that achieved with 0.5% LA and 0.05% PA (Fig. 1a). After 2 h of contact time in the water phase, both SBS and LA were more effective ($P < 0.05$) than PA, with >4-log reductions of *Salmonella*, whereas after 6 h of SBS and LA treatment, *Salmonella* was completely eliminated. PA at 0.05% was effective ($P < 0.05$) after 12 h of contact time and reduced *Salmonella* by >6 log CFU/mL. After 24 h of contact time, all three chemicals tested in the water phase completely eliminated *Salmonella*.

Initial *Salmonella* recovery was ~7 log CFU/mL in the water phase but only ~4 log CFU/mL in the fat phase (Fig. 1b). In the fat phase after 0 and 2 h of contact time, no differences ($P > 0.05$) in *Salmonella* counts were found between the treatments and the control. However, at 6 h no *Salmonella* was recovered from any of the three treated fat

phase samples, but *Salmonella* was recovered from the positive control (~1.5 log CFU/mL), indicating that all three antimicrobials at given concentrations were effective ($P < 0.05$) after 6 h of treatment (Fig. 1b). After 12 h, we were unable to recover *Salmonella* even from the positive control.

Combinations of SBS with each organic acid were tested in chicken fat (Table 1). In the water phase, the 0.1% SBS + 0.05% BA combination reduced ($P < 0.05$) the *Salmonella* counts after 2 h, whereas the 0.05% SBS + 0.075% BA combination was effective only after 12 h (Fig. 2a). Both of these combinations were effective after 12 h, with >5.5-log reductions in *Salmonella* and complete mitigation after 24 h. The water phase combination of 0.1% SBS + 0.1% LA reduced ($P < 0.05$) *Salmonella* counts at 0 h. The combination of 0.05% SBS + 0.15% LA caused significant reduction after 2 h (Fig. 3a) and reduced the counts by ~6 log CFU/mL after 6 h, whereas 0.1% SBS + 0.05% ProA resulted in ~5.4-log reduction ($P < 0.05$) after 6 h and complete elimination after 12 h. This combination was more effective than 0.05% SBS + 0.075% ProA, which resulted in an ~3.5-log reduction

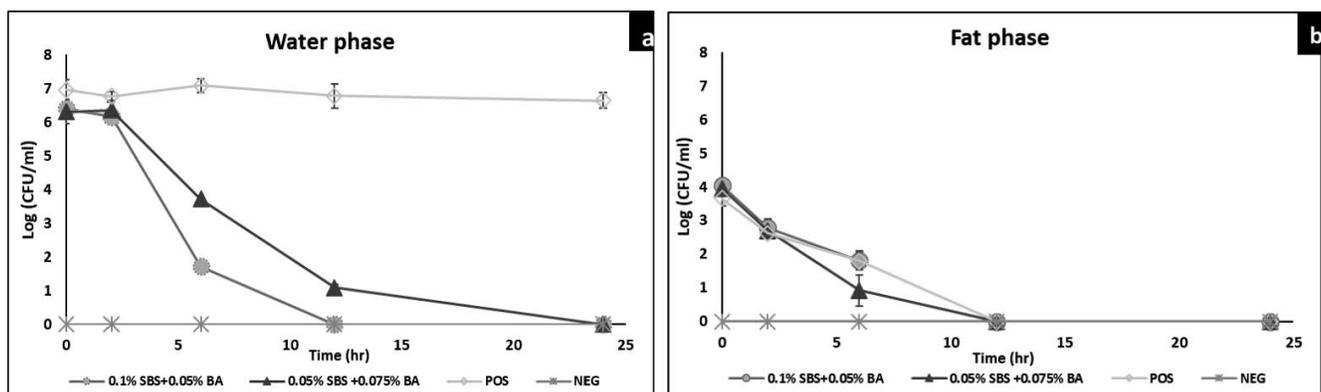


FIGURE 2. Effects of sodium bisulfate (SBS) plus butyric acid (BA) combinations against *Salmonella Typhimurium* (ATCC 14028) evaluated in the water phase (a) and fat phase (b) of a chicken fat system at 45°C. Treatments from each phase were plated on TSA at different times. POS, positive control (*Salmonella* only, no antimicrobials added); NEG, negative control (no *Salmonella*, no antimicrobials).

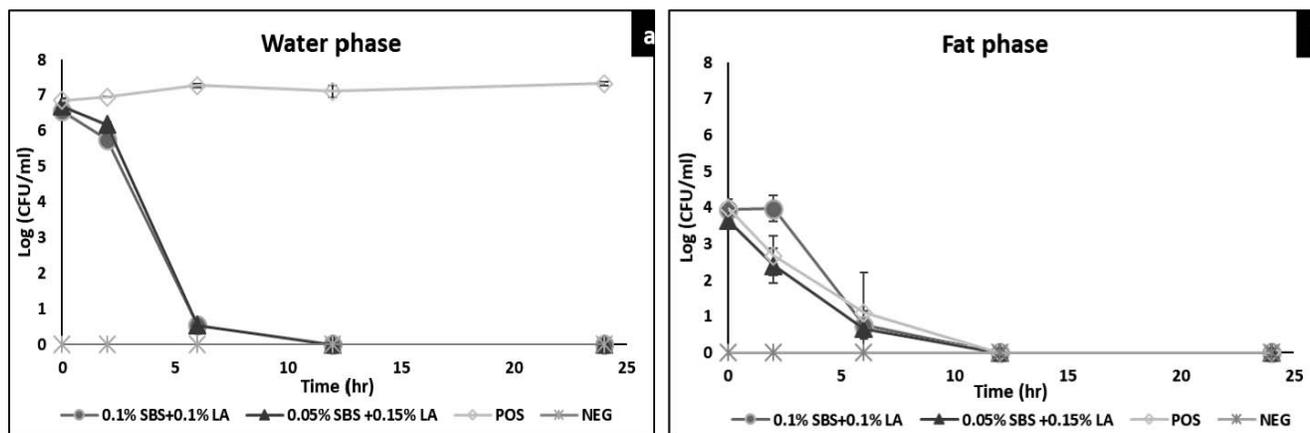


FIGURE 3. Effects of sodium bisulfate (SBS) plus lactic acid (LA) combinations against *Salmonella Typhimurium* (ATCC 14028) evaluated in the water phase (a) and fat phase (b) of a chicken fat system at 45°C. Treatments from each phase were plated on TSA at different times. POS, positive control (*Salmonella* only, no antimicrobials added); NEG, negative control (no *Salmonella*, no antimicrobials).

after 6 h and a 5.2-log reduction after 12 h (Fig. 4a). Both combinations completely eliminated *Salmonella* after 24 h.

In the fat phase, except for the 0.1% SBS + 0.05% ProA treatment (Fig. 4b), the reduction of pathogens by treatment combinations were not different from that of the positive control. The *Salmonella* counts in the 0.1% SBS + 0.05% ProA treatment were greater than ($P < 0.05$) those of the positive control.

DISCUSSION

SBS is used as an antimicrobial agent and litter acidifier for farm animals and in poultry houses, where 3-log reductions in bacterial loads have been reported (32). We found that 0.5% was the MIC of SBS against *Salmonella Typhimurium*, comparable to the results of Micciche et al. (28), who reported 0.4% SBS as the MIC against *Salmonella*. We found the MIC of 0.5% LA against *Salmonella Typhimurium* at pH 3.8, which was comparable to the results of Oh and Marshall (31), who also reported 0.5% as the MIC of LA against *Listeria monocytogenes*. In another study, Theobald (36) reported that the MIC of LA as

0.3% for *Salmonella Typhimurium* and 0.4% for *Escherichia coli*. Matsuda et al. (27) reported that at pH 4.5, LA had a MIC of 1% against *E. coli*. The lower MIC in our study may be due to the lower pH (3.8) and possibly due to the different bacterial species. The MICs of BA and ProA were both 0.25% at pH 5.2 and 4.9, respectively. Our result for ProA was comparable to that reported by BASF Corp. (3) in which 0.25% was identified as the MIC against various bacteria such as *E. coli*. In another study, Ushijima et al. (39) reported MICs of ProA at pH 6.8 against *Salmonella* and *E. coli* as 1.25 to 2.5% in TSB. The lower MIC of the ProA in our study could be related to the lower pH (4.9), because lowering the pH increases the sensitivity of the bacteria to fatty acids (39). The MIC of PA, an inorganic acid, was 0.25% at pH 3.8. To the best of our knowledge, no MIC studies of PA have been published. The MBC of PA against *Enterococcus faecalis* was reported as 2.5% by Arias-Moliz et al. (2). For combination treatments of SBS and organic acids, concentrations tested started at a mixture of two chemicals at half their MICs. Inhibition was achieved by the combinations of SBS and organic acids

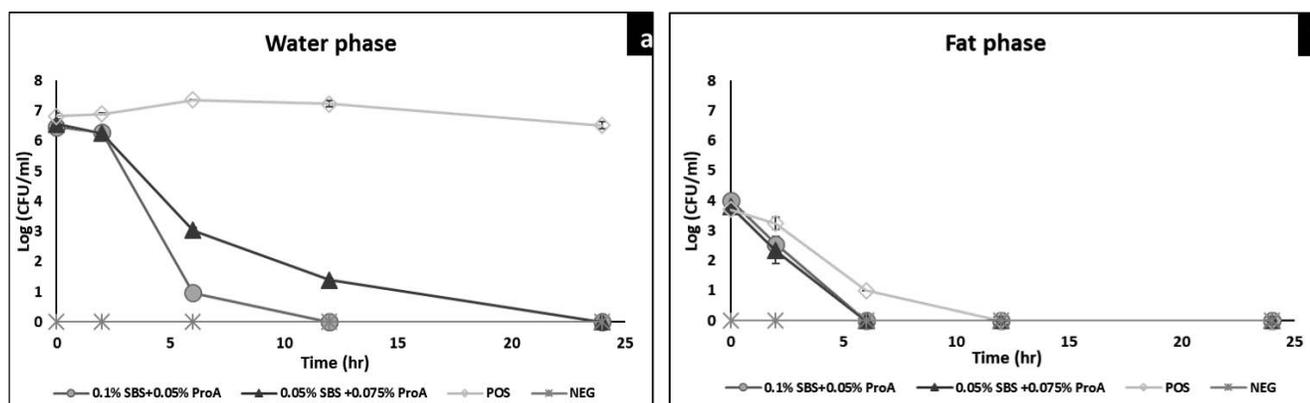


FIGURE 4. Effects of sodium bisulfate (SBS) plus propionic acid (ProA) combinations against *Salmonella Typhimurium* (ATCC 14028) evaluated in the water phase (a) and fat phase (b) of a chicken fat system at 45°C. Treatments from each phase were plated on TSA at different times. POS, positive control (*Salmonella* only, no antimicrobials added); NEG, negative control (no *Salmonella*, no antimicrobials).

when each of the chemicals was mixed in proportion lower than their individual MICs. This finding indicates a potential synergism between SBS and the organic acids. During the combination studies, the selection of different combinations of concentrations of each chemical lead to the identifications of two combinations that were inhibitory and hence designated as the MIC.

Throughout the challenge study, ~3% moisture was maintained in the rendered fat to reflect the moisture, insolubles, and unsaponifiables common in commercial in fats (30, 35). The greater efficacy of 0.5% SBS compared with 0.5% LA at 0 h might be related to the greater acidity and lower pK_a of SBS (1.99) compared with LA (3.86). PA has been used to wash hides in the beef industry and is a potent antimicrobial agent against *E. coli* (5).

In our study, the recovery of *Salmonella* in the fat phase was lower than that in the water phase. Lamb (24) also reported the loss of about 3 log CFU/mL for a *Salmonella* cocktail after 24 h in fats and oils. After 12 h of the treatment, we were not able to recover *Salmonella* even from the positive control in the fat phase, possibly because fats are known to have antibacterial activity (19). The rendered chicken fat used in this study had ~6% free fatty acid from oleic acid, and oleic acids have antimicrobial activity against *L. monocytogenes* and *Salmonella* (41). Another possible explanation for low *Salmonella* recovery could be sedimentation of bacterial pathogens in the bottom of the tube in the water phase. Further research is needed to determine the specific mechanism behind the low recovery of *Salmonella* in the fat phase.

The combinations of all organic acids and SBS were more effective against *Salmonella* in the water phase than in chicken fat, which could partly be related to the lower pK_a of SBS. The 0.1% SBS + 0.1% LA combination was more potent than 0.05% SBS + 0.15% LA and than SBS with other organic acids. This effect may be explained by the fact that the MIC of LA was higher (0.5%) than that of the other two organic acids (each 0.25%), and this combination contained a higher concentration of LA. The differences observed in *Salmonella* reduction between the two combinations of the same chemicals in which both resulted in the same MIC against the same organism needs to be explained. The MIC reported was the concentration at which no growth of bacteria could be seen. Even when both the treatment combinations produce clear wells (no visual growth), the actual bacterial counts might be different because at low levels of bacteria, the liquid growth medium lacks visual turbidity.

Commercial mixtures of organic acids are often used to inhibit pathogenic bacteria such as *Salmonella* in livestock and poultry feed and feed ingredients (40). Blends of organic acids have an array of pK_a values and potentially a broad spectrum of activity (20), which could explain why mixtures of SBS (pK_a of 1.99) and organic acids (pK_a of 3.86 for LA, 4.76 for BA, and 4.87 ProA) in our study had antibacterial effects against *Salmonella*. The inclusion of pH controls using the corresponding pH values of organic acids is recommended for future studies.

In the SBS+BA combination treatment, we observed some charring in the water phase as a dark brown ring. A

darkened or charred layer in fat has also been observed when the temperature of the acid-fat mixture was high or when the acid was too strong (14). The reason why BA caused this effect but the other acids did not is not clear and warrants further study.

The use of SBS as an antimicrobial agent and feed additive may help to reduce the cost of antibacterial treatments because SBS is significantly less expensive than commercial organic acids. SBS in combination with organic acids does not adversely affect stainless steel, which is important because antimicrobials are added to food and feed in mixing bins primarily composed of stainless steel. Organic acids cause corrosion of stainless steel, which is one of the major causes of mild steel pipeline failures (9). The effect of acetic acid on CO₂ corrosion of mild steel has been reported (17, 18). Similar issues have been found with formic acid and PA (12). Although SBS is a stronger acid than the organic acids, it has less of a damaging effect on stainless steel. For example, use of 10% SBS on stainless steel resulted in no or negligible corrosion (11).

In summary, use of SBS alone or in combination with organic acids was effective for mitigating *Salmonella* in rendered chicken fat. The findings also suggest that the combination of SBS with organic acids has a potential synergistic effect against *Salmonella*. Future work exploring the cause for lower recovery of *Salmonella* in the fat phase is recommended to better understand the effectiveness of antimicrobials in lipids.

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