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Research Note

Validation of a Lactic Acid– and Citric Acid–Based Antimicrobial Product for the Reduction of *Escherichia coli* O157:H7 and *Salmonella* on Beef Tips and Whole Chicken Carcasses

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ABSTRACT

The objectives of this study were to determine the effects of a lactic acid– and citric acid–based antimicrobial product on the reduction of *Salmonella* on whole broiler carcasses during processing and the reduction of *Salmonella* and *Escherichia coli* O157:H7 on beef trim. Freshly harvested broiler carcasses were inoculated with an inoculum of *Salmonella* strains to yield a 10^5 CFU/ml pathogen load on the surface of the carcass. The beef tips were inoculated as well with an inoculum of either *E. coli* O157:H7 or *Salmonella* to yield 10^4 CFU/100 cm^2. After 30 min for attachment, the broiler carcasses were treated with Chicxide applied for 5 s via a spray or immersed in Chicxide for 5, 10, or 20 s. Broiler carcasses were rinsed in poultry rinse bags with 400 ml of Butterfield's phosphate buffer in which *Salmonella* was enumerated from the diluents and Butterfield's phosphate. Chicxide significantly reduced *Salmonella* by 1.3 log CFU/ml with spray treatment and 2.3 log CFU/ml for all dip treatments. Following 30 min of attachment, the beef tips were placed into a spray cabinet with either Beefxide or sterilized water (control) and sprayed at 1 ft/2.5 s chain speed at 40 lb/in^2. The external surface of each beef tip was swabbed (100 cm^2) to determine pathogen loads. Beefxide significantly reduced *E. coli* O157:H7 by 1.4 log CFU/100 cm^2 and *Salmonella* by 1.1 log CFU/100 cm^2 ($P < 0.05$) compared with the control samples.

The poultry and beef industries have the challenges of controlling *Salmonella* and *Escherichia coli* O157:H7 within processing and manufacturing plants *(8, 11, 13, 28, 29)*. Poultry and poultry products have been identified by some researchers as the most important source of transmission of *Salmonella* to the human population *(7)*. *Salmonella* is the most widespread bacterium and is associated with the foodborne illness salmonellosis *(13)*. Cross-contamination by *Salmonella* on birds and carcasses may occur during transportation and processing *(5, 25)*. A 2007 study reported 88% of chicken carcasses with the presence of *Salmonella,* with 80% of these *Salmonella* isolates resistant to one or more antibiotics *(5)*. These results are not consistent with the 2008 U.S. Department of Agriculture (USDA) Baseline Data report, which shows only 12% contamination on chicken carcasses *(5, 24)*. It has also estimated that an average of 4% of broilers entering a processing plant are *Salmonella* positive and that 35% of broilers exiting the processing environment are positive for *Salmonella (18)*. In 2007 the Food Safety and Inspection Service (FSIS) reported that 0.68% of beef trim was contaminated with *E. coli* O157:H7 and 1.28% was contaminated with *Salmonella (17)*. Other studies using more aggressive sampling regimens have revealed that the presence of *E. coli* O157:H7 on beef carcasses can be as high as 40% *(1)*. Additionally, the number of recalls and outbreaks associated with *E. coli* O157:H7 contamination in beef products skyrocketed in 2007 and 2008 compared with previous years *(17)*. The beef industry is still in pursuit of a product that can provide effective reductions of *E. coli* O157:H7 and *Salmonella,* can easily be implemented into a processing line, and is cost-effective *(11)*.

Antimicrobials are commonly used in the industry to reduce pathogen loads. The most common antimicrobial treatment used in the poultry industry for decontamination of poultry is chlorine (sodium hypochlorite) because it is inexpensive, safe, and easy to use *(19)*. However, failure to optimize the disinfectant properties of chlorine (improper pH, concentration, or composition of incoming water) may reduce its antimicrobial efficacy and can result in offensive and harmful odors such as chlorine gas and trichloramines *(22)*. Carcass washes with organic acids and hot water washes are the most common intervention techniques within the cattle industry. However, the use of organic acids and hot water washes has not been a common practice with individual muscle cuts in the cattle industry *(11)*. Lactic acid and citric acid at concentrations of 1 to 3% have been shown to reduce *E. coli* O157:H7, *Salmonella* serotypes, and *Listeria monocytogenes* when sprayed onto beef and

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poultry carcasses by causing intracellular acidification (loss of homeostasis) *(32)*. Citric acid has the highest inhibitory effect due to its ability to diffuse through the cell membrane, penetrating the weak nondissociated acid, and lactic acid decreases the ionic concentration within the bacterial cell membrane of the exterior cell wall of the bacterial organism. This leads to an accumulation of the acid within the cell cytoplasm, acidification of the cytoplasm, disruption of the proton motive force, and inhibition of substrate transport *(32)*.

Chicxide and Beefxide (Birko Corp., Denver, CO) are a buffered blend of natural L(+) lactic acid and citric acid. Chicxide is intended for use as an antimicrobial treatment for poultry in compliance with FSIS Directive 7120.1, which allows organic acids at 2.5% to be used as a processing aid, and Beefxide is currently pending USDA approval *(30)*.

The objectives of these studies were to determine the effects of Chicxide on broiler carcasses in reducing *Salmonella* during broiler processing and to determine the effects of Beefxide in reducing *E. coli* O157:H7 and *Salmonella* on beef tips.

MATERIALS AND METHODS

Inoculum preparation. An inoculum cocktail containing four *E. coli* O157:H7 strains (A4 966, A5 528, A 1 920, and 966), all originally isolated from cattle, and a *Salmonella* inoculum cocktail containing three strains (Typhimurium ATCC 14028, Heidelberg Sheldon 3347-1, and Enteritidis phage type 13) were prepared separately. All cultures were obtained from the Texas Tech University Food Microbiology Laboratory Stock Collection (Lubbock). Individual strains were propagated in Trypticase soy broth at 37°C for 18 to 24 h. After growth, a concentrated culture containing all four strains of *E. coli* O157:H7 and a separate one containing all three strains of *Salmonella* were prepared as described by Smith et al. *(27)*. These *E. coli* O157:H7 and *Salmonella* inocula were frozen at −80°C and held until processing day, when they were serially diluted to achieve a 10^5 to 10^6 CFU/ml concentration. An additional tube of culture was sampled on the day of processing and was verified for initial inoculation load.

Whole chicken carcass treatment. A total of 40 broilers were raised together in a litter-lined floor pen for 40 days at the Texas Tech University farms *(20)*. Feed was removed from the birds 12 h prior to processing, but they were allowed access to water until 2 h prior to processing. The birds were transported to the Texas Tech University Poultry Processing facility and were conventionally processed. Following harvest and evisceration, the broilers were placed in a cooler at 3°C and transported to the Pathogen Processing facility at Texas Tech University.

Three carcasses were sampled for naturally occurring *Salmonella* and the other 25 carcasses were randomly assigned to treatments or controls. The 25 carcasses were inoculated by placing harvested carcasses individually into a poultry rinse bag containing 400 ml of autoclaved water with the *Salmonella* inocula and shaken by hand for 5 min to yield a surface inoculum level of 10^6 CFU/ml. After inoculation, carcasses were covered with aluminum foil and held at refrigerated temperature for 30 min to facilitate bacterial attachment. After 30 min, five carcasses were randomly assigned to each of the treatment groups. The treatments were none (control), a 5-s spray of Chicxide, a 5-s dip, a 10-s dip, and a 20-s dip. The spray treatment carcasses were hung with poultry hooks and passed back and forth through a continuous spray of Chicxide for the designated time at 40 lb/in^2 at ambient temperature. For the immersed treatments, a 25-gal tank was used to immerse carcasses in Chicxide solution at ambient temperature. Application of the Chicxide for all treatments was used at a concentration of 2.5% lactic acid solution, and the solutions were verified before and after each treatment for the lactic acid level by using a commercially available lactic acid testing kit along with the presence of *Salmonella* to ensure that the Chicxide solution was not serving as a source of cross-contamination *(30)*.

After the carcasses were treated, the samples were rinsed using the USDA whole bird rinse guidelines *(31)*. The poultry rinse bags were placed in an ice-filled cooler, and within 1 h the samples were transported to the Food Microbiology Laboratory for analysis. The poultry rinse bags were massaged for 1 min using a stomacher, and serial dilutions were then performed. Dilutions were plated onto xylose lysine Tergitol 4 (XLT4) plates with a thin tryptic soy agar (TSA) overlay for recovery of injured *Salmonella* cells from treatment *(6, 16)*. XTL4 plates were incubated at 37°C, and counts were determined after 48 h.

Beef bottom sirloin butt treatment. USDA Select beef tips (Beef Bottom Sirloin Butt, Tri-Tip, Boneless IMPS 185C) were obtained from a commercial processing facility and transported to the Pathogen Processing facility at Texas Tech University within 24 h of harvest. Upon arrival, beef tips were inoculated with either an inoculum cocktail of *E. coli* O157:H7 or a *Salmonella* cocktail (two separate inoculations) by immersing the beef tips in a pathogen-inoculated buffer solution at a concentration of 10^4 CFU/ml. A total of 5 beef tips per treatment per pathogen were prepared for a total of 30 beef tips as follows: noninoculated control, noninoculated with treatment spray, *E. coli* O157:H7 control, *E. coli* O157:H7 treated, *Salmonella* control, and *Salmonella* treated. Inoculated beef tips were placed on stainless steel racks, covered with aluminum foil, and held at refrigerated temperatures (37°C) for 30 min to facilitate attachment. The noninoculated controls were immediately subjected to microbial analysis, while the *E. coli* O157:H7 and *Salmonella* controls were treated in a sanitizing spray cabinet with sterile water. The noninoculated with treatment spray samples, *E. coli* O157:H7 treated samples, and *Salmonella* treated samples were separately placed in the sanitizing spray cabinet and sprayed with 2.5% Beefxide at a rate of 1 ft/2.5 s at 40 lb/in^2 *(30)*. Equipment was cleaned and sanitized between each treatment combination by using a protocol adopted by the Pathogen Processing facility at Texas Tech University proven to eliminate pathogens present on multiple types of material. Additionally, environmental samples were obtained before, during, and after the project was completed to ensure adequate cleaning and no cross-contamination.

After treatment or control spray, the external surface of each beef tip was swabbed (100 cm^2) to determine pathogen loads on the surface of the product. The swab was placed into a sterile Whirl-Pak bag with 10 ml of peptone buffer. The noninoculated control and noninoculated treatment spray bags with swabs were serially diluted and plated onto MacConkey agar and plate count agar to determine *E. coli* counts and total aerobic plate counts, respectively (Difco, Becton Dickinson, Sparks, MD). The MacConkey agar plates and plate count agar were incubated at 37°C for 24 h. The treatment samples containing *E. coli* O157:H7 (control samples and treated samples) were serially diluted and plated onto MacConkey agar with a thin layer of TSA for recovery of injured cells *(6, 16)*. Samples containing *Salmonella* (control samples and treated samples) were serially diluted and plated onto

xylose lysine deoxycholate agar with a thin layer of TSA (Difco, Becton Dickinson) to increase recovery of injured *Salmonella*.

Statistical analysis. The data were analyzed using a descriptive analysis (PROC MIXED) in an SAS program. Data were log transformed to satisfy the normality assumption with a completely randomized statistical design. Verification of normality was confirmed using PROC UNIVARIATE within SAS. If a plate revealed no colonies, a count of 1 CFU/100 cm^2 or 1 CFU/ml was recorded in the data set for statistical program analysis purposes. Significance was reported as P values of <0.05.

RESULTS

Naturally occurring *Salmonella* was not isolated from the chickens that were harvested at the Texas Tech Poultry Laboratory Facility. The control samples indicated an initial concentration of 6.5 log CFU/ml of *Salmonella* on the chicken carcasses. Spraying the carcasses for 5 s with Chicxide resulted in a reduction of 1.3 log CFU/ml, while immersing the carcasses for 5 s yielded a 2.3-log CFU/ml reduction ($P < 0.05$). There were no significant differences ($P > 0.05$) between immersed carcasses for 5, 10, or 20 s, with all yielding a 2.3-log CFU/ml reduction.

The beef tips had an initial aerobic plate count of 3.5 log CFU/100 cm^2 and *E. coli* count of 1.5 log CFU/100 cm^2. After the beef tips were immersed into Beefxide, the aerobic plate counts decreased significantly, by 1.5 log CFU/100 cm^2 ($P < 0.0001$), while *E. coli* decreased by 0.4 log CFU/100 cm^2 ($P > 0.05$). The control sample indicated an initial *E. coli* O157:H7 concentration of 5.5 log CFU/100 cm^2 and *Salmonella* on the beef tips. After treatment, the *E. coli* O157:H7 population was reduced by 1.4 log CFU/100 cm^2, and the *Salmonella* population was reduced by 1.1 log CFU/100 cm^2 ($P < 0.05$).

DISCUSSION

The most widely used chemical compound in the poultry industry to reduce *Salmonella* is chlorine; however, misuse of this compound can render it ineffective and result in discoloration of the product *(21)*. There are many studies that report that chlorine is effective in reducing *Salmonella* and *Campylobacter* by as much as 1 to 2 log on poultry carcasses *(19),* while other studies using other organic acids to spray or dip poultry carcasses can reduce *Salmonella* as much as 3 log *(3, 8, 13, 19, 32)*. A specific example was the use of 2% lactic acid sprayed on chicken carcasses by Yang et al. *(33),* which resulted in a 2-log CFU per carcass reduction of *Salmonella*. In general, carcass rinse applications that decrease *Salmonella* by 2 log CFU/ml are considered effective, since most carcasses are considered to have <100 *Salmonella* cells *(14)*. Poultry quality is a concern when using different organic acid washes. In an earlier study, the quality effects of acetic, citric, lactic, malic, mandelic, or tartaric acids at 0.5, 1, 2, 4, and 6% concentrations were tested on broilers, revealing that in simulated dip application, all the acids decreased lightness and increased redness and yellowness values in the skin of the broiler carcasses with increasing acid concentration *(2)*. Our observations when using Chicxide (which contains both lactic acid and citric acid) at a 2.5% concentration were that no quality defects were noticed at the approved usage level, but at a concentration above the approved 2.5% a yellowing of the skin of the broiler carcasses was detected.

In 1994, the USDA-FSIS instituted a zero-tolerance policy for *E. coli* O157:H7 in ground beef *(29)*. Therefore, organic acids such as lactic acid and citric acid are currently used on beef carcasses to reduce the incidence of *E. coli* O157:H7 and *Salmonella* on retail meat. A study evaluating lactic acid's ability to reduce *E. coli* O157:H7 on beef subprimal cuts revealed that the surface populations of the cuts were reduced by 0.93 to 1.10 log CFU/100 cm^2 *(11)*. Other studies have indicated that hot water rinses followed by an organic acid treatment (either lactic or acetic acid) performed better at reduction of *E. coli* O157:H7 and *Salmonella* serotypes than trimming or washing alone on beef carcasses *(10–12, 15, 26)*. Overall, lactic acid reduced levels of *E. coli* O157:H7 significantly better than acetic acid, while lactic acid and citric acid performed equally well in their ability to reduce *Salmonella (10)*. Additionally, lactic acid at 2.0% concentration was shown to reduce *Salmonella* by 1.0 log CFU/100 cm^2 on beef trim and *E. coli* O157:H7 by 2.0 log CFU/100 cm^2 *(11)*. At 3%, lactic acid as a preevisceration step reduced *E. coli* loads by 35% *(4)*. *Salmonella* Typhimurium on beef after immediate treatment has been reduced by 0.09 to 1.14 log when 1 to 3% lactic acid is spray applied *(24)*. Lactic acid has also been shown to reduce the total bacteria counts and psychrotropic bacteria on beef carcasses *(26)*. After 7 days of ripening on beef carcasses, 2% lactic acid reduced the total bacteria load by 1.2 log and the psychrotropic load by 1.32 log when stored at 4 to 6°C *(9)*. Our results indicate that the use of these two products achieve pathogenic reductions similar to those obtained with lactic acid sprays.

With the recent increase in the number of recalls and outbreaks associated with *E. coli* O157:H7–contaminated beef, the industry is in need of an intervention to reduce this pathogen on the product itself and to prevent cross-contamination from the product to other products. Beefxide may be a viable option in providing the industry with another product to further reduce pathogen loads. Additionally, with concerns of increased cases of salmonellosis in the United States, Chicxide may be a viable option for the industry to decontaminate poultry products.

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