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Original Research Article

REDUCTION OF *CAMPYLOBACTER* ON POULTRY THIGHS USING SEQUENTIAL TREATMENTS OF ANTIMICROBIALS

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Running Title: Sequential Dips to Reduce *Campylobacter*

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Abstract

Campylobacter is a major concern for poultry processors, as USDA performance standards have become stricter. This study evaluated CMS PoultrypHresh™, a low pH processing aid, and peracetic acid using consecutive and sequential dip treatments to reduce *Campylobacter* in thighs. Thighs (n=3/treatment group) were inoculated with a *C. coli* marker strain (10^8) and each dipped into bags containing 1 L of treatment 1 for 6 s. Thighs were allowed 5 s to drip, placed onto foil for 60 s, and dipped into treatment 2 for 6 s. After 5 s drip time, each was placed in a bag with 150 mL buffered peptone water and hand shaken for 60 s; controls same procedure, no treatment. Rinsates were serially diluted, plated onto Campy Cefex agar with 200 ppm gentamicin and incubated microaerobically for 48 h at 42°C. Procedures were replicated 5 times. Significant reductions compared to untreated using consecutive dips of PoultrypHresh™ and PAA were 98.2% and 99.3%, respectively. Treatments of PoultrypHresh™ then peracetic acid reduced *Campylobacter* 99.2% from untreated thighs. Peracetic acid then PoultrypHresh™ showed significant reductions compared to all other treatments (99.9% from untreated). Treating with this sequence may allow processors to meet the strict performance standards on *Campylobacter* in broiler parts.

Introduction

Campylobacter is the main cause of bacterial gastroenteritis in the world and researchers have shown it to be present in high levels in retail poultry. [1],[2] Of *Campylobacter* illnesses, 50 - 70% were caused by consumption of poultry or poultry products. [3],[4] Symptoms of *Campylobacter* include fever, abdominal pain and diarrhea within 2 - 5 days of ingesting contaminated product. [5],[6] Stricter performance standards have been implemented by the USDA, aimed at reducing incidence of *Campylobacter* in processing facilities. [7] FSIS estimated 46% of poultry facilities would not be able to meet new requirements; therefore, processors must give serious attention to finding efficient antimicrobial treatments to be used throughout the plant. It is mandatory *Campylobacter* prevalence remain below 7.7% on poultry parts (4 of 52 samples), allowing only a small margin of error. [7]

A variety of antimicrobials have been evaluated for use on poultry at various points

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throughout processing procedures. It is essential chemicals be cost effective, reduce pathogen prevalence and not cause organoleptic damage to poultry carcasses or parts. [8] Antimicrobials vary in levels of efficacy, treatment concentration, contact time and application method. Chemical efficacy is affected by microbial load, composition of flora, organic material or possibly changed from residual effects of prior treatments. [9] Stopforth et al. [10] determined a single intervention could not significantly reduce pathogen presence on finished carcasses. Instead, a multi-hurdle approach is required at various intervention points throughout processing for increased pathogen reduction on poultry products. [10],[11] There was no previous research found evaluating consecutive, sequential treatments and how they affect pathogens. The purpose of this study is to evaluate the effectiveness of a low acid antimicrobial, CMS PoultrypHresh™, and whether dipping carcasses in consecutive or sequential treatments along with peracetic acid (PAA) leads to further reductions in the prevalence of *Campylobacter* on poultry thighs.

Materials and Methods

Bacterial Marker Strain

Cox et al. [12] developed a marker strain of *Campylobacter* resistant to the antibiotic gentamicin, *Campylobacter coli* (CC^{GR}), which was used in this study. Campy Cefex agar (Sigma, St. Louis, MO) plates were prepared using 200 ppm gentamicin (CCGen) to eliminate the growth of wild type *Campylobacter* that may be naturally present on poultry thighs. Initial cultures were streaked from storage in an -80°C freezer, maintained in Bolton's broth with 15% glycerol and no supplements. Cultures were streaked onto CCGen agar and incubated in sealed bags under microaerobic (5% O_2, 10% CO_2, 85% N_2) conditions at 42°C for 48 h. A sterile swab was used to remove colonies and mix them into a 9 mL tube of Bolton's broth, placed in sealed bags and incubated microaerobically for 24 h at 42°C. Tubes were evaluated for colorimeter analysis by a Spectronic 200E (Thermo Fisher Scientific, Madison, WI) and approximately a 10^8 cfu/mL CC^{GR} inoculum was prepared. Inocula were confirmed using serial dilutions, plated on CCGen and incubated for 48 h at 42°C.

Treatment Procedures

Skin-on poultry thighs were purchased from a local grocery (n=18) and divided into the six treatment groups. A 6.5 cm^2 section of skin was inoculated on each thigh by spreading 0.1 mL of 10^8 CC^{GR} marker strain. Each thigh was inoculated individually and given a 5 min attachment period before treatment. Thighs were individually dipped into Ziploc bags containing either 1 liter of water, PAA (600 ppm), or PoultrypHresh (pH 1.5) for 6 s. Thighs were removed and placed on tin foil squares for 60 s. Each thigh was then dipped into the second dip treatment for 6 s, drained 5 s, placed into individual sealable bags with 150 mL buffered peptone water (BPW), and hand shaken and massaged for 60 s. Thighs were removed, and rinses placed on ice for approximately 15 min before diluting. Three thighs served as controls in each replication and were inoculated as described, remained untreated and placed directly into rinse bags with BPW after the attachment period. The entire experiment was replicated 5 times.

Plating and Incubation

Rinsates were serially diluted and a plate spreader used to disperse onto CCGen agar plates in duplicate. Plates were incubated at 42°C for 48 h. Characteristic colonies were counted, and cfu/mL log transformed.

Statistical Analysis

The study was constructed of five replicates of skin-on thighs using 18 individual thighs (N=18; n=3). Each replicate consisted of 3 of each untreated, consecutive water dips, consecutive PoultrypHresh™ (pH 1.5) dips, consecutive PAA (600 ppm) dips, PoultrypHresh™ followed by PAA dip, and PAA followed by PoultrypHresh™ dip. Duplicate counts were averaged, and numbers were transformed by $\log_{10}$. Data was analyzed using Statistica software (Statistica, 2013). A General Linear Model was conducted to determine whether sequential dips were statistically different ($P < 0.05$). Means were separated with a Tukey Multiple Comparison test; statistical significance was assigned at $P \leq 0.05$.

RESULTS

Consecutive water dips were used to determine rinsing effect of water alone. A 0.8 $\log_{10}$ cfu/mL *Campylobacter* reduction was observed, although contamination remained high at 4.9 $\log_{10}$ cfu/mL (Table 1). Treating with consecutive PoultrypHresh™ dips significantly ($P \leq 0.05$) reduced *Campylobacter* from 5.64 $\log_{10}$ cfu/mL untreated and 4.87 $\log_{10}$ cfu/mL water dipped to 3.90 $\log_{10}$ cfu/mL (Table 1). This equates to a reduction of 98.2% from untreated and 89.3% from water dipped thighs (Table 1). Dipping with consecutive PAA dips showed slightly higher reductions; reducing *Campylobacter* 99.3% (2.1 $\log_{10}$ cfu/mL) from untreated and 95.7% (1.4 $\log_{10}$ cfu/mL) from water dipped samples (Table 1).

Dipping in PoultrypHresh™ followed by PAA demonstrated findings similar to consecutive PAA dips. Interestingly, thighs dipped in PAA followed by PoultrypHresh™, showed reductions significantly ($P \leq 0.05$) greater than any other dipping combination. *Campylobacter* was reduced 99.9% (> 3 $\log_{10}$ cfu/mL reduction) from untreated thighs and 99.6% (2.4 $\log_{10}$ cfu/mL reduction) from water treated (Table 1). This pattern was observed in all 5 replications of the study; therefore, the order of chemicals used in dipping sequences was significantly ($P \leq 0.05$) correlated to *Campylobacter* reductions.

DISCUSSION

Results can be compared to a study by Landrum et al. (2018) evaluating PoultrypHresh™ on broiler thighs, where *Campylobacter* reductions were higher compared to untreated from this study, 2.2 $\log_{10}$ cfu/mL and1.7 $\log_{10}$ cfu/mL, respectively. Comparing the water dipped thighs to untreated, the research by Landrum et al. (2018) demonstrated a 1.4 $\log_{10}$ cfu/mL reduction, whereas this study a 1.0 $\log_{10}$ cfu/mL. Differences between the two studies were the dip time, (25 s compared to two 6 s dips) and air agitation, not used in this study. Shorter dip times of 6 s were chosen for this research as a more practical time of exposure in a modern processing facility. The higher dip times used by Landrum et al. (2018) exhibited better results, seemingly due to the longer exposure time, although the effects of air agitation could have also assisted in higher reductions. The improved microbial

reductions using air agitation have been shown in previous research. [14] Therefore, air agitation is a concept that may require more research to heighten reductions of microbial contamination.

PAA is a low pH organic peroxide mixture of acetic acid and hydrogen peroxide currently being used throughout the United States in multiple processing facilities. [8],[15],[16] The mode of action for PAA is a disruption to the cell membrane permeability, altering protein synthesis, leading to bacterial death. [17] PAA reduced the presence of *Campylobacter* slightly more than consecutive PoultrypHresh™ dips, although differences were not significant. Findings by Bauermeister et al. [8] showed a 1.5 $\log_{10}$ cfu/mL *Campylobacter* reduction using an extremely low concentration of only 200 ppm PAA. As PAA is approved for use throughout processing at concentrations up to 2000 ppm, reductions could be even greater with increased acid levels. [18] Bauermeister [19] used 200 ppm PAA, only demonstrating a 1.5 $\log_{10}$ cfu/mL reduction; therefore, other factors could be associated to its efficacy. King et al. [20] determined that the effects of PAA may vary and greatly depend on bacteria level and how they are attached to the surface. Nagel et al. [21] evaluated higher levels of PAA at 400 and 1000 ppm and reduced *Campylobacter* levels by more than 2.0 $\log_{10}$ cfu/mL.

Scientists have determined a multi-hurdle approach is necessary for adequate pathogen reduction, which uses multiple intervention points throughout processing procedures such as the inside-outside bird washer, brush washer, cabinet washer, or dip tank before and/or after chilling. [22] Using this approach, the facility does not rely only on a single step intervention, but instead incorporates many applications for reducing foodborne pathogen prevalence prior to entering secondary processing [10],[23],[24] No research was found, however, demonstrating the usefulness of consecutive, sequential chemical hurdles for pathogen reduction. This study demonstrates the possibility that consecutive application of chemicals in a specific sequential order may reduce *Campylobacter* prevalence.

Table 1. Average Log_{10} cfu/mL of *Campylobacter coli* recovered by replicate from sequentially dip treated thighs with no treatment, water-water, PpH-PpH, PAA-PAA, PpH-PAA, or PAA-PpH (mean ± standard error).

Treatment		Average Log_{10} cfu/mL	Reduction compared to Untreated (%)	Reduction compared to Water (%)
1st Dip	2nd Dip			
Untreated		$5.64^{a} \pm 0.04$	-	-
Water	Water	$4.87^{b} \pm 0.05$	83.0	-
PpH[1]	PpH	$3.90^{c} \pm 0.06$	98.2	89.3
PAA[2]	PAA	$3.50^{d} \pm 0.08$	99.3	95.7
PpH	PAA	$3.53^{d} \pm 0.08$	99.2	95.4
PAA	PpH	$2.52^{e} \pm 0.09$	99.9	99.6

[1] PpH = CMS PoultrypHresh™
[2] PAA = peracetic acid

CONCLUSIONS

Findings from this study demonstrated PoultrypHresh™ and PAA could reduce the prevalence of *Campylobacter* on poultry parts when used separately. However, when evaluated sequentially, PAA followed by PoultrypHresh™ can significantly reduce pathogen presence. Such findings may be extremely beneficial and lead to better intervention strategies in future antimicrobial applications.

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