Reduction of *Salmonella* Infantis on skin-on, bone-in chicken thighs by cetylpyridinium chloride application and the impact on the skin microbiota

L. A. Wythe,[*] D. K. Dittoe,[*] K. M. Feye,[†] E. G. Olson,[*] L. M. Perry,[‡] and S. C. Ricke[*,1]

[*]*Meat Science and Animal Biologics Discovery Program, Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI 53706;* [†]*Department of Food Science and Center for Food Safety, University of Arkansas, Fayetteville, AR 72704; and* [‡]*Safe Foods Corporation, Little Rock, AR 72114*

ABSTRACT *Salmonella* Infantis has been the etiological agent of numerous foodborne outbreaks of nontyphoidal *Salmonella*. Consequently, there is an emergent need to mitigate *Salmonella* Infantis among poultry. Thus, this study evaluated the efficacy of cetylpyridinium chloride (**CPC**) versus peroxyacetic acid (**PAA**), on bone-in, skin-on chicken thighs for the reduction of *Salmonella* and changes in the microbiota. Exactly 100 skin-on, bone-in chicken thighs (2 trials, 0 and 24 h, k = 5, n = 5, N = 50) were inoculated with 10^8 CFU/mL of a nalidixic acid resistant strain of *S.* Infantis for an attachment of 10^6 CFU/g. Thighs were treated with 20 s part dips (350 mL): a no inoculum, no treatment control (**NINTC**); no treatment control (**NTC**); tap water (**TW**); TW+CPC; TW+PAA. Following treatment, thighs were rinsed in 150 mL of nBPW, and rinsates were collected. Rinsates were spot plated for *Salmonella* and aerobic bacteria (**APC**). Log_{10} transformed counts were analyzed using a mixed-effects model (random effect = trial) with means separated using Tukey's HSD ($P \leq 0.05$). The genomic DNA of rinsates was extracted, and the 16S rDNA was sequenced on an Illumina MiSeq. Microbiota data were analyzed using QIIME2, with data considered significant at $P \leq 0.05$ (main effects) and Q≤0.05 (pairwise differences). Treatment × time interactions were observed for both *Salmonella* and APC ($P < 0.05$). The treatment of thighs with PAA and CPC reduced *Salmonella* and APC in respect to the controls. Numerically, thighs treated with CPC had less *Salmonella* (4.29 log_{10}CFU/g) and less APC (4.56 log_{10}CFU/g) at 24 h than all other treatments ($P > 0.05$). Differences in diversity metrics were not consistently observed between treatments; however, in trial 2, the NTC treated thighs were different than those treated with CPC ($P < 0.05$; Q < 0.05). In both trials, ANCOM, the analysis of microbiome compositional profiles, revealed shifts at both the phylum and order levels with thighs being different in the relative abundances of *Proteobacteria* ($P < 0.05$). In conclusion, treatment of skin-on poultry parts with CPC may reduce the risk of foodborne outbreaks caused by *Salmonella* Infantis.

Key words: poultry, peroxyacetic acid, cetylpyridinium chloride, *Salmonella* Infantis, 16S rDNA

2021 Poultry Science 101:101409
https://doi.org/10.1016/j.psj.2021.101409

INTRODUCTION

Poultry producers in the United States (US) process billions of pounds of poultry in a year, with approximately 112.5 pounds of poultry consumed per capita in 2019 (NAMI, 2020; NCC, 2020). However, salmonellosis is a common risk with the consumption of poultry (Henchion et al., 2014; Kalaba et al., 2017). As such, *Salmonella enterica* is a public health concern, infecting approximately 1.35 million people in the United States annually (CDC, 2019a). Currently, poultry processors utilize acidifiers to mitigate foodborne pathogens such as *Salmonella enterica* throughout poultry processing. However, due to the acid tolerance response seen in certain *Salmonella enterica* serovars to organic and inorganic acids, alternative interventions need to be employed (Kieboom and Abee, 2006). One prevalent serovar, *Salmonella* Infantis, which has been isolated in poultry feed, live chickens, and raw products, has led to significant outbreaks in the US (CDC, 2019b; Shariat et al., 2020). In the past few years, an emerging strain of *S.* Infantis has been reported globally (Aviv et al., 2014). These emerging strains have been reported to have enhanced biofilm formation, adhesion, and invasion into avian and mammalian host cells with increased resistance to antibiotics and other antimicrobials (Aviv et al., 2014).

Therefore, there is a need for alternative interventions capable of reducing *S.* Infantis. Currently, peroxyacetic

Received May 12, 2021.
Accepted July 22, 2021.

[1]Corresponding author: sricke@wisc.edu

acid (**PAA**), an organic peroxide that dissociates into acetic acid and hydrogen peroxide, is one of the more widely utilized chemical intervention in poultry processing (Dittoe et al., 2019a, b). Undeniably, PAA has been proven to be an effective antimicrobial for reducing *Salmonella* serovars across various industries (3 Baldry, 1983; Jolivet-Gougeon et al., 2006; Dittoe et al., 2019a, b). However, previous evidence has suggested up-regulation of virulence genes coinciding with the acid-tolerance response genes in various *Salmonella* enterica serovars (Garcia-Del Portillo et al., 1993; Ryan et al., 2015; Lianou et al., 2017; Szmolka et al., 2018). Due to the complex nature of processing and the complex matrices of poultry carcasses, the final concentrations of PAA that pathogens may be treated with could be sub-inhibitory and thus increase the risk of biocide tolerance (Foster and Hall, 1991; Finstad et al., 2012; Cadena et al., 2019; Lin et al., 1995). Therefore, potential alternatives such as quaternary ammonium compounds (**QAC**), including the surfactant cetylpyridinium chloride (**CPC**) require continued evaluation. By binding to the anionic sites on the surface membranes of bacteria, various levels of QACs can cause slow or rapid cell death by damaging the cell walls then leaking or solubilizing cellular components, based on low or high concentrations (Morente et al., 2013). The use of CPC among inoculated poultry parts has demonstrated comparable reduction of *Salmonella* serovars to that of PAA treated parts (Scott et al., 2015; Chylkova et al., 2017; Zhang et al., 2018). Therefore, the current study was conducted to determine the effects of CPC (Cecure®, Safe Foods Corporation™, Little Rock, AR) on reducing a marker strain of *S.* Infantis as compared to the standard poultry processing antimicrobial peracetic acid PAA (Promoat®, Safe Foods Corporation™, Little Rock, AR) on inoculated skin-on parts. Due to the buffering effect of the skin that may reduce the efficacy of antimicrobials, thus impacting the survival of attached *Salmonella* by increasing cell attachment and protecting attached cells, skin on parts were utilized in the current study (Tan et al., 2014a,b).

Increasing attention has been geared toward understanding the impact that antimicrobials have on poultry carcass microbiota, especially as next-generation sequencing methods become less costly and more readily accessible. Recent explorations into poultry carcass microbiota, processing reuse water microbiota, and biomapping processing have reported useful insights for poultry processors (Feye et al., 2020a,b). Identifying the indigenous poultry carcass microbiota can have important implications for food safety and quality, particularly in understanding the relationship between indigenous bacteria and pathogens on carcasses and throughout the processing plant (Rothrock et al., 2016; Kim et al., 2017; Handley et al., 2018; Wages et al., 2019; Feye et al., 2020a,b). Analyzing the effects of antimicrobials and the relationships between pathogens and indigenous microbiota on various poultry products offer potential specific intervention strategies for improving their shelf-life and safety. Thus, the first objective of the current study was to compare the antimicrobials PAA and CPC on *S.* Infantis inoculated skin-on chicken thighs. The second objective was to determine whether shifts in microbiota on chicken thighs occurred when treated with PAA or CPC when inoculated with *S.* Infantis using short-read sequencing targeting the V4 region of 16S rDNA.

MATERIALS AND METHODS

Chicken Part Procurement

The current experiment was conducted as 2 separate trials with the application of antimicrobials followed by sampling for microbiological analyses. Each trial consisted of 50 skin-on chicken thighs (2 trials, 5 treatments, 0 h and 24 h, 5 replicates per treatment) with an average weight of 253 g obtained postharvest from a commercial poultry processing facility as a component of standard poultry slaughter operations. As parts were collected from an off-campus commercial poultry processing plant, this study was exempt from the University of Arkansas (**UA**) Institutional Animal Care and Use Committee oversight. Immediately postprocessing, the chicken thighs were transported directly to the UA Center for Food Safety, Fayetteville, AR. Thighs were stored at 4°C overnight until the start of the study.

Preparation of Nalidixic Acid Resistant Salmonella

Fresh and pure *S.* Infantis (CDC H3517) culture was obtained and grown overnight at 37°C in Mueller Hinton Broth (MHB; Hardy Diagnostics, Irving, TX). The strain used was associated previously with an outbreak from alfalfa sprouts (Dong et al., 2003). The *S.* Infantis strain was made nalidixic acid resistant as per Olson et al. (2020). The antibiotic-resistant strain and nalidixic acid supplemented (64 μg/mL) Xylose Lysine Dextrose (NA+XLD; HiMedia, West Chester, PA) was used in this study as a marker strain for the determination of effects specifically on *S.* Infantis.

Inocula Preparation

The day before each individual trial began, 500 mL of MHB was inoculated with the NA resistant *S.* Infantis and grew overnight in a shaking incubator. Immediately following the overnight incubation, approximately 50 mL of the inocula were aseptically transferred into separate 50 mL conical tubes and centrifuged (Eppendorf 5810R, Eppendorf, Hamburg, Germany) at 18,000 × g for 3 min. The supernatant was decanted and washed with 1 × PBS 2 times. After the final wash, the pellets were resuspended in 1 × PBS. A representative part from each treatment for 0 h and 24 h time points were inoculated with the same inocula with there being 5 replications for each trial. Therefore, at each trial, there were 10 thighs inoculated per poultry rinsate

bag with there being approximately 100 mL of 8 $\log_{10}$ CFU per mL of *S.* Infantis. Thus, the chicken parts were inoculated using 1 mL of inoculum per 25 g of parts, with the mean weight of each thigh at 253.16 ± 2.96 g. The inocula were hand massaged into the poultry thighs for 1 min and allowed to adhere for 60 min at 4°C.

Treatments were prepared in the laboratory following manufacture specifications for poultry processing in 15 L of tap water. Due to the stability of the products and the short duration of the antimicrobial dips, room temperature water was used in the current study. Concentrations were confirmed with titration kits provided by the manufacturer at 5,000 ppm (0.5%) of CPC (Cecure) and 600 ppm of PAA (Promoat). Exactly 400 mL of each treatment were aliquoted into sterile, individual collection bags. A randomly selected chicken part was added using sterile forceps and agitated for 15 s to allow for complete contact. The part was then aseptically transferred to a second sterile, empty rinsate bag, and the remaining treatment was allowed to drip off for 2 to 3 min before the remaining liquid in the collection bag was decanted.

The treatments utilized were as follows: a no inoculum, no treatment control (**NINTC**); a no-treatment control (**NTC**); a tap water control (**TW**); PAA + TW (600 ppm); CPC + TW (5,000 ppm). There was no difference in inoculation for NTC thighs and thighs selected for other treatment groups. The thighs treated with TW were used as a mechanical control to account for the rinsing effect the treatments may have had on loosely attached *Salmonella* (Lillard, 1989; Zhang et al., 2018). Following the CPC dip, per 21 CFR § 173.375 (2,020), the parts were aseptically moved to a sterile rinsate bag with tap water and dipped until fully submerged for 10 s to rinse residual CPC from the parts (USDA-FSIS, 2020). Parts were subsequently moved to individual dry, sterile rinsate bags.

After treatment, parts were either stored at 4°C for 24 h or were immediately prepared for microbial analysis (0 and 24 h). At the designated time point, individual parts were rinsed using 150 mL of neutralizing buffered peptone water (nBPW, Hardy Diagnostics, Irving, TX) in sterile rinsate bags. The parts were manually agitated using a 180° arcing motion for 1 min (United States Department of Agriculture- Federal Safety Inspection Service USDA-FSIS, 2013). The parts were then aseptically removed from the bags and discarded, and the subsequent rinsates were used for *Salmonella* and total aerobic plate count (**APC**) as well as 16S rDNA sequencing.

Salmonella and Aerobic Bacteria Enumeration

An aliquot of 1 mL of each sample was transferred to individual 2 mL microcentrifuge tubes. These tubes were stored at −80°C until DNA extraction and sequencing analysis could be completed. Another 20 μL from each sample was serially diluted (1:10) to 10^{-7} in 180 μL of 1 × PBS in a flat bottom 96 well plate. The rinsate samples were then spot plated with 10 μL of each serial dilution on NA+XLD for *S.* Infantis and Tryptic Soy Agar (TSA; Millipore Corporation, Billerica, MA) for total aerobic plate counts. Plates were left to dry entirely before inverting and were incubated aerobically at 37°C for 24 h. Only black colonies on the NA+XLD plates were counted as *Salmonella* Infantis (Scott et al., 2015).

DNA Extraction

The 1 mL aliquots of reserved rinsate samples were centrifuged (Heraeus Pico 21, Thermo Fisher Scientific, Langenselbold, Germany) for 10 min at 5,000 x g to pellet the rinsates. Genomic DNA from these pellets was extracted using a QIAamp DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA), following the standard protocol provided with the kit. The concentration and purity of the DNA were measured for each extracted sample using a NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE). The extracted samples were then diluted to 10 ng/μL of DNA. Samples were stored at −20°C until further analysis occurred.

Illumina MiSeq Library Preparation and Sequencing

A library targeting the V4 region of 16S rDNA was prepared as per Kozich et al. (2013). Following the MiSeq v2 (500 cycles) Reagent cartridge (Illumina, San Diego, CA) manufacturer protocol, the pooled library was combined with a PhiX Control v3 (Illumina), 0.2 N fresh NaOH, and HT1 buffer (Illumina), for a final concentration of 12 pM. The final library was loaded on a MiSeq v2 (500 cycles) reagent cartridge for sequencing, and the procedures were monitored via the Illumina BaseSpace website. Resulting sequences (fastq files) were downloaded from Illumina BaseSpace and uploaded to a GitHub depository (https://github.com/RickeLab-UW/Microbiome-of-Poultry-Thigh-Rinsates-Inoculated-with-Salmonella-Infantis.git). Data were also uploaded to NCBI Sequence Read Archive (**SRA**) under the accession PRJNA729057.

Microbiological Statistical Analysis

Each chicken part was randomly assigned a treatment, a time point, and a trial before analysis. Rinsates from both trials were used for microbiological data, and then separated by trial during microbiome analysis. Data were collected at 0 h and 24 h. The colony forming units (**CFU**) of *Salmonella* and APC were $\log_{10}$ transformed and reported as either $\log_{10}$ CFU of *S.* Infantis per g chicken ($\log_{10}$ CFU/g) or $\log_{10}$ CFU of total aerobic bacteria per g chicken, respectively. Data were analyzed through RStudio (RStudio Team, 2020), using a linear mixed-effects model through the lmerTest packages (Kuznetsova et al., 2017), with the fixed (main) effects evaluated being treatment and time and the random effect being trial. Means were evaluated and separated with Tukey's HSD using the emmeans and

multcomp packages (Hothorn et al., 2008; Lenth, 2021). Significance was set at $P \leq 0.05$.

QIIME2 Analysis

The FASTQ files of sequencing output were downloaded from the Illumina Biospace and sequence read analysis was performed using Quantitative Insights into Microbial Ecology (QIIME2) pipeline version 2020.2 (Bolyen et al., 2019). Sequences from both trials were imported as separate datasets and followed similar coding paths. Demultiplexed sequences were imported as paired-end sequences using Casava 1.8 (Callahan et al., 2016). Sequences were subsequently denoized and filtered for quality with DADA2 with the q2-dada2 plugin (Callahan et al., 2016). Chimeras were filtered with consensus in the q2-dada2 plugin. Amplicon sequence variants (**ASVs**) were aligned with mafft using the q2-alignment plugin and were used to create a phylogenetic tree with fasttree2 using the q2-phylogeny plugin (Price et al., 2010). Sequences were rarified to the same number of random reads from each sample for diversity analyses, with a sampling depth set at 450 sequences for trial 1 and a sampling depth of 300 for trial 2.

The microbiome metrics for α diversity, β diversity, and Principal Coordinate Analysis were determined using the q2-diversity plugin. For α diversity metrics Faith's Phylogenetic Diversity (Faith's PD), Shannon Diversity, and Pielou's Evenness were utilized, while Weighted UniFrac and Jaccard Dissimilarity were applied for β diversity (Jaccard, 1912; Faith et al., 1987; Lozupone and Knight, 2005). Pairwise differences were determined for α diversity metrics using Kruskal-Wallis, whereas the pairwise differences for β diversity metrics were determined using PERMANOVA and PERMDISP. The α diversity interactions between treatment and time for trial 1 were analyzed using the parametric ANOVA. The β diversity interactions for trial 1 were analyzed using ADONIS, a nonparametric, multivariate analysis of variance that accounts for both Euclidean and non-Euclidean distances in ecological diversity (Anderson, 2001). Interactions and main effects were considered significant at a $P \leq 0.05$, while pairwise differences were considered significant at a $Q \leq 0.05$.

Operational taxonomical units (**OTUs**) were classified using the q2-feature-classifier with SILVA.138 (99% OTUs full-length sequences) classifier from phylum to genus at a 97% confidence interval (McDonald et al., 2012; Bokulich et al., 2018). Analysis of composition of microbiomes (**ANCOM**) was performed to define specific quantitative differences of taxa across treatments and time for trial 1, and treatment only for trial 2 (Mandal et al., 2015).

RESULTS

Microbiological Analysis

The first objective of the current study was to determine the effects of CPC (Cecure) application versus PAA (Promoat) application in reducing the population levels of *Salmonella* Infantis on chicken parts. With trial analyzed as a random effect in a mixed-effect model, there was a treatment by time interaction between the *Salmonella* level and total aerobic bacteria ($\log_{10}$ CFU/g; $P < 0.05$; Figure 1). Only one sample from NINTC thighs was reported positive for *S.* Infantis at 24 h; therefore, the mean $\log_{10}$ CFU of *S.* Infantis per gram of thigh was only 0.17 at 24 h post-treatment (Figure 1A). Nonetheless, both groups of NINTC thighs at 0 h and 24 h (0.00 and 0.17 $\log_{10}$ CFU/g, respectively) were statistically indistinguishable from each other and different from all other groups ($P < 0.05$, Figure 1A). Thighs treated with TW (6.41 and 5.71 $\log_{10}$ CFU/g at 0 h and 24 h, respectively) were not statistically different from NTC thighs (6.33 and 7.16 $\log_{10}$ CFU/g at 0 h and 24 h, respectively) at either time point. At 0 h, thighs treated with PAA (5.19 $\log_{10}$ CFU/g) and those treated with CPC (5.38 $\log_{10}$ CFU/g) were not statistically different from each other. Thighs treated with PAA at 0 h (5.19 $\log_{10}$ CFU/g) were different from the NTC thighs, while those treated with CPC (5.38 $\log_{10}$ CFU/g) were not different from those treated as NTC ($P < 0.05$, Figure 1A). At 24 h, thighs treated with CPC (4.29 $\log_{10}$ CFU/g) were numerically lower in total *Salmonella* than those treated with PAA (4.96 $\log_{10}$ CFU/g). In addition, thighs treated with PAA (4.96 $\log_{10}$ CFU/g) at 24 h post-treatment were not significantly different from those treated with TW (5.71 $\log_{10}$ CFU/g), while thighs treated with CPC (4.29 $\log_{10}$ CFU/g) were different. The only antimicrobial treatment that was significantly different in *Salmonella* load at 24 h from their 0 h counterparts were those treated with CPC ($P < 0.05$; 5.38 and 4.29 $\log_{10}$ CFU/g). However, the mean *Salmonella* load on thighs treated with PAA were relatively stable, with thighs treated with PAA at 24 h not being different from those treated with PAA at 0 h (5.19 and 4.96 $\log_{10}$ CFU/g).

There was a significant interaction between treatment and time on the total aerobic bacteria recovered from thighs inoculated with *S.* Infantis ($P < 0.05$; Figure 1B). Total aerobic microbial loads on thighs from all treatment groups were not significantly different between treatment groups at 0 h (5.47, 6.59, 6.33, 5.77, 5.20 $\log_{10}$ CFU/g; Figure 1B). At 24 h, the total aerobic microbial populations of thighs treated with NTC and TW (7.15 and 7.06 $\log_{10}$ CFU/g) were different than those treated with CPC and PAA, with CPC and PAA not being different (4.56 and 5.59 $\log_{10}$ CFU/g). At the same time (24 h), there was no difference between thighs treated with NINTC (4.21 $\log_{10}$ CFU/g) and those treated with CPC and PAA. Numerically, at 0 h, thighs treated with PAA had the lowest numerical mean load of APC (5.20 $\log_{10}$ CFU/g); whereas, at 24 h, thighs treated with CPC had the lowest numerical load of APC (4.56 $\log_{10}$ CFU/g).

Microbiome Analysis

The second objective of the current study was to determine the overall effect of these treatments on the

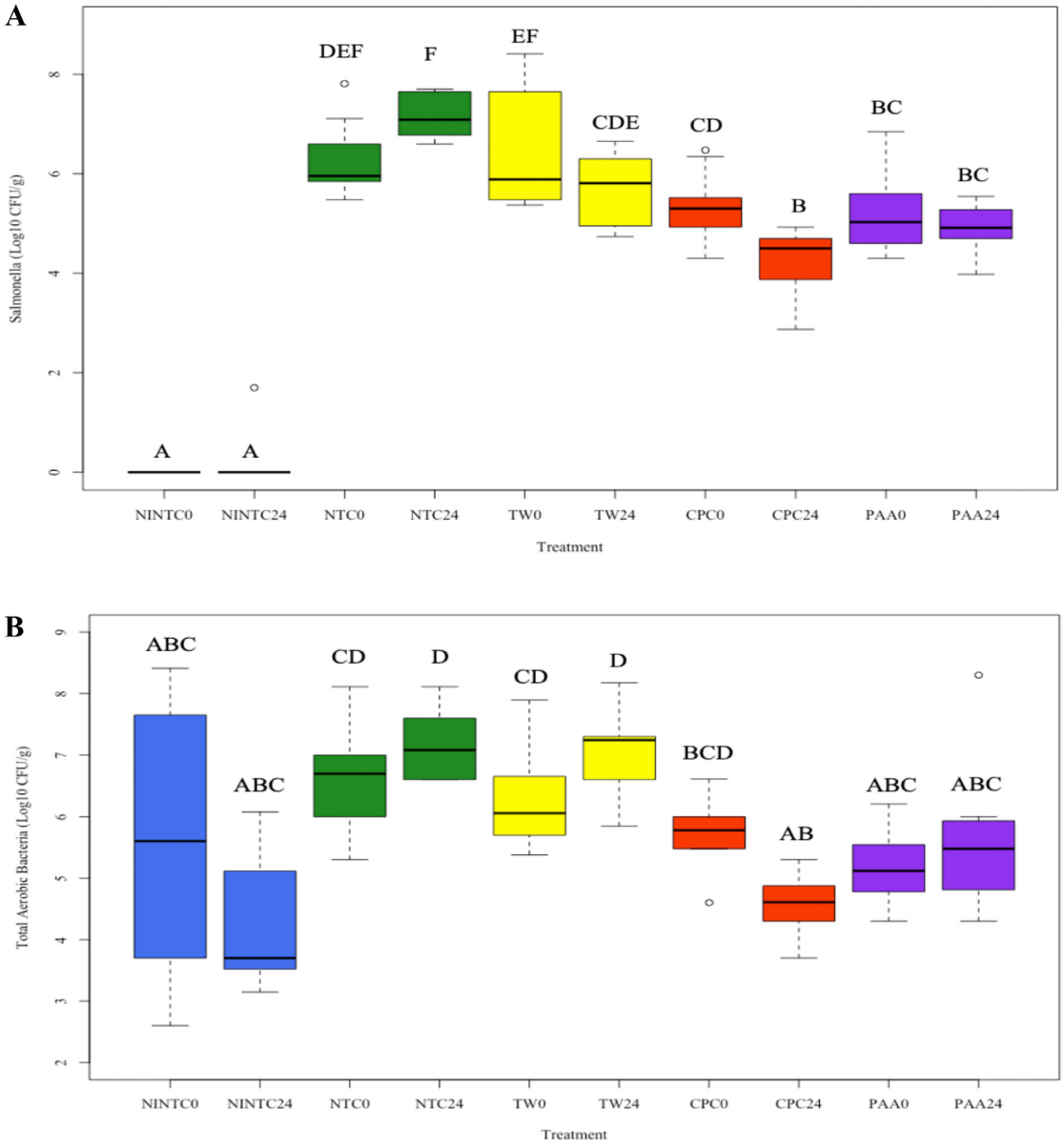

Figure 1. Boxplots of microbiological counts of nalidixic resistant *Salmonella* (64 ng/μL) and APC counts at 0 h and 24 h. (A) A treatment by time interaction on the total nalidixic resistant *Salmonella* (Log_{10} CFU/g) on thighs inoculated with a nalidixic resistant strain of *S.* Infantis (N = 50, n = 5, k = 5, $P < 0.05$). (B) A treatment by time interaction on the total aerobic bacteria (Log_{10} CFU/g) on thighs inoculated with a nalidixic resistant strain of *S.* Infantis (N = 50, n = 5, k = 5, $P < 0.05$). The treatments utilized were: a no inoculum, no treatment control (NINTC); a no treatment positive control (NTC); tap water (TW); PAA + TW (600 ppm); CPC + TW (5,000 ppm), all applied as a 20 s part dip via rinsate. Means with different superscripts are considered significantly different (a,d).

skin microbiota. The rinsates from the first trial were used to examine if shifts occurred over time in response to treatment, while the rinsates from the second trial were used to evaluate the 24 h time point exclusively. The current study employed quantitative and qualitative α and β metrics to analyze sequences while comparing phylogenetic and nonphylogenetic diversity differences in microbiota composition. The α diversity metrics utilized in this study were Pielou's Evenness, Shannon's Diversity, and Faith's PD; the β metrics used were Jaccard Dissimilarity and Weighted UniFrac.

The main effects of treatment, time, and trial, and the interactions between, were evaluated for α and β metrics using ANOVA and ADONIS (Table 1, $P < 0.05$). There was not a significant 3-way interaction for any α or β metrics. The following interactions were significant (Table 1, $P < 0.05$): treatment by time for Weighted UniFrac; treatment by trial for Jaccard; and time by trial for Jaccard. The main effect of treatment was significant for Pielou's evenness, Shannon's Diversity, and Faith's PD (Table 1, $P < 0.05$). The main effect of time was not significant for any metrics (Table 1, $P > 0.05$). The main effect of trial was significant for Pielou's evenness and Shannon's Diversity. Because the 2 trials were independent and the main effect of trial was influencing the results, the trials were split in the analysis pipeline.

Trial 1

The main effects of treatment and time, and the interaction between them, were evaluated for α and β metrics

Table 1. Overall ANOVA (α diversity) and ADONIS (β diversity) results for main effects of treatment, time, trial, and the interactions between them ($P < 0.05$).[1]

Metric	Treatment	Time	Trial	Treatment × Time	Treatment × Trial	Time × Trial	Treatment × Trial × Time
Evenness[2]	**3.17E-20**	0.331	**0.040**	0.247	0.222	0.628	0.401
Shannon's Diversity[2]	**2.72E-20**	0.834	**0.037**	0.155	0.251	0.282	0.744
Faith's PD[2]	**1.85E-08**	0.951	0.279	0.412	0.693	0.057	0.814
Weighted UniFrac[3]	**0.001**	0.629	0.081	**0.001**	0.539	0.274	0.929
Jaccard[3]	**0.001**	0.240	**0.001**	0.261	**0.008**	**0.033**	0.460

[1]Bolded P values are significant ($P < 0.05$).
[2]α diversity metrics. These metrics are quantitatively evaluated using ANOVA for significance in evenness, evenness and richness, and weighted phylogenetic diversity within samples through Pielou's Evenness, Shannon's Diversity, and Faith's Phylogenetic Diversity, respectively.
[3]β diversity is evaluated through ADONIS to determine significance in qualitative dissimilarity and weighted phylogenetic differences between samples through Jaccard Dissimilarity and Weighted UniFrac.

Table 2. Trial 1 ANOVA (α diversity) and ADONIS (β diversity) results for main effects of treatment and time, and the interaction between the two ($P < 0.05$).[1]

Metric	Treatment[1]	Time[1]	Treatment × Time
Evenness[2]	0.060	0.879	0.848
Shannon's Diversity[2]	**3.19E-03**	0.465	0.184
Faith's PD[2]	0.778	0.957	0.522
Weighted UniFrac[3]	**0.001**	**0.028**	0.801
Jaccard[3]	**0.001**	**0.003**	0.778

[1]Bolded P values are significant ($P < 0.05$).
[2]α diversity metrics. These metrics are quantitatively evaluated using ANOVA for significance in evenness, evenness and richness, and weighted phylogenetic diversity within samples through Pielou's Evenness, Shannon's Diversity, and Faith's Phylogenetic Diversity, respectively.
[3]β diversity is evaluated through ADONIS to determine significance in qualitative dissimilarity and weighted phylogenetic differences between samples through Jaccard Dissimilarity and Weighted UniFrac.

using ANOVA and ADONIS (Table 2, $P < 0.05$). An interaction between treatment and time was not significant for either α or β metrics. The main effect of treatment was significant for Shannon's Diversity ($P < 0.05$) but not other α metrics ($P > 0.05$). Time was not significant across α diversity assessment ($P > 0.05$). Treatment and time were both significant for Weighted UniFrac and Jaccard Distance ($P < 0.05$).

Using Kruskal-Wallis to determine the main effect of treatment among α metrics, there was a significant main effect of treatment on Shannon's Diversity (Figure 2), but there were no significant pairwise differences between the treatment groups (Supplemental Table 1; $P < 0.05$, $Q > 0.05$). The main effect of treatment was trending toward significance ($P = 0.057$) for Pielou's Evenness, and there was no effect of treatment on Faith's Phylogenetic Diversity Index (Supplemental Table 2; $P > 0.05$). Thus, samples from trial 1 were not different in evenness, richness, nor phylogenetically weighted diversity.

Although there was no interaction between treatment and time using ADONIS, there was a main effect for both treatment and time ($P < 0.05$). Therefore, main effect of treatment and subsequent pairwise differences in β diversity between groups were determined using PERMANOVA and PERMDISP. In addition, due to the reporting of the main effect of time for both Jaccard and Weighted UniFrac using ADONIS as significant ($P < 0.05$), PCoA plots were separated by time for both β diversity metrics (Figure 3). For Jaccard Dissimilarity, PERMANOVA, and PERMDISP were significant for the main effect of treatment ($P < 0.05$, Supplemental

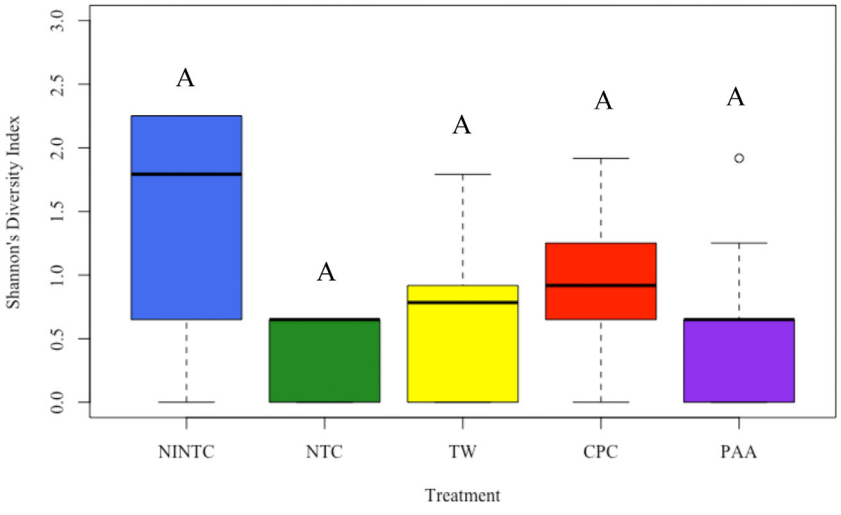

Figure 2. Trial 1 α box plot for Shannon Diversity group significance (N = 50, n = 10, k = 5, $P < 0.05$, $Q > 0.05$). Shannon Diversity, a measure of richness and evenness, was the only α metric in which the main effect was significant through Kruskal-Wallis ($P = 0.019$). However, no differences occurred between treatment groups ($Q > 0.05$). The treatments utilized were: a no inoculum, no treatment control (NINTC); a no treatment control (NTC); a tap water control (TW); PAA + TW (600 ppm); CPC + TW (5,000 ppm). Rinsate samples from NINTC thighs were different from samples from thighs from all other groups. No significant differences were found between rinsates samples from other thighs ($Q > 0.05$).

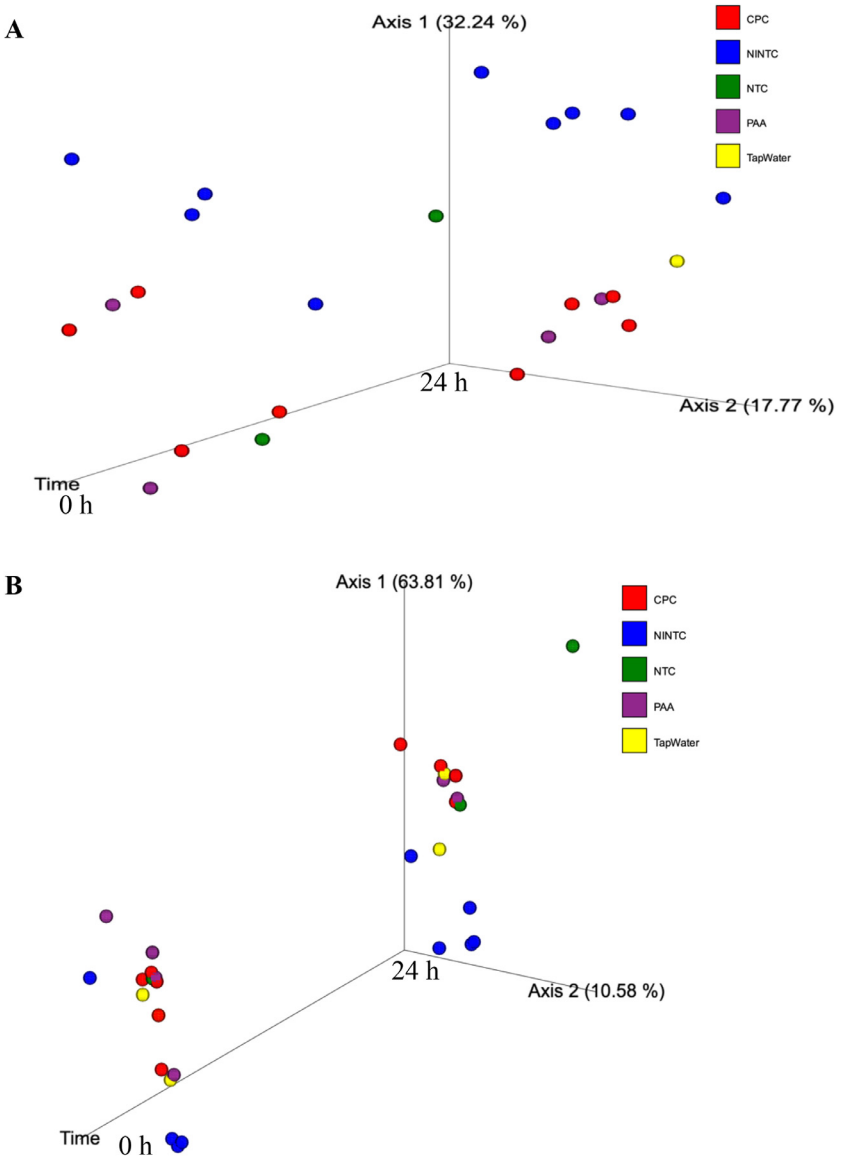

Figure 3. Trial 1 PCoA Emperor Plots of (A) Jaccard and (B) Weighted UniFrac. Because the main effect of treatment and time were significant for both metrics using ADONIS ($P < 0.05$), time (0 h and 24 h) was used as the third axis, shown by the separated clusters.

Table 2). For Weighted UniFrac, PERMANOVA was significant for the main effect ($P < 0.05$), but PERMDISP was not ($P > 0.05$). In both Jaccard Dissimilarity and Weighted UniFrac, NINTC thigh samples were different from thighs from all other groups for PERMANOVA (Q < 0.05, Supplemental Table 2). Significant differences were not found between rinsate samples from thighs of other treatment groups (Q > 0.05). Using PERMDISP, the dispersion of the data was significant between NINTC thighs and thighs from other groups through Jaccard Dissimilarity (Q > 0.05).

Trial 2

Unlike trial 1, trial 2 focused specifically on the 24 h time point to provide a more defined snapshot into the microbiota response. Since treatment was the only main effect of interest, ANOVA and ADONIS were not used for this trial. Kruskal-Wallis was instead used to determine significance of the main effect of treatment for the α diversity metrics. Treatment was significant for Pielou's Evenness and Shannon's Diversity ($P < 0.05$, Supplemental Table 3; Figure 4) but was not significant for Faith's PD ($P > 0.05$, Supplemental Table 3; Figure 4). The microbiota on noninoculated thighs (NINTC) was different in evenness and richness (Pielou's Evenness and Shannon's Diversity) from the microbiota on thighs from all other groups ($P < 0.05$). The microbiota from NTC thighs was different in evenness and richness from the microbiota on thighs from all treated thighs. There were no statistical differences in microbiota evenness and richness in thighs treated with TW, CPC, or PAA (Q > 0.05, Supplemental Table 3; Figure 4).

Pairwise differences in β diversity between treatment groups were again evaluated through PERMANOVA and PERMDISP. The main effect of treatment was significant in PERMANOVA for Jaccard Dissimilarity and Weighted UniFrac ($P < 0.05$, Figure 5;

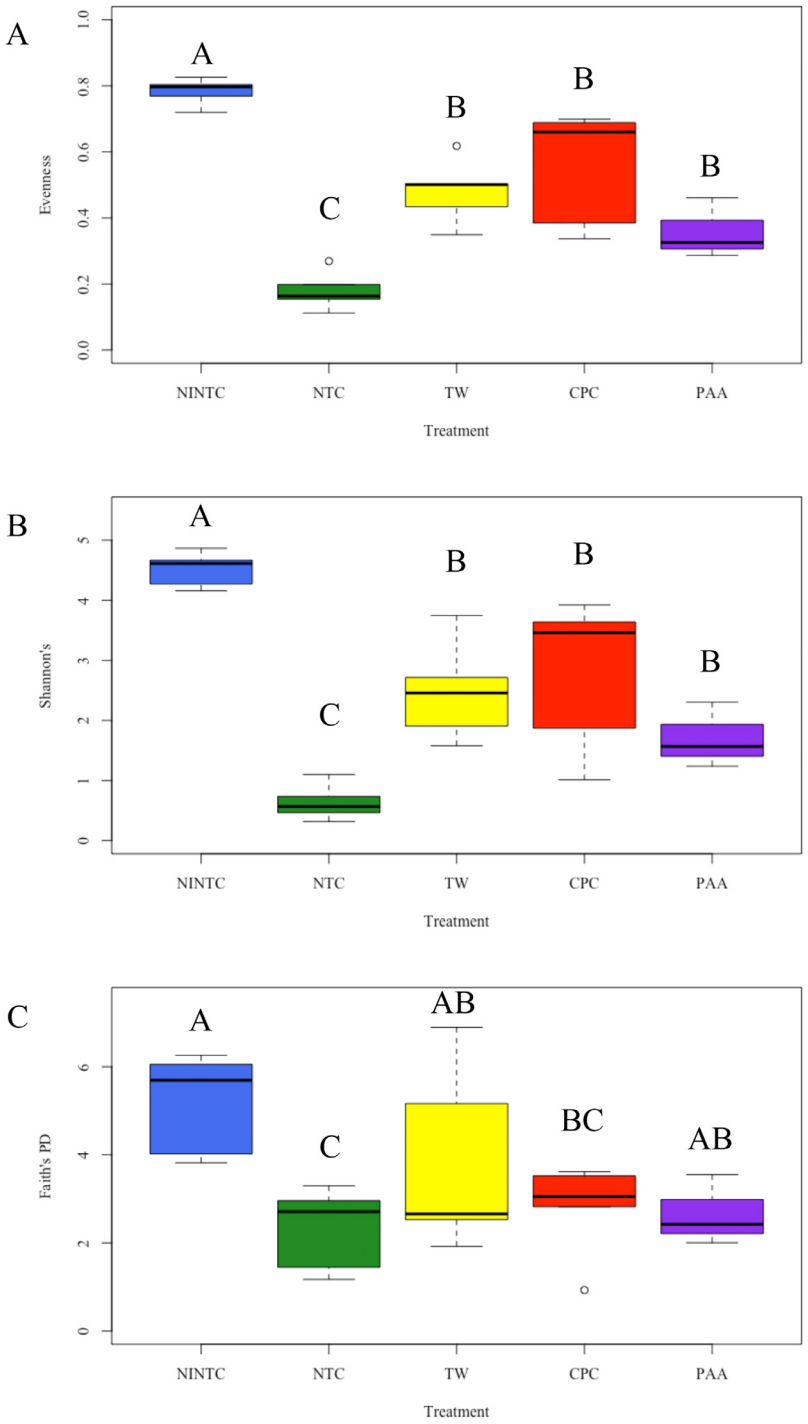

Figure 4. Trial 2 α group significance of (A) Pielou's Evenness, (B) Shannon's Diversity, and (C) Faith's PD ($P < 0.05$, N = 25, n = 5, k = 5). Pairwise comparisons are included in supplementary data. The treatments utilized were: a no inoculum, no treatment control (NINTC); a no treatment control (NTC); a tap water control (TW); PAA + TW (PAA, 600 ppm); CPC + TW (CPC, 5,000 ppm).

Supplemental Table 4). Treatment was significant for PERMDISP for Weighted UniFrac ($P < 0.05$), but not for Jaccard ($P > 0.05$). In evaluating pairwise differences through PERMANOVA, the microbiota on NINTC thighs were statistically different in Jaccard Dissimilarity to the microbiota on NTC thighs ($Q < 0.05$) but were not different from the microbiota on thighs from all other groups. The microbiota on NTC thighs were significantly different from the microbiota on thighs treated with CPC and TW ($Q < 0.05$). There were no differences in Jaccard Dissimilarity between microbiota on thighs treated with CPC, PAA, or TW ($Q > 0.05$). Because the main effect of treatment was not significant for PERMDISP in Jaccard, dispersions in the data did not account for any of these differences ($P < 0.05$). In evaluating pairwise differences through PERMANOVA, the microbiota on NINTC thighs was different in Weighted UniFrac from the microbiota on NTC thighs and the microbiota on thighs treated with PAA and TW ($Q < 0.05$). The microbiota on thighs treated with CPC and on NINTC thighs were not different ($Q > 0.05$). The microbiota on NTC thighs were different from the

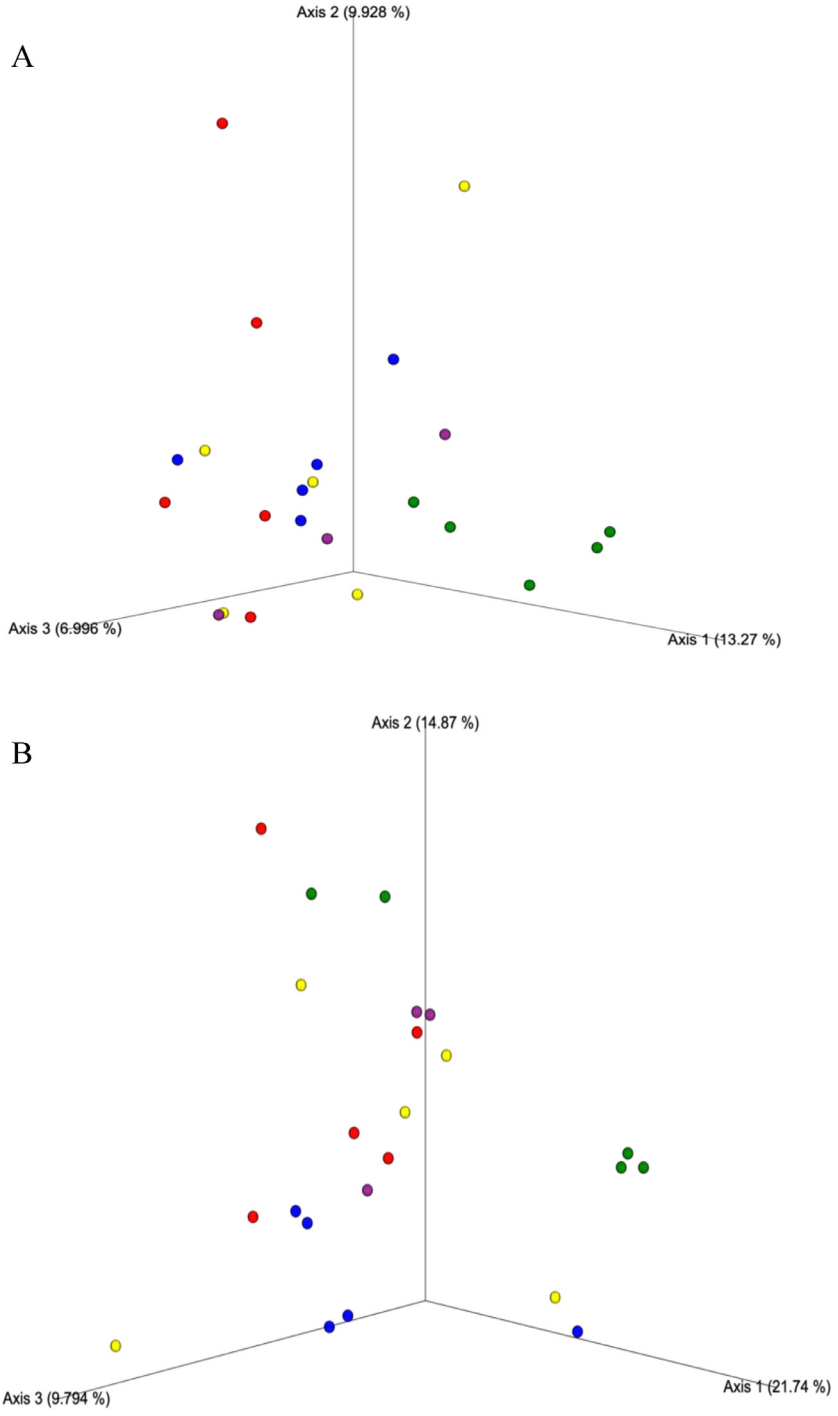

Figure 5. Trial 2 PCoA Emperor Plots of (A) Jaccard and (B) Weighted UniFrac. Treatment was significant through ADONIS for trial 2 ($P < 0.05$).

microbiota on thighs treated with CPC and TW, but not PAA. There were no significant differences in the diversities on thighs treated with TW, PAA, or CPC (Q > 0.05). The only significant pairwise differences in PERMDISP for Weighted UniFrac was between the microbiota of the NINTC treated thighs and the NTC thighs ($P < 0.05$, Q < 0.05). The pairwise differences between NINTC thighs and thighs treated with PAA, and between NTC thighs and thighs treated with CPC, were trending toward significance ($P < 0.05$, Q > 0.05).

ANCOM Compositional Diversity Analysis

Trial 1 ANCOM results revealed significant shifts due to treatment and due to treatment over time at both the phylum and genus levels ($P < 0.05$, Figures 6 and 7). At the phyla level, due to treatment and time, all taxa reported at the phylum level were significantly different ($P < 0.05$, W ≥ 0, Figure 6A). The most abundant phyla on NINTC thighs at 0 h and 24 h were *Firmicutes* ($P < 0.05$, W = 1), and the most abundant phyla on all other thighs at both time points were *Proteobacteria* ($P <$

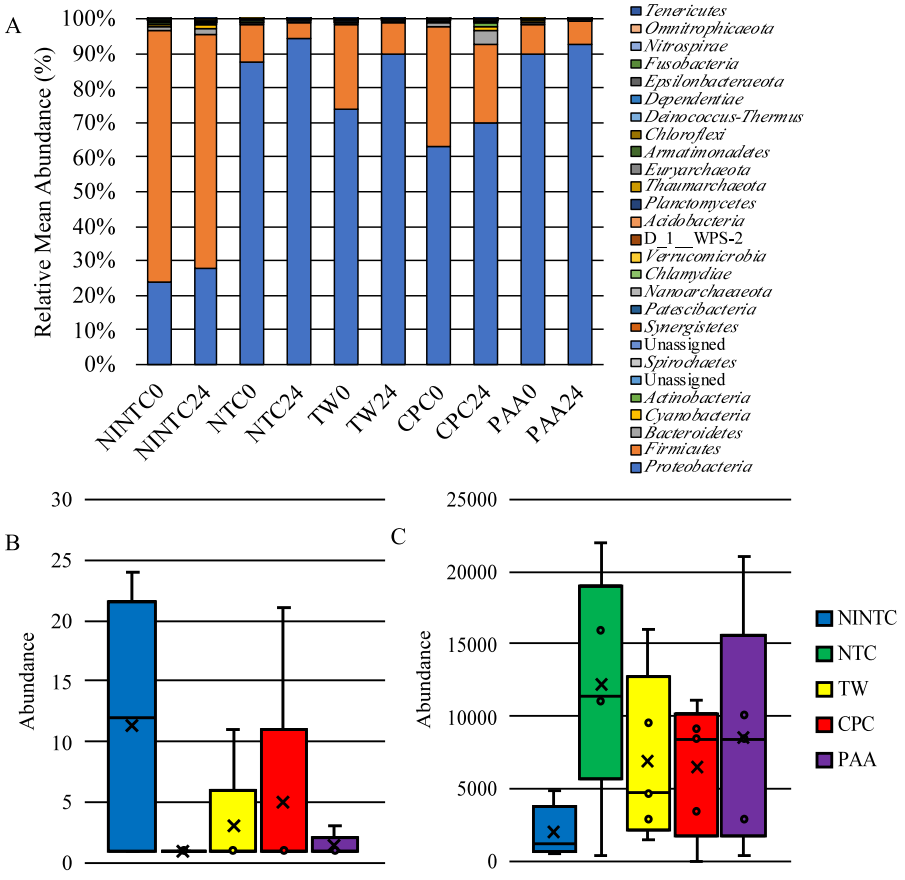

Figure 6. Significantly different taxa at the phyla level during trial 1 ($P < 0.05$, N = 50, n = 5, k = 5). (A) Interaction of treatment and time using ANCOM, in which all reported phyla were significantly different (W ≥ 0). Main effect of treatment using ANCOM, in which *Epsilonbacteraeota* (B; W = 10) and *Proteobacteria* (C; W = 7) were significantly different.

0.05, W = 2). At that same level, among the main effect of treatment, *Proteobacteria* and *Epsilonbacteriota* were significantly different ($P < 0.05$, W = 7, 10, respectively, Figure 6B; Supplemental Figure 1) in relative abundance. *Proteobacteria* ($P < 0.05$, W = 7) and *Firmucutes* ($P > 0.05$, W = 1) had the highest numerical relative abundances on all thighs, with *Firmicutes* most prevalent on NINTC thighs and *Proteobacteria* the most prevalent on all inoculated thighs. Of the inoculated thighs, those treated with CPC had the lowest numerical relative abundance of *Proteobacteria* and the highest numerical relative abundance of *Firmicutes*.

At the genus level, the only significantly different taxa were *Ruminococcaceae* UCG-014 ($P < 0.05$, W = 25, Figure 7A; Supplemental Figure 2). The NINTC thighs had the highest OTU reads of *Ruminococcaceae* UCG-014 at both time points. Thighs treated with CPC at 24 h had the highest OTU reads of *Ruminococcaceae* UCG-014 of the inoculated thighs but reads were relatively similar otherwise for inoculated thighs at both time points. Among the main effect of treatment, *Enterobacteriaceae* was significantly different in relative abundance ($P < 0.05$, W = 313, Figure 7B). The NTC thighs had the highest number of OTU reads of *Enterobacteriaceae* and thighs treated with CPC had the lowest number of OTU reads.

Trial 2 ANCOM revealed significant shifts at the phyla levels in microbiota due to treatment only. Only 4 phyla were significantly different in relative abundance: *Proteobacteria, Firmicutes, Bacteroidetes*, and a previously unassigned taxonomy ($P < 0.05$, W = 3, 4, 2, and 8, respectively; Figure 8; Supplemental Figure 3). Similar to trial 1, NINTC thighs had the lowest relative abundance of *Proteobacteria* ($P < 0.05$, W = 3) and the highest of *Firmicutes* ($P < 0.05$, W = 4). Thighs treated with CPC mirrored this pattern of relative abundances of *Proteobacteria* and *Firmicutes* as the NINTC thighs. There were no significant differences at the genus level ($P > 0.05$), therefore the order level was analyzed, and significant shifts were determined (Supplemental Figure 4). All reported taxa were significantly different at the order level ($P < 0.05$; Figure 9). Numerically, thighs treated with CPC had the lowest relative abundances of *Enterobacteriales* for the inoculated thighs ($P < 0.05$, W = 11), and greater relative abundances of *Lactobacillales* ($P < 0.05$, W = 1) and *Bacillales* ($P < 0.05$, W = 1), compared to the other inoculated thighs. Thighs treated with CPC, PAA, and TW also exhibited similar relative abundances of *Bacillales*, yet more than the NTC thighs ($P < 0.05$, W = 1).

DISCUSSION

The current study was performed to explore the impact of a 20 s application of CPC and PAA as

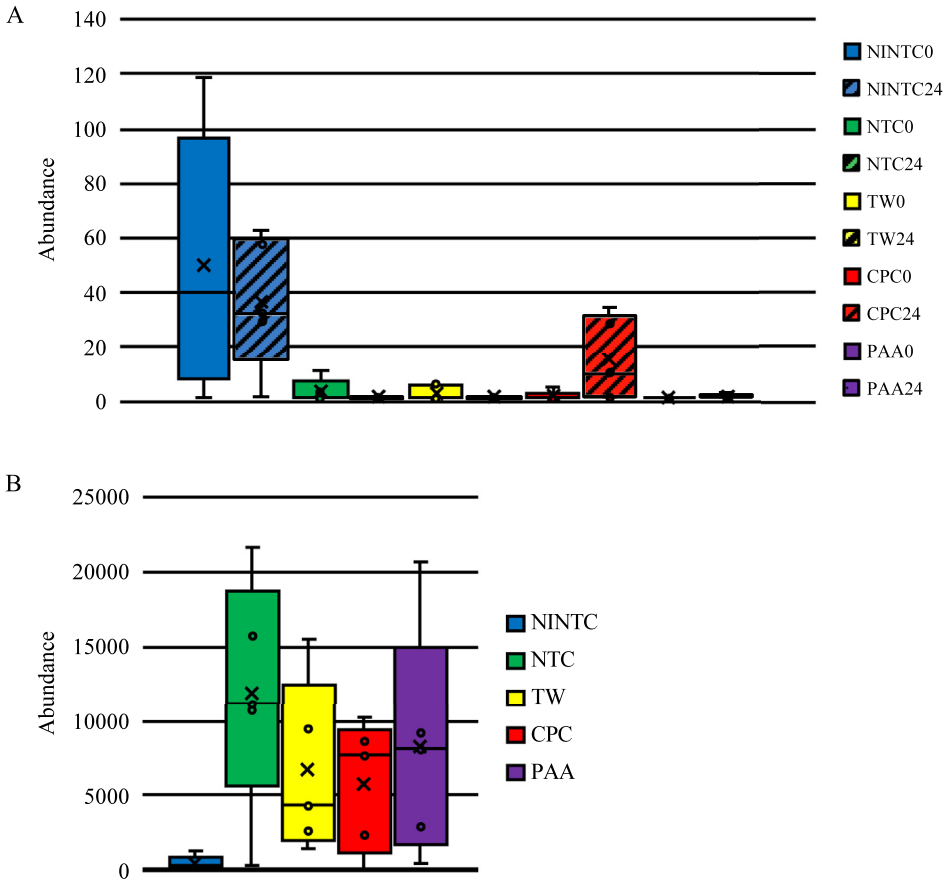

Figure 7. Significantly different taxa at the genus level during trial 1 ($P < 0.05$, N = 50, n = 5, k = 5). (A) Interaction of treatment and time using ANCOM, in which *Ruminococcaceae* UCG-014 was significantly different in relative abundance (W = 134). (B) Main effect of treatment using ANCOM, in which *Enterobacteriaceae* was significantly different in relative abundance (W = 313).

antimicrobial part dips on the microbiota of skin-on chicken thighs when inoculated with *Salmonella* Infantis. Quaternary ammonium cation surfactants such as CPC are commonly utilized across food and health industries as an antimicrobial surfactant (NCBI, 2021). Surfactants have been demonstrated to lower the surface tension of water on the skin of poultry and increase the solubility of inorganic compounds, which may decrease cell viability and the ability of bacterial cells to attach to poultry skin (Kim and Day, 2007). In the current study, when skin-on chicken thighs were treated with CPC, the reduction of *S.* Infantis seen in response to CPC was not different to that of the treatment of thighs with PAA at 0 h. However only PAA treated thighs were significantly different from NTC thighs. Nonetheless there was a possible inhibitory effect over time on thighs treated with CPC. Previous reports have suggested CPC disrupts cell viability and attachment to skin, which may explain the current study's effect showing a reduction of loads of *Salmonella* and total aerobic bacteria over time (Yegin et al., 2019). Nonetheless, it remains unknown if the current inhibitory effect occurred because the surfactant specifically prevents attachment to the skin or if the agent alters *Salmonella* adaptability.

In the current study, thighs treated with CPC and PAA were not significantly different in the reduction of *S.* Infantis or overall aerobic bacterial load at 0 h. At 0 h, the total *Salmonella* load on thighs treated with CPC was not different from NTC thighs, while thighs treated with PAA were. At 24 h thighs treated with CPC were numerically lower in total aerobic microorganisms than thighs treated with PAA, and thighs from both groups were different from the NTC thigh group. It is also interesting to note similar behavior in total *Salmonella* on thighs treated with TW and thighs treated with CPC. In the current methodology, thighs treated with CPC were also followed by a TW rinse, per 21 CFR § 173.375 (U. S. Food and Drug Administration, 2020). The mechanical rinsing control treatment did inhibit over time similar to the CPC treatment, but this behavior was not seen again in total aerobic load. Thighs treated with TW were also significantly different from NTC thighs at 24 h but were not different at 0 h. Thus, the additional mechanical rinsing of poultry parts may have then played a role in removing loosely attached *Salmonella* when treated with CPC, which would not be done in a typical processing procedure by PAA (Lillard, 1989; USDA-FSIS, 2021).

Previously reported findings are inconsistent on the effectiveness of PAA and CPC as antimicrobials applied to poultry carcasses, varying based on the method of use (i.e., dip or spray), using a water dip or a pressurized water spray after CPC treatment, concentration, and as use of meat and point of inoculation (Scott et al., 2015; Chylkova et al., 2017; Moore et al., 2017; Zhang et al.,

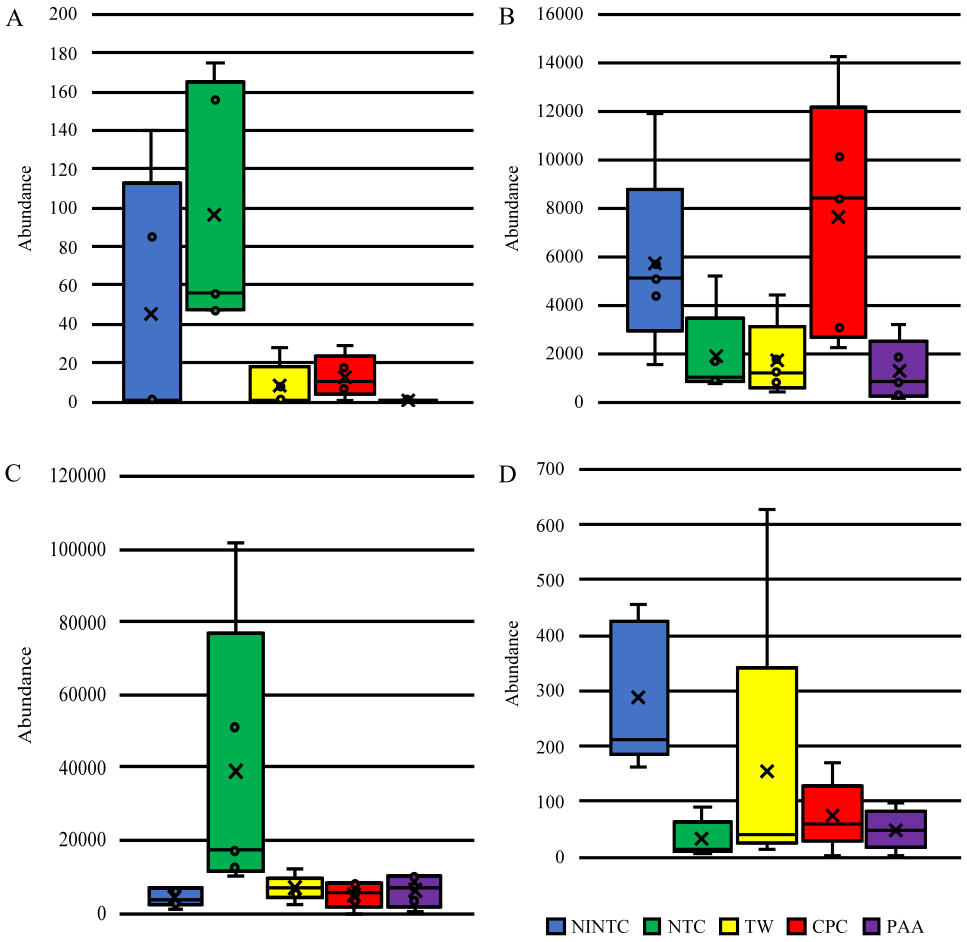

Figure 8. Significantly different taxa at the phyla level during trial 2 ($P < 0.05$, N = 25, n = 5, k = 5). Main effect of treatment using ANCOM, in which there were 4 phyla reported as significantly different: an unassigned taxonomy (A; W = 8), *Firmicutes* (B; W = 4), *Proteobacteria* (C; W = 3), and *Bacteroidetes* (D; W = 2).

2018). As such, Scott et al. (2015) determined that PAA reduced *Salmonella* over time while CPC was not different at 0 h or 24 h when chicken wings were inoculated with a five-strain *Salmonella* inoculum (Montevideo, Typhimurium, Heidelberg, Enteritidis, and Newport) and treated with either PAA or CPC. In addition, Zhang et al. (2018) found that CPC treatment in a post-

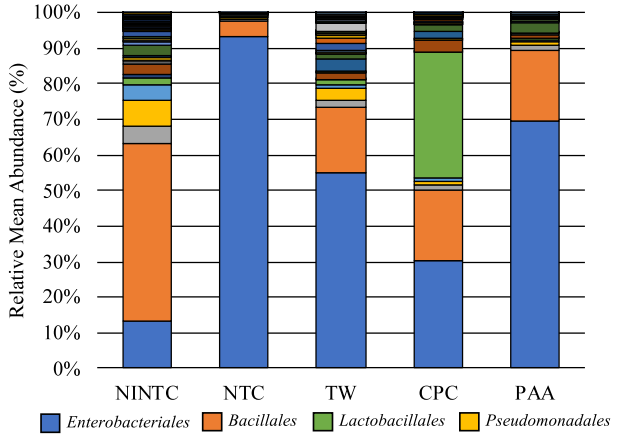

Figure 9. Significantly different taxa at the order level during trial 1 ($P < 0.05$, N = 25, n = 5, k = 5). Main effect of treatment using ANCOM, in which all reported orders were significantly different (W ≥ 0).

cut-up immersion tank at 0.35% and 0.60% reduced *Salmonella* on chicken breasts by 2.5 and 3.5 log CFU/sample and reduced *Campylobacter* by 4 and 5 log CFU/mL. Alternatively, PAA at 0.07% and 0.1% reduced *Salmonella* and *Campylobacter* by only 1.5 log CFU/mL (Zhang et al., 2018). In an in vitro simulated chiller tank for 90 min at 4°C, Chylkova et al. (2017) concluded that CPC at 2,000 ppm effectively reduced several species of *Salmonella* as well as *Escherichia coli*. However, Moore et al. (2017) found that the treatment of ground chicken frames that had been inoculated with *Salmonella* Heidelberg and *Campylobacter jejuni* with CPC (6,000 ppm or 0.6%) and PAA (1,000 ppm) resulted in a 0.9 and 1.4 $\log_{10}$ CFU/g of *Salmonella* at 24 h post-treatment. In summation, validating the best methods for utilizing CPC may be useful to explore in future studies for poultry processors to reduce pathogenic contamination successfully.

While traditional microbiological techniques have historically been utilized for pathogen detection in poultry processing, the increasing accessibility of next-generation sequencing has initiated a more in-depth approach for understanding the role of microbial ecosystems on carcasses and throughout processing plants (Rothrock et al., 2016; Kim et al., 2017; Handley et al., 2018;

Wages et al., 2019; Feye et al., 2020a). While previous research has explored shifts in carcass microbiota throughout commercial poultry processing and reported possible relationships between indigenous pathogens and nonpathogen microbial taxa present on poultry meat and skin, these studies stop short of pathogen inoculation (Kim et al., 2017; Handley et al., 2018; Wages et al., 2019). In fact, the inoculation of chicken thighs at such a level in the current study, although providing valuable information, impeded the background microbiota from behaving more naturally as what would be seen in a commercial scenario. There was a bottleneck of OTU reads across trials because of inoculation, especially since the current sequencing pipeline could not quantify the ecological differences of the microbiota and are instead presented on a relative basis (Feye et al., 2020a). This is evident by the differences in significance between trials for α and β diversity.

Trial 1 Shannon's Diversity was significant for the main effect but not for any pairwise differences (Figure 2, Supplemental Table 1). Pielou's Evenness was also trending toward significance for the main effect (Supplemental Table 1). Shannon's Diversity measures species richness and evenness, or the number of species and their abundances, within samples and then compares these diversities between samples (Pielou, 1966). Differences occurring between the noninoculated and inoculated thighs were expected as those inoculated had a higher microbial count due to the loosely attached microorganisms on the thighs (Lillard, 1989). Although differences in Shannon's Diversity were trending toward significance, especially between NTC and NINTC treated thighs, the overall species richness and evenness between the groups were not significantly different, revealing the background microbiota were similar in richness and evenness. The lack of significance for Faith's PD (Supplemental Table 1), which weighs ecological differences phylogenetically, is further evidence of the background microbiota having similar taxonomical distribution between thighs during this trial and may explain why time was not significant. For trial 2, there were more significant differences in α diversity between groups at 24 h. The noninoculated thighs were different in all three diversity metrics from inoculated thighs, with the exception of TW-treated thighs through Faith's PD (Figure 4, Supplemental Table 3). Similarly, NTC thighs were different from all other thighs (inoculated or not) in all diversity metrics, except for thighs treated with CPC through Faith's PD (Figure 4, Supplemental Table 3). By 24 h post-treatment, the microbiota may have stabilized as the loosely attached microorganisms and dead cells may not have been as present in the rinsates, explaining the significant differences in trial 2 that were not as evident in the first trial (Lillard, 1989).

Similarly, β diversity results varied between trials. For trial 1, NINTC thighs were different in Jaccard and Weighted UniFrac from all inoculated thighs, but there were no differences between inoculated groups (Figure 3, Supplemental Table 2). For trial 2, there were differences in Jaccard between noninoculated thighs and only NTC thighs. For Weighted UniFrac, the noninoculated thighs were different from all other inoculated thighs except for thighs treated with CPC. Furthermore, there were significant differences between NTC thighs and thighs treated with tap water and thighs treated with CPC for Jaccard and Weighted UniFrac. As previously stated, the mechanical rinsing due to tap water for both treatments may have more successfully removed loosely attached cells from the chicken parts, and by 24 h post-treatment this effect was more apparent for background microbiota. The differences in the current results by looking at treatment and time versus only looking at the 24 h time point between the 2 trials may provide insight for future microbiome shelf-life studies. By using sequencing analysis pipelines in multiple ways, future studies may gain a multitude of insights that following only one pipeline may not otherwise give.

The use of ANCOM provides the ability to quantitatively evaluate relative differences in composition due to treatment and over time. The current research looked at variations in taxa at the phylum, order, and genus levels. Because this research was looking at shifts in the microbiota of inoculated pieces, an overwhelming proportion of OTU reads from the inoculated *Salmonella* species dominated the results, which is apparent in the current ANCOM results. There was background *Proteobacteria* (and subsequently *Enterobacteriaceae*) on NINTC thighs during both trials; however, the influence on comparisons of taxon due to inoculation is apparent on inoculated thighs. Nonetheless, thighs treated with CPC had greater relative diversities in OTUs of indigenous microbial taxa during both trials and time points than the other inoculated thighs, including the greatest relative abundance of *Firmicutes* and the subsequent orders within.

During trial 1, there was a significant difference in relative abundance of *Enterobacteriaceae* when evaluating treatment only. Figure 6A shows a similar change in numerical abundance of OTU reads of *Enterobacteriaceae* as shown in the microbiological data (Figure 1A), suggesting treatment and the inoculated *S.* Infantis influenced these reads. It is interesting to note a wider distribution of OTU reads of *Enterobacteriaceae* due to PAA treatment compared to CPC treatment (Figure 6A). Alternatively, ANCOM results for treatment by time for this trial revealed *Ruminococcaceae* UCG-014 as the only significantly different genus. Interestingly, thighs treated with CPC at 24 h had the highest number of OTU reads of this taxon of the inoculated thighs.

By analyzing the 24 h time point only, the results of trial 2 were advantageous in revealing the effects of treatment on background taxa as the background microbiota may have stabilized by this time point as revealed by the α and β diversity results. Furthermore, because current sequencing analysis techniques cannot differentiate the DNA of live, recoverable, or dead bacterial cells (Feye et al., 2020a), the 0 h time point samples during trial 1 may have contributed to the lack of significance

between α and β diversity metrics. In addition, during trial 2, of the significantly different taxa determined through ANCOM at the order level, thighs treated with CPC had the lowest abundance of *Enterobacteriales* in relation to the abundances of background taxa such as *Lactobacillales* (Supplemental Figure 4). Also, thighs treated with CPC, PAA, and TW had greater relative abundances of *Bacillales* in relation to other taxa than the NTC thighs. The inoculation of thighs with *Salmonella* Infantis also resulted in a high relative abundance of *Enterobacteriales* among NTC thighs and those treated with PAA. Nonetheless, thighs treated with CPC or TW had greater relative abundances of background indigenous microorganisms despite inoculation. It is also interesting to note that there was a 1.2 $\log_{10}$ CFU/mL numerical difference between the NINTC thighs at 0 and 24 h post-treatment. This numerical difference is not typical of shelf-life; however, these time points were not significantly different as seen in Figure 1B. The lack of difference is most likely due to the distribution of the data which may be attributed to the background microbiota present on the obtained thighs. Due to the overwhelm of *Salmonella* in the sequencing pipeline, it was not possible to explore the endogenous microbiota thoroughly. Further explorations into inoculated carcass microbiota through shelf-life studies may provide more insight into the impact CPC may have had in these results.

CONCLUSIONS

The current study revealed a possible over-time inhibitory effect on *Salmonella* Infantis when treated with CPC that was not observed among the thighs treated with PAA. The treatment of chicken thighs with CPC resulted in an increased reduction in *Salmonella* over time (0 and 24 h), while thighs treated with PAA remained static. Further studies assessing skin invasion and attachment by using transcriptomic or reverse transcriptase-qPCR analyses to explore CPC use on *S.* Infantis and other *Salmonella* strains may provide more insight into the exact roles of cationic surfactants inhibiting *Salmonella* species. These assays can further explore cellular behavior and responses, including genetic up- and down-regulations, which the current study did not assess.

Treatment of thighs with CPC also allowed the microbiota to overcome the bottleneck of *Proteobacteria* and *Enterobacteriales* that was most likely due to the inoculation of thighs with *Salmonella* Infantis. As a limitation of short-read sequencing, the current sequencing pipelines cannot quantitatively describe population differences between specific taxa or define species (Grim et al., 2017; Feye et al., 2020a). This limitation is especially problematic for enteric pathogens such as *Salmonella enterica* serovars, as they have few copies of the 16S region and are similar to other enteric species such as *E. coli* (Grim et al., 2017). Despite this, the current results show significant shifts due to treatment. As sequencing technologies and analysis pipelines become more refined and quantitative, exploring indigenous microbiota in response to pathogenic microbial presence may help provide better insight into indicator taxa in addition to traditional microbiological data.

Finally, all parts used for this study were gathered from an outside processing facility, setting no control measures regarding the feed, farms, flocks, trucks, or processing day. Variations in skin microbiota can depend on many factors and are not limited to antimicrobial treatment. The current results reveal that only one or 2 trials may provide a small snapshot into the skin microbiota, as several confounding variables may exist in the pipeline between the farm and the processing plant. In the future, it will be essential to repeat these types of studies over multiple trials with sufficient replication for more in-depth analyses.

ACKNOWLEDGMENTS

The authors thank Hayley Thompson and Madison Looper for their technical assistance on this project. The authors would also like to thank Safe Foods for its support and assistance in completing this research.

DISCLOSURES

Lindsey Mehall Perry is employed by Safe Foods. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101409.

REFERENCES

Anderson, M. J. 2001. A new method for nonparametric multivariate analysis of variance. Austral. Ecol. 26:32–46.

Aviv, G., K. Tsyba, N. Steck, M. Salmon-Divon, A. Cornelius, G. Rahav, G. A. Grassl, and O. Gal-Mor. 2014. A unique megaplasmid contributes to stress tolerance and pathogenicity of an emergent Salmonella enterica serovar Infantis strain. Environ. Microbiol. 16(4):977–994, doi:10.1111/1462-2920.12351 In press.

Baldry, M. G. C. 1983. The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid. J. Appl. Bacteriol. 54:417–423.

Bokulich, N. A., B. D. Kaehler, J. R. Rideout, M. Dillon, E. Bolyen. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6:90.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37:852–857.

Cadena, M., T. Kelman, M. L. Marco, and M. Pitesky. 2019. Understanding antimicrobial resistance (AMR) profiles of Salmonella biofilm and planktonic bacteria challenged with disinfectants commonly used during poultry processing. Foods 8:275.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-Resolution sample inference from Illumina amplicon data. Nat. Methods 13:581–583, doi:10.1038/nmeth.3869.

Center for Disease Control and Prevention (CDC). 2019a. *Salmonella*. Accessed May 11, 2021. https://www.cdc.gov/salmonella/index.html

Center for Disease Control and Prevention (CDC). 2019b. Outbreak of multidrug-resistant *Salmonella* infections linked to raw chicken products. Accessed May 11, 2021. https://www.cdc.gov/salmonella/infantis-10-18/index.html

Chylkova, T., M. Cadena, A. Ferreiro, and M. Pitesky. 2017. Susceptibility of *Salmonella* biofilm and planktonic bacteria to common disinfectant agents used in poultry processing. J. Food Protect. 80:1072–1079.

Dittoe, D. K., J. A. Atchley, K. M. Feye, J. A. Lee, C. J. Knueven, and S. C. Ricke. 2019a. The efficacy of sodium bisulfate salt (SBS) alone and combined with peracetic acid (PAA) as an antimicrobial on whole chicken drumsticks artificially inoculated with *Salmonella* Enteritidis. Front. Vet. Sci. 6:6.

Dittoe, D. K., K. M. Feye, B. Peyton, D. Worlie, M. J. Draper, and S. C. Ricke. 2019b. The addition of Viriditec^{TM} aqueous ozone to peracetic acid as an antimicrobial spray increases air quality while maintaining *Salmonella* Typhimurium, non-pathogenic *Escherichia coli*, and *Campylobacter jejuni* reduction on whole carcasses. Front. Microbiol. 9:3180.

Dong, Y., A. L. Iniquez, B. M. M. Ahmer, and E. W. Triplett. 2003. Kinetics and strain specificity of rhizosphere and endophytic colonization by enteric bacteria on seedlings of *Medicago sativa* and *Medicago truncatula*. Appl. Environ. Microbiol. 69:1783–1790.

Faith, D. P., P. R. Minchin, and L. Belbin. 1987. Compositional dissimilarity as a robust measure of ecological distance. Vegetatio 69:57–68.

Feye, K. M., D. R. Thompson, M. J. Rothrock Jr., M. H. Kogut, and S. C. Ricke. 2020a. Poultry processing and the application of microbiome mapping. Poult. Sci. 99:678–688.

Feye, K. M., A. C. Micchichi, P. M. Rubinelli, C. J. Knueven, D. R. Thompson. 2020b. The effect of acid sanitizers on the microbiome of re-use water. Front. Sustain. Food Syst. 4:85.

Finstad, S., C. A. O'Bryan, J. A. Marcy, P. G. Crandall, and S. C. Ricke. 2012. *Salmonella* and broiler processing in the United States: relationship to foodborne salmonellosis. Food Res. Int. 45:789–794.

Foster, J. W., and H. K. Hall. 1991. Inducible pH homeostasis and the acid tolerance response of *Salmonella* typhimurium. J. Bacteriol. 173:5129–5135.

Garcia-del Portillo, F., J. W. Foster, and B. B Finlay. 1993. Role of acid tolerance response genes in *Salmonella* Typhimurium virulence. Infect. Immun. 61:4489–4492.

Grim, C. J., N. Daquigan, T. S. Lusk Pfefer, A. R. Ottesen, J. R. White, and K. G. Jarvis. 2017. High-resolution microbiome profiling for detection and tracking of *Salmonella enterica*. Front. Microbiol. 8:1587.

Handley, J. A., S. H. Park, S. A. Kim, and S. C. Ricke. 2018. Microbiome profiles of commercial broilers through evisceration and immersion chilling during poultry slaughter and the identification of potential indicator microorganisms. Front. Microbiol. 9:345, doi:10.3389/fmicb.2018.00345.

Henchion, M., M. McCarthy, V. C. Resconi, and D. Troy. 2014. Meat consumption: Trends and quality matters. Meat Sci. 98(3):561–568, doi:10.1016/j.meatsci.2014.06.007. In press.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical J. 50:346–363.

Jaccard, P. 1912. The distribution of the flora in the Alpine Zone. New Phytologist 11:37–50.

Jolivet-Gougeon, A., F. Sauvager, M. Bonnaure-Mallet, R. R. Colwell, and M. Cormier. 2006. Virulence of viable but non-culturable *S.* Typhimurium LT2 after peracetic acid treatment. Inter. J. Food Microbiol. 112:147–152.

Kalaba, V., B. Golic, Ž. Sladojević, and D. Kalaba. 2017. Incidence of *Salmonella* Infantis in poultry meat and products and the resistance of isolates to antimicrobials. IOP Conf. Ser.: Earth Environ. Sci. 85:012082 (Abstr.).

Kieboom, J., and T. Abee. 2006. Arginine-dependent acid resistance in *Salmonella enterica* serovar Typhimurium. J. Bacteriol. 188:5650–5653.

Kim, S. A., S. H. Park, S. I. Lee, C. M. Owens, and S. C. Ricke. 2017. Assessment of chicken carcass microbiome responses during processing in the presence of commercial antimicrobials using a next generation sequencing approach. Sci. Rep. 7:43354, doi:10.1038/srep43354.

Kim, D., and D. F. Day. 2007. A biocidal combination capable of sanitizing raw chicken skin. Food Control 18:1272–1276.

Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina Sequencing Platform. Appl. Environ. Microbiol. 79(17):5112–5120, doi:10.1128/AEM.01043-13.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest package: tests in linear mixed effects models. J. Stat. SoftW 82:1–26.

Lenth, R. V. 2021. Emmeans: Estimated Marginal Means, aka Least-Squares Means. R packages version 1.5.4. Accessed April 14, 2021. https://CRAN.R-project.org/package=emmeans.

Lianou, A., G.-J. E. Nychas, and K. P. Koutsoumanis. 2017. Variability in the adaptive acid tolerance response phenotype of *Salmonella enterica* strains. Food Microbiol. 62:99–105.

Lillard, H. S. 1989. Factors affecting the persistence of *Salmonella* during the processing of poultry. J. Food Prot. 52:829–832.

Lin, J., I. S. Lee, J. Frey, J. L. Slonczewski, and J. W. Foster. 1995. Comparative analysis of extreme acid survival in *Salmonella typhimurium, Shigella flexneri*, and *Escherichia coli*. J. Bacteriol. 177:4097–4104.

Lozupone, C., and R. Knight. 2005. UniFrac: A new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71(12):8228–8235, doi:10.1128/AEM.71.12.8228-8235.2005.

Mandal, S., W. Van Treuren, R. A. White, M. Eggesbø, R. Knight, and S. D. Peddada. 2015. Analysis of composition of microbiomes: a novel method for studying microbial composition. Microb. Ecol. Health Dis. 26:27663, doi:10.3402/mehd.v26.27663.

Mcdonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. Desantis. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 6:610–618.

Morente, E. O., M. A. Fernández-Fuentes, M. J. G. Burgos, H. Abriouel, R. P. Pulido, and A. Gálvez. 2013. Biocide tolerance in bacteria. Int. J. Food Microbiol. 162:13–25.

Moore, A., R. Nannapaneni, A. Kiess, and C. S. Sharma. 2017. Evaluation of USDA approved antimicrobials on the reduction of *Salmonella* and *Campylobacter* in ground chicken frames and their effect on meat quality. Poult. Sci. 96:2385–2392.

National Center for Biotechnology Information (NCBI). 2021. PubChem compound summary for CID 31239, cetylpyridinium chloride. Accessed May 11, 2021. https://pubchem.ncbi.nlm.nih.gov/compound/Cetylpyridinium-chloride.

National Chicken Council (NCC). 2020. Per capita consumption of poultry and livestock, 1965 to estimated 2019, in pounds. Accessed May 11, 2021. https://www.nationalchickencouncil.org/statistic/per-capita-consumption-poultry/

North American Meat Institute (NAMI). 2020. The United States meat industry at a glance. Accessed May 11, 2021. https://www.meatinstitute.org/index.php?ht=d/sp/i/47465/pid/47465.

Olson, E. G., L. A. Wythe, D. K. Dittoe, K. M. Feye, and S. C. Ricke. 2020. Application of Amplon in combination with peroxyacetic acid for the reduction of nalidixic acid−resistant *Salmonella* Typhimurium and *Salmonella* Reading on skin-on, bone-in tom turkey drumsticks. Poult. Sci. 99:6997–7003.

Pielou, E. C. 1966. Shannon's formula as a measure of specific diversity: its use and misuse. The Am. Nat. 100:463–465.

Price, M. N., P. S. Dehal, and A. P. Arkin. 2010. FastTree 2 - Approximately maximum-likelihood trees for large alignments. PLOS ONE 5(3), doi:10.1371/journal.pone.0009490. In press.

Rothrock, M. J., A. Locatelli, T. C. Glenn, J. C. Thomas, A. C. Caudill. 2016. Assessing the microbiomes of scalder and chiller tank waters throughout a typical commercial poultry processing day. Poult. Sci. 95:2372–2382.

RStudio Team. 2020. RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA. http://www.rstudio.com/.

Ryan, D., N. B. Pati, U. K. Ojha, C. Padhi, S. Ray. 2015. The acid tolerance response of *Salmonella* Typhimurium: global

transcriptome and mutagenic analysis. Appl. Environ. Microbiol. 81:8054–8065.

Scott, B. R., X. Yang, I. Geornaras, R. J. Delmore, D. R. Woerner. 2015. Antimicrobial efficacy of a sulfuric acid and sodium sulfate blend, peroxyacetic acid, and cetylpyridinium chloride against *Salmonella* on inoculated chicken wings. J. Food Protect. 78:1967–1972.

Szmolka, A., M. Szabó, J. Kiss, J. Pászti, E. Adrián. 2018. Molecular epidemiology of the endemic multiresistance plasmid pSI54/04 of Salmonella Infantis in broiler and human population in Hungary. Food Microbiol. 71:25–31.

Tan, S. M., S. M. Lee, and G. A. Dykes. 2014a. Buffering effect of chicken skin and meat protects *Salmonella enterica* strains against hydrochloric acid but not organic acid treatment. Food Control. 42:329–334.

Tan, S. M., S. M. Lee, and G. A. Dykes. 2014b. Fat contributes to the buffering capacity of chicken skin and meat but enhances the vulnerability of attached *Salmonella* cells to acetic acid treatment. Food Res. Int. 66:417–423.

United States Department of Agriculture- Federal Safety Inspection Service (USDA-FSIS). 2013. Salmonella and Campylobacter verification program for raw meat and poultry products. FSIS Directive 10(250.1). Accessed May 11, 2021. https://www.fsis.usda.gov/sites/default/files/media_file/2021-03/10250.1_0.pdf.

United States Department of Agriculture- Federal Safety Inspection Service (USDA-FSIS). 2020. List of approved on line processing (OLR) and off line reprocessing (OFLR) antimicrobial systems for poultry. Accessed May 11, 2021. https://www.fsis.usda.gov/wps/wcm/connect/4788a166-513f-432c-9e00-f3bcec646558/olr-oflr-tables.pdf?MOD=AJPERES.

U. S. Food and Drug Administration. 2020. Cetylpyridinium chloride, 21 CFR part 173.375

United States Department of Agriculture- Federal Safety Inspection Service (USDA-FSIS). 2021. Safe and suitable ingredients used in the production of meat, poultry, and egg products. Accessed July 15, 2021. https://www.fsis.usda.gov/sites/default/files/media_file/2021-02/7120.1_table_2.pdf.

Wages, J. A., K. M. Feye, S. H. Park, S. A. Kim, and S. C. Ricke. 2019. Comparison of 16S rDNA next sequencing of microbiome communities from post-scalder and post-picker stages in three different commercial poultry plants processing different classes of broilers. Front. Microbiol. 10:972, doi:10.3389/fmicb.2019.00972.

Yegin, Y., J. K. Oh, M. Akbulut, and T. Taylor. 2019. Cetylpyridinium chloride produces increased zeta-potential on *Salmonella* Typhimurium cells, a mechanism of the pathogen's inactivation. N. P. J. Sci. Food 3, doi:10.1038/s41538-019-0052-x.

Zhang, L., L. J. Garner, S. R. Mckee, and S. F. Bilgili. 2018. Effectiveness of several antimicrobials used in postchill decontamination tank against *Salmonella* and *Campylobacter* on broiler carcass parts. J. Food Prot. 81:1134–1141.