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Article

Reduction of Bacterial Load on Broiler Carcasses Using Low-Volume Fluidic Nozzles in Combination with 60 °C Water at 450 Psi Pressure

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Abstract: With the changing regulations in poultry processing, increased pressure is placed on integrators to reduce the number of human enteropathogenic bacteria on the final carcass and/or parts. Reducing the total number of bacteria on broiler carcasses before entering the evisceration side of the processing plant is projected to reduce the number of bacteria on the carcasses after chilling. This study was designed to evaluate the efficacy of a prototype wash cabinet using low volume, fluidic nozzles in combination with high pressure (450 psi) and hot water (60 °C) to remove bacteria from pre-scald, post-scald, or post picked carcasses. Carcasses (n = 5) from each location were obtained from a commercial processing plant, placed into individual sterile sample bags, placed into an insulated container, and transported to the U.S. National Poultry Research Center Pilot Plant within 30 min of collection. Carcasses were hung in standard shackles and sampled pre-wash with pre-moistened, cellulose swabs. All carcasses were washed in the prototype wash cabinet with 60 °C water at 450 psi at a line speed of 52 birds/minute on 15.24 cm centered shackles. Post-wash breast sponge samples were collected identical to pre-wash swabs. Buffered peptone water (**BPW**) was added, sponges stomached and serially diluted before plating onto total aerobic count (**TAC**), *Enterobacteriaceae* (**ENT**) and *Escherichia. coli* (**EC**) Petrifilm® cards. All PetriFilm® cards were incubated at 37 °C for 24 ± 2 h. After incubation, bacterial counts were recorded and converted to $\log_{10}$ CFU/swab. Samples were processed for *Campylobacter* species using the Tempo® CAM protocol. Four replications were conducted on separate dates. Paired *t*-tests were used to compare numbers recovered from breast swabs collected before and after the wash cabinet, significance reported at $p < 0.05$. Pre-scald samples had significant reductions of 2.50, 2.01, and 1.73 $\log_{10}$ colony-forming units/carcass (CFU/carcass) for TAC, Ent, and EC Petrifilm®, respectively, and a 2.21 CFU/mL reduction of *Campylobacter* species using Tempo® CAM. Post-scald, there were significant reductions of 2.09, 1.23, and 0.90 CFU/carcass for TA, Ent, and EC Petrifilm®, respectively, and a 1.14 CFU/mL reduction of *Campylobacter* species using Tempo® CAM. Post-pick, significant reductions of 0.73, 1.53, and 0.99 CFU/carcass for TA, Ent, and EC Petrifilm®, respectively, and a 0.86 CFU/carcass reduction of *Campylobacter* species using Tempo® CAM were reported. These data indicate that hot water at high pressure can reduce total bacterial load on carcasses and reduce pathogenic bacteria on carcasses prior to evisceration.

Keywords: broilers; bacterial load; *Campylobacter*; intervention strategy

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1. Introduction

In the United States, processors are under increasing pressure from regulatory agencies to reduce the prevalence of human enteropathogens on whole carcasses and cut-up parts [1].

Cross contamination of positive carcasses can occur in a commercial plant during both slaughter, evisceration, and further processing. During live production and handling, dirt and fecal matter adhere to and/or accumulate on the feet, feathers, and skin of broilers. The presence of this material is dependent on many factors: litter condition, flock health, transport conditions, weather conditions, and duration of feed/water withdrawal. Lahellec and Colin [2] determined that external contamination of broilers can increase during the last week of production and at the time of processing, with cases of birds not exhibiting *Salmonella* contamination during rearing becoming and exhibiting contamination at pre-slaughter and during initial processing. Katoula and Pandya [3] determined that broilers entering the processing plant can be highly contaminated with human enteropathogens warranting additional modifications to reduce the microbial populations on the broilers.

Campylobacter spp. is a leading cause of acute bacterial gastroenteritis in most developed countries [4]. Handling and consumption of poultry is considered to be a major source for human illnesses [5,6]. Healthy broilers often carry *Campylobacter* spp. which can contaminate carcasses during slaughter [7]. Reduction of *Campylobacter* spp. on broiler carcasses has been linked to significant decreases in associated human diseases. Lowering *Campylobacter* counts on products by 1 log unit has been estimated to decrease human risk by 50–90% [8]. Zwiefel et al. [9] demonstrated that across three abattoirs, bacterial counts were reduced at the beginning of slaughter (scalding and plucking), but only minor changes occurred in the following stages of first processing, with the counts remaining mainly consistent from plucked to chill carcass. Physical removal of *Campylobacter* spp. before the scald tank could increase the efficiency of scalding lowering levels.

Broilers enter the processing facilities transported in crates often packed 15–17 birds per section under all weather conditions which provides conditions ripe for cross-contamination. Stern et al. [10] found after transport significant increases in the levels of *Campylobacter* recovered from feathered carcasses, in some cases reaching levels of 6.8 to 8.7 $\log_{10}$ CFU/carcass. After arrival, the broilers are unloaded and shackled, stunned (either by electrical or controlled atmosphere stunning), allowing for humane slaughter, and exsanguinated (unless processed as Kosher or Halal). Mulder et al. [11] demonstrated that transmission of organisms from carcass to carcass is correlated with the external condition of the broiler: contamination of feathers and skin with dust and feces before scalding and defeathering, which is spread to succeeding carcasses during these processing operations.

Limited knowledge or research is available about the effect of interventions applied before scalding on bacterial concentrations after scalding or at the end of processing, with multiple studies looking at the effects of the scalding process itself [11–15]. The scalding process contributes to significant bacterial reductions via high temperatures and dilution effects [12,13]). Shackelford et al. [16] stated that the removal of dirt, fecal material, and fresh feces from carcasses prior to scalding should improve product quality, reduce the quantity of scald water required, and concentrate waste material in smaller volumes of effluent. To achieve this carcass cleaning, large brush systems were developed and implemented by processors to remove this material prior to the carcasses entering the scald tank [15,16]. Shackleford et al. [16] did not assay for the reduction of bacterial loads on the carcasses during the use of the brush system designed, focusing mainly on the removal of dirt and fecal material from the carcasses. Pascholewicz et al. [15] demonstrated an average reduction of only 0.3 log for both *Escherichia coli* and *Enterobacteriaceae*. In a survey of commercial poultry processing plants, Berrang and Bailey [17] were not able to demonstrate a significant reduction of *Campylobacter, E. coli*, *Salmonella*, or coliforms after the use of a pre-scald brush system. Many, if not all, of these brush systems have been removed from processing plants due to aging equipment, lack of performance, and/or maintenance issues with cleaning a brush system in constant contact with thousands of carcasses daily.

It has been theorized that the removal of fecal material and bacteria prior to entering the scald tank would reduce the overall levels of bacteria on the broiler carcasses and improve food safety [16]. To this end, the authors have developed a wash cabinet design

incorporating high pressure and high water temperatures using low-volume fluidic nozzles to remove dirt, fecal material, and fresh feces from broiler carcasses. The specific capacity of the wash cabinet will depend upon the line speed of the processing plant. The cabinet design is such that the manifolds can be adjusted to increase the dwell time and carcass coverage to be compatible with the line speeds used in the industry. The purpose of this study was to evaluate the efficacy of a prototype wash cabinet on the reduction of the bacterial loads on carcasses prior to entering the scald tank.

2. Materials and Methods

2.1. Wash Cabinet

The wash cabinet used was designed with a series of six manifolds (three per side) angled downward from entrance to exit, allowing the water to rinse the fecal matter from vent to head of the carcass. A second series of horizontal manifolds (one per side) with the nozzles aimed at the footpad/hock region of the carcass was incorporated to remove fecal material from the feet and hock areas of carcasses. Fluidic nozzles with a 40° spray angle utilizing 0.68 L per minute (lpm) at 40 psi (Spraying Systems Company, Glendale Heights, IL, USA; Patent: US10875035B2) were used. A total of 50 fluidic nozzles with a water pressure of 450 psi were used in the 8 manifolds of the cabinet at 10–15 cm from the carcass. Total water usage using these nozzles at this pressure was approximately 107.9 lpm (Figure 1). Figure 2 provides a diagram of the wash cabinet as used.

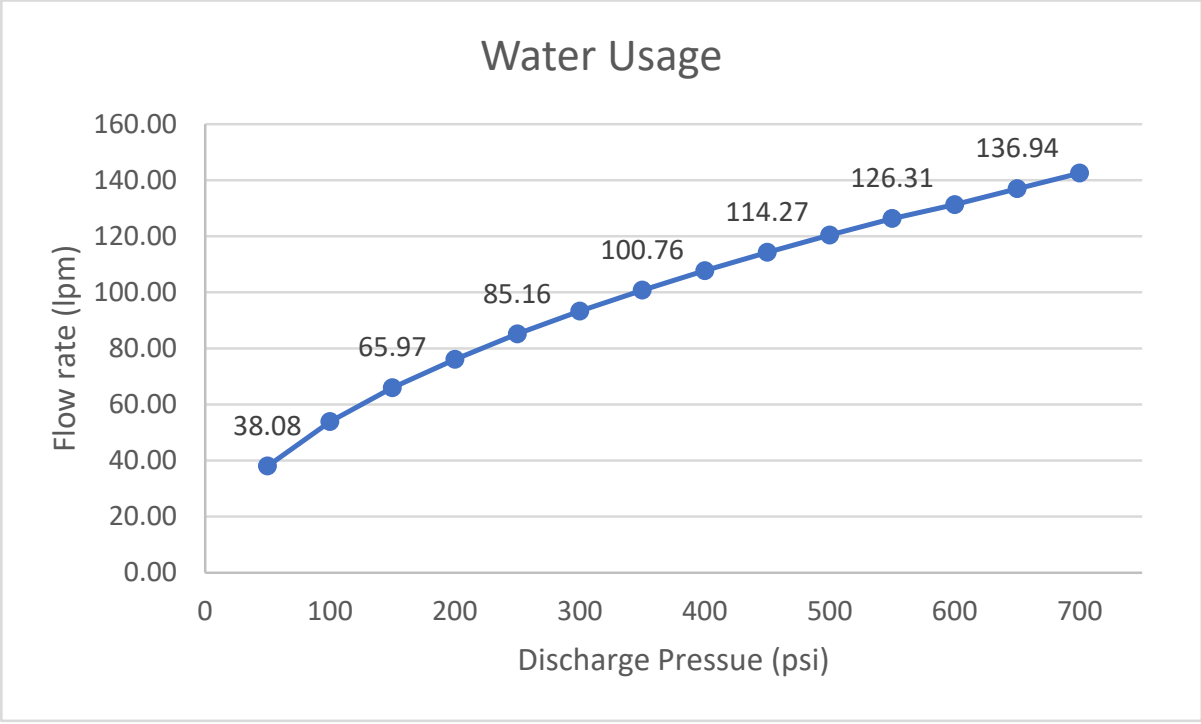

Figure 1. Water usage rates for 50 low-volume fluidic nozzles in liters per minute across the range of the supplied water pump (50–700 psi).

2.2. Broiler Carcasses

Carcasses were collected from a processing plant located in Northeast Georgia. Five carcasses (n = 5) were collected immediately following exsanguination, five carcasses (n = 5) from immediately following scald, and five carcasses (n = 5) from immediately following defeathering for each replication. All carcasses were placed into individual plastic carcass bags (Cryovac, Charlotte, NC, USA) and placed into a rigid cooler (Pelican 70 quart, Torrance, CA, USA) for transport to Pilot Processing Plant at the U.S. National Poultry Research Center, Athens, Georgia. Carcasses were removed from the transport

bags and placed into standard shackles on 15.24 cm (6-inch) centers according to site of collection; one collection site was rinsed per wash cabinet run, three wash cabinet runs per replication. The carcasses were passed through the wash cabinet at a line speed of 52 birds/min with 50 low-volume fluidic nozzles operating at 450 psi using steam heated water at 60 °C. Four replications were conducted over a 4-week period with one replication per week (N = 60). The carcasses were removed after slaughter so consent was required for use.

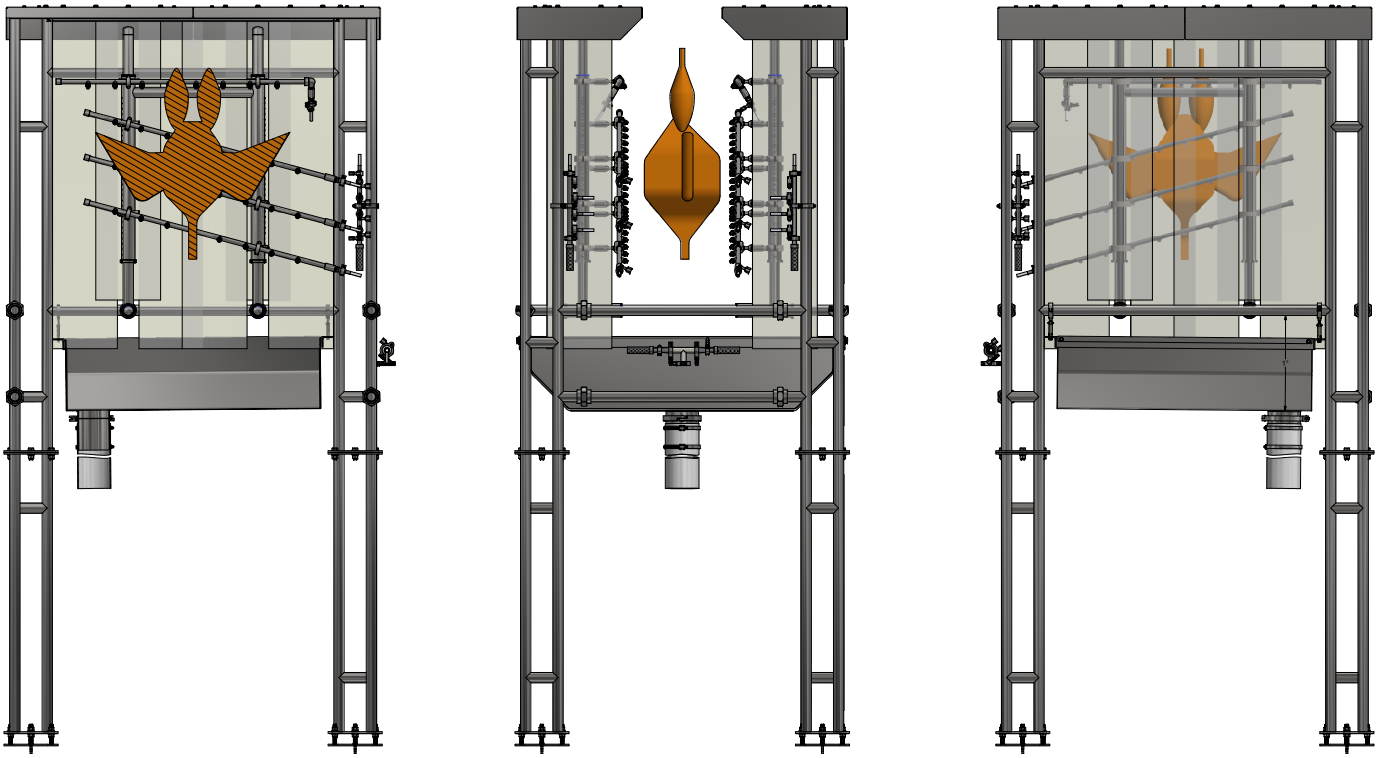

Figure 2. Design of the prototype wash cabinet.

2.3. Sample Collection

Breast swab samples (pre-wash swab) were collected according to the method of Berrang et al., [18] using cellulose sponge swabs (Whirlpak Cellulose Speci-Sponge Bags, Whirlpak, Fort Atkinson, WI, USA) moistened with 10 mL of sterile buffered peptone water (**BPW**, Becton-Dickinson, Sparks, MD, USA). Briefly, the moistened swab was wiped down the skin of the sternal feather tracts from the top of the keel to the bottom of the rib cage (approximately 30 cm^2 three times. Identical breast swab samples (post-wash swab) were collected immediately after the wash cabinet using the procedure above, ensuring that pre-wash and post-wash samples were paired for statistical analysis. All swabs were placed onto ice for transportation to the laboratory.

2.4. Microbiological Evaluation

Ten mL of BPW was added to each breast swab. Swabs were stomached for 60 s in a paddle blender (Stomacher 80, Seward Inc., Port St. Lucie, FL, USA). Serial dilutions were made as appropriate from the stomached swab samples and plated in duplicate onto Total Aerobic Count, *Escherichia coli*, and Enterobacteriaceae Petrifilm® cards (**TAC, EC, and ENT**, Petrifilm®, 3M Corporation, St. Paul, MN, USA). All inoculated cards were incubated 22 ± 2 h at 37 °C. Plates were counted according to manufacturer's instructions, counts recorded. The number of colony-forming units detected per mL(**CFU/mL**) of swab rinsate was used to determine the number of bacteria per carcass. The number of CFUs per carcass was log transformed and the transformed data was used to calculate means and standard error.

A 1.0 mL aliquot of all samples was collected and individually processed using the TEMPO® *Campylobacter* Enumeration card (TEMPO® CAM, bioMerieux, Inc., Durham, NC, USA) according to manufacturers and FSIS Microbiological Laboratory Guidebook 3.02 (USDA, FSIS, 2015 [19]) protocols. Cards were incubated under microaerobic conditions at 42 °C for 48 h. After incubation, the TEMPO® CAM cards were read with the TEMPO® reader according to manufacturer's protocol. Positive cards were confirmed by wet mount microscopy. Results are reported as CFU/carcass. The number of CFUs per carcass was log transformed with the transformed data used to calculate means and standard error.

2.5. Statistical Analysis

Recorded transformed bacterial counts were averaged for each location over the 4 replications for pre-wash and post-wash enumerations. Bacterial reductions were calculated from the averaged $\log_{10}$ CFU/carcass and are reported as Δ. Standard error was calculated for Δ. Paired *t*-tests were used to determine significance ($p < 0.05$) between pre-wash and post-wash bacterial counts for each location. Paired student's *t*-test was used to compare the bacterial load on the carcasses collected from the three locations before the wash cabinet was used.

3. Results

For the pre-scald samples, the average total aerobic bacterial count (TAC) was 9.02 $\log_{10}$ CFU/carcass before the wash cabinet and 6.52 $\log_{10}$ CFU/carcass after for a significant reduction ($p < 0.05$) of 2.50 $\log_{10}$ CFU/carcass. A significant reduction of 2.01 $\log_{10}$ CFU/carcass for Enterobacteriaceae (ENT) with 6.04 $\log_{10}$ CFU/carcass being detected before the wash cabinet and 4.03 $\log_{10}$ CFU/carcass after the wash cabinet. Using the TEMPO® CAM protocol, a significant reduction of 2.21 $\log_{10}$ CFU/carcass was measured with enumerations of 3.87 and 1.66 $\log_{10}$ CFU/carcass being recorded before and after the wash cabinet use, respectively. *E. coli* (EC) demonstrated the lowest level of reduction with a significant reduction of 1.73 $\log_{10}$ CFU/carcass recorded with an enumeration of 4.95 $\log_{10}$ CFU/carcass pre-wash and 3.22 $\log_{10}$ CFU/carcass post-wash (Table 1).

Table 1. Average reduction of bacterial load on broiler carcasses washed with a prototype wash cabinet using 60 °C water at 450 psi immediately before the scald tank.

	TAC *	**ENT**	**EC**	***Campylobacter*–Tempo®**
Pre-Wash	9.02	6.04	4.95	3.87
Post-Wash	6.52	4.03	3.22	2.21
Δ	−2.5	−2.01	−1.73	−2.21
p-value ($p < 0.05$)	2.15×10^{-13}	1.30×10^{-7}	1.26×10^{-6}	2.10×10^{-13}
Std Err	0.14	0.25	0.21	0.12

* Results are reported as $\log_{10}$ CFU per carcass for total aerobic count (TAC), *Enterobacteriaceae* (ENT), *Escherichia coli* (EC), and *Campylobacter*. Δ symbol stands delta which is reference to change in numbers.

Using post-scald carcasses, a similar pattern for reductions was observed with TAC having the largest reduction and EC the lowest level of reduction. A total reduction of 2.09 $\log_{10}$ CFU/carcass was observed for TAC, with 5.90 and 3.81 $\log_{10}$ CFU/carcass for pre-wash and post-wash enumeration, respectively. The wash cabinet removed 1.23 $\log_{10}$ CFU/carcass of ENT with an initial load of 4.14 $\log_{10}$ CFU/carcass before the cabinet and 2.91 $\log_{10}$ CFU/carcass after the cabinet. *Campylobacter* were reduced by a total of 1.14 $\log_{10}$ CFU/carcass with an initial load of 1.41 $\log_{10}$ CFU/carcass prior to the carcass wash and 0.27 $\log_{10}$ CFU/carcass after the wash. Similarly, EC had an initial load of 2.91 $\log_{10}$ CFU/carcass before the wash cabinet and a final load of 2.01 $\log_{10}$ CFU/carcass after the wash for a total reduction of 0.90 $\log_{10}$ CFU/carcass. All reductions were statistically significant at $p < 0.05$ (Table 2).

Table 2. Average reduction of bacterial load on broiler carcasses washed with a prototype wash cabinet using 60 °C water at 450 psi immediately after the scald tank.

	TAC *	ENT	EC	*Campylobacter*–Tempo®
Pre-Wash	5.90	4.14	2.91	1.41
Post-Wash	3.81	2.91	2.01	0.27
Δ	−2.09	−1.23	−0.90	−1.14
p-value ($p < 0.05$)	9.75×10^{-5}	1.26×10^{-7}	6.64×10^{-6}	5.37×10^{-5}
Std Err	0.43	0.16	0.15	0.22

* Results are reported as $\log_{10}$ CFU per carcass for total aerobic count (TAC), *Enterobacteriaceae* (ENT), *Escherichia coli* (EC), and *Campylobacter*. Δ symbol stands delta which is reference to change in numbers.

Post-pick carcasses yielded the lowest levels of reduction across the four assays and did not follow the same pattern seen in the pre-scald and post-scald experiments. ENT exhibited the highest reduction with 1.53 $\log_{10}$ CFU/carcass removed with the pre-wash load being 2.61 $\log_{10}$ CFU/carcass and the post-wash load being 1.08 $\log_{10}$ CFU/carcass. EC demonstrated the next largest reduction with 0.99 $\log_{10}$ CFU/carcass EC removed. *Campylobacter* were reduced by a total of 0.86 $\log_{10}$ CFU/carcass. TAC demonstrated the least reduction with only 0.73 $\log_{10}$ CFU/carcass being removed from the carcasses by the wash cabinet (Table 3).

Table 3. Average reduction of bacterial load on broiler carcasses washed with a prototype wash cabinet using 60 °C water at 450 psi immediately after the picker.

	TAC *	ENT	EC	*Campylobacter*–Tempo®
Pre-Wash	3.86	2.61	1.73	1.85
Post-Wash	3.13	1.08	0.74	0.99
Δ	−0.73	−1.53	−0.99	−0.86
p-value ($p < 0.05$)	3.69×10^{-4}	1.58×10^{-5}	3.79×10^{-3}	1.36×10^{-6}
Std Err	0.17	0.27	0.30	0.012

* Results are reported as $\log_{10}$ CFU per carcass for total aerobic count (TAC), *Enterobacteriaceae* (ENT), *Escherichia coli* (EC), and *Campylobacter*. Δ symbol stands delta which is reference to change in numbers.

The bacterial counts collected prior to the wash cabinet were compared to determine the basic reductions produced by the scald tank or the defeathering machines without the use of the wash cabinet (Table 4) and to determine the overall value of the wash cabinet at reducing bacteria on the carcass. Significant bacterial reduction was found to occur in the scald tank with reductions of 3.12, 2.45, 2.05, and 1.90 $\log_{10}$ CFU/carcass for TAC, *Campylobacter*, EC, and ENT, respectively. The feather-picking machine significantly reduced the number of bacteria on the carcasses by 2.04, 1.53, and 1.18 CFU for TAC, ENT, and EC, respectively, with an increase of 0.44 $\log_{10}$ CFU/carcass for *Campylobacter*. Overall, the reduction of bacterial load on carcasses with these two processing steps was 5.16, 3.43, 3.22, and 2.02 $\log_{10}$ CFU/carcass for TAC, ENT, EC, and *Campylobacter*, respectively (Table 5).

Table 4. Average initial bacterial counts from broiler carcasses without processing through the prototype wash cabinet.

	TAC *	ENT	EC	*Campylobacter*–Tempo®
Pre-Scald	9.02	6.04	4.95	3.87
Post-Scald	5.90	4.14	2.91	1.41
Post-Pick	3.86	2.61	1.73	1.85

* Results are reported as CFU $\log_{10}$ per carcass for total aerobic count (TAC), *Enterobacteriaceae* (ENT), *Escherichia coli* (EC), and *Campylobacter*.

Table 5. Delta, *p*-values and standard error for bacterial reduction of the scalder and picker on the average initial bacterial load from the processing plant as determined from unwashed broiler carcasses.

Pre-Scald versus Post-Scald				
	TAC *	ENT	EC	*Campylobacter*–Tempo®
Δ	3.12	1.90	2.05	2.45
p-value ($p < 0.05$)	1.4×10^{-26}	1.70×10^{-12}	3.08×10^{-6}	1.34×10^{-7}
Std Err	0.13	0.20	0.31	0.28
Pre-Scald versus Post-Pick				
	TAC *	ENT	EC	*Campylobacter*–Tempo®
Δ	5.16	3.43	3.22	2.02
p-value ($p < 0.05$)	3.91×10^{-28}	1.85×10^{-11}	1.13×10^{-5}	6.21×10^{-7}
Std Err	0.19	0.22	0.30	0.19
Post-Scald versus Post-Pick				
	TAC *	ENT	EC	*Campylobacter*–Tempo®
Δ	2.04	1.53	1.18	−0.44
p-value ($p < 0.05$) ‡	7.46×10^{-16}	6.62×10^{-7}	5.94×10^{-3}	0.13
Std Err	0.16	0.29	0.30	0.18

* Results are reported as $\log_{10}$ CFU per carcass for total aerobic count (TAC), *Enterobacteriaceae* (ENT), *Escherichia coli* (EC), and *Campylobacter*. ‡ *p*-values greater that 0.05 are not significant within columns. Δ symbol stands delta which is reference to change in numbers.

4. Discussion

Interventions in poultry processing are usually focused either on the farm or on the evisceration side of the processing plant. Studies on the farm usually focus on antimicrobial intervention to prevent colonization of *Salmonella* spp. and *Campylobacter* spp. in the broilers, while the intervention strategies in the processing plant involve antimicrobial chemicals added to various operations on the evisceration side of the processing plant. The delineation between the slaughter side and the evisceration side of the plant is generally separated by means of a physical barrier/wall between the live and dead portions of the plant as a method to reduce contamination. This is, also, generally the point of transfer from a kill shackle line to an evisceration shackle line [20].

Prior to the scald tank, limited intervention strategies were available in modern processing plants. Brush systems have been used to remove fecal material from carcasses prior to the scald tank but have become less prevalent. Our system used 60 °C water at 450 psi to remove fecal material and associated bacteria. These parameters were selected based on preliminary experiments using ambient temperature water at lower pressures and heated water at lower pressures which were less efficient at reducing bacteria.

On the evisceration side of the processing plant, many interventions are used to reduce the bacterial load. These interventions involve the use of multiple chemicals as antimicrobial interventions [21] often applied during online reprocessing, inside-outside bird washers, chillers, and post-chill operations. The various approved antimicrobials include acidified sodium chlorite, bromine, chlorine dioxide, organic acids, peracetic acid (PAA), trisodium phosphate, electrolyzed water, and hypochlorous acid (chlorine), with companies proposing novel chemicals for approval as they become available.

McKee [22] identified carcass picking and evisceration as major sites in processing where bacteria can be transferred from the intestines to the skin. Berrang et al. [23] demonstrated that the feather picker at a pilot processing plant increased the *Campylobacter* contamination of 120 carcasses from 0.80% to 79.17% for commercial broilers and linked this increase to fecal material expressed during the mechanical feather removal. Multiple studies have demonstrated that *Campylobacter* can be recovered after carcass picking and evisceration [24–26]. Rivera-Perez et al. [27] demonstrated a 0.3 log CFU/carcass

increase in *Salmonella* following mechanical defeathering. Our data indicating an increase of *Campylobacter* following the picker is consistent with the data from Berrang et al. [18].

When the wash cabinet was placed immediately prior to the scald tank, data show an average reduction of 2.5, 2.21, 2.01, and1.73 $\log_{10}$ CFU/carcass for TAC, *Campylobacter*, ENT, and EC. The brush system of Pascholewicz et al. [15] demonstrated a 0.3 log reduction for *E. coli*, while Berrang and Bailey [17] demonstrated a 0.2 log reduction for *E. coli.* The wash cabinet demonstrated a five- to eight- fold increase in the removal of the *E. coli* from the carcasses. A major benefit of using the wash cabinet prior to the scald tank would be the removal of fecal material, dust, dirt, etc. from the carcasses, allowing the scald tank to be more efficacious in reducing the bacterial load on the carcasses.

Counter-current-flow scalding is one intervention strategy used in commercial processing plants during slaughter. Cason et al. [13] demonstrated that using a three stage (compartmented) scald tank with counterflow designs (clean water being added to the tank at the point where carcasses exit the tanks) lowered the mean CFU/mL of coliforms and *E. coli* in water samples collected after 8 h of processing at a commercial plant and decreased the prevalence of salmonellae in water of the last tank. While this study focused on carcass microbial loads, removal of bacteria before the carcasses enter the scald tank should improve the overall efficacy of the scald tank as an intervention strategy.

Carcasses entering the plant had a TAC load of 9.02 $\log_{10}$ CFU/carcass, an ENT load of 6.04 $\log_{10}$ CFU/carcass, an EC load of 4.95 $\log_{10}$ CFU/carcass and a *Campylobacter* load of 3.87 $\log_{10}$ CFU/carcass. When the bacterial reductions demonstrated using the wash cabinet are combined with the normal reductions (Table 4) seen in the scald tank (determined by the unwashed samples collected immediately prior to and immediately after the scald tank), reductions of 5.61, 4.66, 3.91, and 3.78 $\log_{10}$ CFU/carcass were seen for TAC, *Campylobacter*, ENT, and EC, respectively. The authors are unaware of any studies which have demonstrated intervention strategies capable of reducing bacteria at these levels.

When the cabinet was used after the scald tank, the levels of reduction are lower, with an average reduction of 2.09, 1.23, 1.14, and 0.90 $\log_{10}$ CFU/carcass for TAC, ENT, *Campylobacter*, and EC, respectively. Cabinet use immediately after the scald tank was slightly less efficacious at removing the bacterial load from the carcasses than before the scald tank, but the lower initial load of bacteria on the carcass could be a confounding factor. Use of the cabinet at this location in the slaughter process does not improve the efficacy of the scald tank to remove bacteria because the fecal material, dust, and dirt would enter the tanks and contribute to saturation of the scald tanks as currently experienced [15].

Using the cabinet after the defeathering process was the least efficacious for removing bacteria from the carcasses, with an average reduction of 1.53, 0.99, 0.86, and 0.73 $\log_{10}$ CFU/carcass for ENT, EC, *Campylobacter*, and TAC, respectively. As previously hypothesized, this could be due to the lower initial loads on the defeathered carcasses, or it could be a result of the bacteria entering the open feather follicles which prevents removal. Berrang et al., [28] in an experiment with similar time between defeathering and a spray re-scald (73 °C for 20 s, no value was provided for pressure), demonstrated non-significant reductions for TAC, EC, coliform bacteria, and *Campylobacter*, in contrast with the present study, where the reduction demonstrated was significant for all four bacterial classes assayed. Again, using the cabinet in this location does not improve the efficacy of the scald tank, as the authors predict, when the cabinet is used before the scald tank by the removal of dirt, dust, fecal matter, and bacteria.

With the potential for a cleaner scald tank from use of this type of intervention strategy, more intervention strategies become available for use in the actual scald tank. McKee et al. [29] demonstrated that the use of alkaline additives was effective in reducing *Salmonella* Typhimurium on broiler carcasses, particularly when hard scald temperatures are used. An additional benefit would be the ability of the processing plant to reduce the amount of overflow water currently used to remove sludge and particulate material from the scald tanks. This reduction of water would reduce the amount of water used, the amount processed as wastewater, and reduce the energy required for maintaining

the temperature of the scald water. There could be several other benefits not evident at this time.

While current line speeds in commercial plants are higher by a factor of two or three, the predicted level of bacterial load reduction should not be much less because the dirt, dust, fecal matter, and bacteria will still be removed from the carcasses. This cabinet has the ability to reduce the bacterial load of the scald tank water, broiler carcasses, and finished product being provided to the consumer. The limitations of working in a pilot plant are slower line speeds and the inability to process large numbers of carcasses, which prevents the measurement of water quality factors such as total soluble solids, total dissolved solids, conductance, and turbidity that would be measurable in a commercial processing plant. Further work is planned to assess the efficacy of this cabinet system in processing plants across the poultry industry for removal of bacteria, fecal material, dust, and dirt from carcasses entering the scald tank.

5. Conclusions

Rules and regulations for improving the microbial quality of poultry meat and meat products will continue to become more restrictive as governments and consumers strive to reduce foodborne illnesses linked to poultry. No single intervention step short of producing irradiated or fully cooked, ready-to-eat products will prevent the risk associated with consuming poultry products. This cabinet used before the scald tank should provide an improved product by serving as another effective intervention strategy focused during the slaughter process to eliminate potential microbial cross-contamination and reduce the incidences of non-compliance for fecal contamination.

Small scale (pilot plant) studies have several limitations which should be underscored here. First, the quantity of animals/carcasses available for use are often limited. The pilot processing plant at the U.S. National Poultry Research Center has a maximum line speed of 52 birds/minute whereas a commercial plant can operate between 104–170 birds/minute or higher. A third limitation is the inability to utilize the number of carcasses that would allow for the meaningful collection of water quality samples from the scald tanks. A commercial plant will process between 6000–10,000 birds/hour while the USNPRC pilot plant has a maximum capacity of 300–500 birds/day. Further studies are needed using this intervention in a commercial processing plant where water quality can be determined as well as the bacterial load reduction, both indicator and human enteropathogenic organisms. One last limitation was the inability to recover *Salmonella* spp. from the carcasses in any of the four replications, which prevented the authors from determining the efficacy of the wash cabinet to remove this pathogen from carcasses. Further research is planned to determine the ability to remove inoculated *Salmonella* spp. and *Campylobacter* spp. from carcasses with this wash cabinet.

A cleaner broiler carcass entering the scald tank will enable the processors to provide a cleaner, more wholesome product to consumers.

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References

1. Code of Federal Regulations. Title 9: Animal and Animal Products, Chapter III: Food Safety and Inspection Service, Department of Agriculture, Part 381: Poultry Products Inspection Regulations. 2023. Available online: https://ecfr.gov/current/title-9/chapter-III/subchapter-A/part-381 (accessed on 5 September 2023).
2. Lahellec, C.; Colin, P. Relationship between serotypes of *Salmonellae* from hatcheries and rearing farms and those from processed poultry carcasses. *Br. Poult. Sci.* **1983**, *26*, 179–186. [CrossRef] [PubMed]
3. Katoula, K.L.; Pandya, Y. Bacterial contamination of broiler chickens before scalding. *J. Food Prot.* **1995**, *58*, 1326–1329. [CrossRef] [PubMed]
4. EFSA/ECDC. The European Union summary report on trens and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. *EFSA J.* **2014**, *12*, 3547.
5. Boysen, M.H.; Rosenquist, H.; Larsson, J.T.; Nielsen, E.M.; Sorensen, G.; Nordentoft, S.; Hald, T. Source attribution of human campylobacteriosis in Denmark. *Epidemiol. Infect.* **2014**, *142*, 1599–1608. [CrossRef] [PubMed]
6. Kittl, S.; Heckel, G.; Korczak, B.M.; Kuhnert, P. Source attribution of human *Campylobacter* isolates by MLST and *fla*-typing and association of genotypes with quinolone resistance. *PLoS ONE* **2013**, *8*, e81796. [CrossRef] [PubMed]
7. Skarp, C.P.A.; Hanninen, M.L.; Rautelin, H.I.K. Campylobacteriosis: The role of poultry meat. *Clin. Microbial. Infect.* **2016**, *22*, 103–109. [CrossRef] [PubMed]
8. EFSA. Scientific Opinion on *Campylobacter* in broiler meat production: Control options and performance objectives and/or targets at different stages of the food chain. *EFSA J.* **2011**, *9*, 105–246.
9. Zweifel, C.; Althaus, D.; Stephan, R. Effects of slaughter operations on the microbiological contamination of broiler carcasses in three abattoirs. *Food Cont.* **2015**, *51*, 37–42. [CrossRef]
10. Stern, N.J.; Clavero, M.R.S.; Bailey, J.S.; Cox, N.A.; Robach, M.C. *Campylobacter* spp. in broilers on the farm and after transport. *Poult. Sci.* **1995**, *74*, 937–941. [CrossRef]
11. Mulder, R.W.A.W.; Dorresteijn, L.W.J.; van der Broek, J. Cross-contamination during the scalding and plucking of broilers. *Br. Poult. Sci.* **1978**, *19*, 61–70. [CrossRef]
12. Berrang, M.E.; Dickens, J.A. Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. *J. Appl. Poultry Res.* **2000**, *9*, 43–47. [CrossRef]
13. Cason, J.A.; Hinton, A., Jr.; Ingram, K.D. Coliform, *Escherichia coli* and Salmonellae concentrations in a multiple-tank, counterflow poultry scalder. *J. Food Prot.* **2000**, *63*, 1184–1188. [CrossRef] [PubMed]
14. Yang, H.; Li, Y.; Johnson, M.G. Survival and death of *Salmonella* Typhimurium and *Campylobacter jejuni* in processing water and on chicken skin during poultry scalding and chilling. *J. Food Prot.* **2001**, *64*, 770–776. [CrossRef] [PubMed]
15. Pacholewizc, E.; Lipman, L.J.A.; Swart, A.; Heemskerk, W.J.C. Pre-scald brushing for the removal of solids associated with broiler carcass bacterial contamination. *Poult. Sci.* **2016**, *95*, 2979–2985. [CrossRef] [PubMed]
16. Shackleford, A.D.; Whittemore, A.D.; Papa, C.M.; Wilson, R.L. Development of a prototype carcass cleaning machine. *J. Appl. Poultry Res.* **1992**, *1*, 235–241. [CrossRef]
17. Berrang, M.E.; Bailey, J.S. On-line brush and spray washers to lower numbers of *Campylobacter* and *E. coli* and presence of *Salmonella* on broiler carcasses during processing. *J. Appl. Poultry Res.* **2009**, *18*, 74–78. [CrossRef]
18. Berrang, M.E.; Cox, N.A.; Oakley, B.B. Sampling by sponge wipe or skin excision for recovery of inoculated *Salmonella* and *Campylobacter* from defeathered broiler carcasses. *J. Food Prot.* **2014**, *77*, 824–827. [CrossRef] [PubMed]
19. USDA; FSIS. Microbiological Laboratory Guidebook, Chapter 3.02: Quantitative Analysis of Bacteria in Foods as Sanitary Indicators. 2015. Available online: https://fsis.usda.gov/news-events/publications/microbiology-laboratory-guidebook (accessed on 5 September 2023).
20. Sams, A.R.; McKee, S.R. First processing: Slaughter through chilling. In *Poultry Meat Processing*, 2nd ed.; Owens, C.M., Alvarado, C.Z., Sams, A.R., Eds.; CRC Press, Taylor & Francis Group: Boca Raton, FL, USA, 2010; pp. 25–49.
21. McKee, S.R. *Salmonella* control in poultry processing. In Proceedings of the Abstracts from the 65th Annual Reciprocal Meat Conference, Fargo, ND, USA, 17–20 June 2012.
22. McKee, S.R. *Salmonella and Campylobacter Control during Poultry Processing*; International Poultry Scientific Forum: Atlanta, GA, USA, 2011.
23. Berrang, M.E.; Buhr, R.J.; Cason, J.A.; Dickens, J.A. Broiler carcass contamination with *Campylobacter* from feces during defeathering. *J. Food Prot.* **2001**, *64*, 2063–2066. [CrossRef]
24. Rosenquist, H.; Sommer, H.M.; Nielsen, N.L.; Christensen, B.B. The effect of slaughter operation on the contamination of chicken carcasses with thermotolerant *Campylobacter*. *Int. J. Food Microbiol.* **2006**, *108*, 226–232. [CrossRef]
25. Izat, A.L.; Garnder, F.A.; Denton, J.H.; Golan, F.A. Incidence and level of *Campylobacter jejuni* in broiler processing. *Poult. Sci.* **1988**, *67*, 1568–1572. [CrossRef]
26. Stern, N.J.; Robach, M.C. Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses. *J. Food Prot.* **2003**, *66*, 1557–1563. [CrossRef]

27. Rivera-Perez, W.; Barquero-Calva, E.; Zaomra-Sanabria, R. *Salmonella* contamination risk points in broiler carcasses during slaughter line processing. *J. Food Prot.* **2014**, *13*, 2031–2034. [CrossRef]
28. Berrang, M.E.; Dickens, J.A.; Musgrove, M.T. Effects of hot water application after defeathering on the levels of *Campylobacter*, coliform bacteria, and *Escherichia coli* on broiler carcasses. *Poult. Sci.* **2000**, *79*, 1689–1693. [CrossRef]
29. McKee, S.R.; Townsend, J.C.; Bilgili, S.F. Use of a scald additive to reduce levels of *Salmonella* Typhimurium during poultry processing. *Poult. Sci.* **2008**, *87*, 1672–1677. [CrossRef]