CONSORTIUM

Research Article

LOW PH PROCESSING AID TO LOWER THE PRESENCE OF NATURALLY OCCURRING *CAMPYLOBACTER* ON WHOLE BROILER CARCASSES

Landrum MA[1], NA Cox[2], JL Wilson[1], DE Cosby[2], ME Berrang[2], GR Gamble[2], MJ Da Costa[1], GM Pesti[1] and A Hinton, Jr.[2]

1. University of Georgia, Athens, GA;
2. The U.S. National Poultry Research Center, Athens, GA

History:
Received: April 23, 2018
Revised: May 27, 2018
Accepted: June 8, 2018
Published: June 11, 2018
Collection year: 2018
Status: Published

Identifiers and Pagination:
Year: 2018
Volume: 3
First Page: 7
Last Page: 13
Publisher Id:
10.21065.AdvFoodNutrSci-3-7
DOI:
http://dx.doi.org/10.21065/AdvFoodNutrSci.3.7

Corresponding author:
Dr. Nelson A Cox. 950 College Station Road Athens, GA 30605.
T.: (706)546-3484
F.: (706)546-3633
E.: nelson.cox@ars.usda.gov

Citation:
Landrum et al. Low ph processing aid to lower the presence of naturally occurring campylobacter on whole broiler carcasses. Adv Food & Nutr Sci. 2018; [3]. 7-13

Abstract

Campylobacter is a serious foodborne pathogen for which the USDA-FSIS has established stricter performance standards. Processors must establish effective treatment plans to lower the microbial levels of *Campylobacter* to meet these regulations. This study evaluated the low pH processing aid - CMS PoultrypHresh™ to reduce *Campylobacter* on carcasses (3 groups of 6) collected prior to the chiller that were individually placed into a 38 L container with either 20 L tap water (pH = 7.3) or 20 L of CMS PoultrypHresh™ solution (pH = 1.4) with air agitation. An untreated group was the control. After treatment, drained carcasses were placed into a plastic bag, and rinsed by hand in 400 mL of buffered peptone for 60 s. Rinsates were cultured for *Campylobacter* by direct plating on Campy-cefex agar and enrichment in Bolton's broth. If no *Campylobacter* was detected by direct plating, the incubated broth was plated and incubated under the same conditions. Confirmed *Campylobacter* were detected on 30/36 (83.3%) untreated carcasses, on 25/36 (69.4%) water treated carcasses, and on 2/36 (5.6%) of CMS PoultrypHresh™ treated carcasses. This treatment may be an option for processors to meet requirements and minimize *Campylobacter* to avoid regulatory action from FSIS.

Key words: *Campylobacter*, CMS PoultrypHresh™, broiler, carcass rinse, immersion.

Introduction

USDA-Food Safety and Inspection Service (FSIS) has established new performance standards for the presence of *Campylobacter* and *Salmonella* on fresh poultry. Processors have responded by continuing to develop and test intervention methods that lower the prevalence of foodborne microorganisms in order to be in compliance with new regulations. *Campylobacter* is a major pathogen of concern which causes foodborne illness with gastrointestinal symptoms to more than 1.3 million people in the U.S. each year. [1] *Campylobacter* is most often reported being associated with poultry and has been found on as many as 88% of chicken carcasses. [2] Through the past seven years, USDA-FSIS has encouraged greater control of food safety measures, improved record keeping, proper labeling requirements, correct testing methods, and higher performance standards. [3] These regulations were made to reduce illnesses associated with foodborne pathogens found in poultry products and prevent an estimated 50,000

Competing Interests:
The authors declare no competing interests

Additional information is available at the end of the article.

illnesses each year. [3] More specifically, recent regulatory changes aim to achieve a 32% reduction of illnesses from *Campylobacter* alone. [3] It is essential for broiler processing companies to establish an effective treatment plan to lower microbial levels of *Campylobacter* to pass rigorous performance standards set by USDA-FSIS. FSIS predicts that 46% of broiler companies will not be able to meet proposed 2016 performance standards for *Campylobacter*. [4]

Under regulations enacted in 2016, processing facilities are designated into categories 1, 2, 3, 4 or 5 by whether their microbial performance is consistent, variable, highly variable, passing, or failing to achieve FSIS *Campylobacter* standards. [5] Sampling to evaluate microbial control methods will become routine throughout the year, rather than infrequent sampling on consecutive days. [3] Results will be reported to the public, heightening the importance of reducing microbial levels to reach set standards and avoiding consumer rejection. Being listed on the FSIS website as "failing" to meet standards may have consequences for a company's brand loyalty. The objective of this study was to evaluate a twenty second air agitated immersion in a proprietary antimicrobial treatment, PoultrypHresh™, at a pH of 1.4 as a means to reduce or eliminate viable *Campylobacter* on whole broiler carcasses.

Materials and Methods

Treatment Procedures

Eighteen whole, pre-chill broiler carcasses were randomly collected from the evisceration line of a commercial processing facility prior to chilling, individually bagged, placed on ice and transported to the laboratory. Within 60 min, six whole carcasses were individually dipped for 20 s into 38 L, high density polyethylene plastic containers holding 20 L of tap water at a pH of approximately 7.3. An additional 6 whole carcasses were dipped into similar containers for 20 s with 20 L of PoultrypHresh™ solution at a pH of 1.4. Air agitation of the solution in each bucket was achieved by pumping air (50 psi) from a compressor, through a six way manifold to the bottom of each container by means of plastic tubing (1/4" ID, McMaster-Carr, Elmherst, IL) which was secured to the side of the bucket by a metal pipe placed such that the tubing air exit was at the bottom of each container. After treatment (20 s immersion with agitation), each carcass was removed, and allowed to drip for 5 s before being subjected to a whole carcass rinse procedure in 400 mL of buffered peptone water (BPW) for 60 s using a mechanical rinsing machine. [6] The final six carcasses served as untreated controls, receiving no dip treatment before whole carcass rinse sampling, as described above. Six replications were conducted on 6 separate days, n=36 per treatment.

Campylobacter Detection Methods

Rinsates from each whole carcass were treated as described in the FSIS microbiology laboratory guidebook. [7] Initial rinses were used to spread 0.25 mL on four Campy-Cefex plates which were incubated microaerobically (5% O_2, 10% CO_2, 85% N_2) for 48 h

at 42°C. Thirty mL of each carcass rinsate was also placed into a tissue culture flask with 30 mL of 2X Bolton's broth (2X-BEB) and incubated concurrently with the direct plates under the same conditions. Incubated enrichment broth was plated onto a Cefex agar plate and incubated for 48 h under the same conditions. After incubation, plates were examined for typical *Campylobacter* colonies which were confirmed as members of the genus *Campylobacter* by observation of typical cellular morphology, motility under phase contrast microscopy and by use of a latex agglutination test kit (Microgen Bioproducts Ltd, Camberley, U.K.).

Statistical Analysis

The study was replicated six times using naturally contaminated *Campylobacter* carcasses, totaling 36 whole birds for each treatment (N=108, n=36). In each replicate, 6 untreated, 6 tap waters treated, and 6 treated with PoultrypHresh™ at pH 1.4 were used. Each sample was recorded as either positive or negative for the presence of *Campylobacter* and data was analyzed using the Chi Square test for independence. Data was analyzed using a generalized mixed model (binomial distribution) where treatment was considered as a fixed effect and the treatment nested within the days of collection as a random effect. The GLIMMIX Procedure of SAS (SAS Inst. Inc., Cary, NC) was used: Proc GLIMMIX Data=campy; Class day rep trt; Model status = trt /dist=bin link=logit solution; Random day(trt).

Results and discussion

There was no difference in the presence of *Campylobacter* on the control or water treated samples from replication to replication. The covariance of treatment within replications was not significant (p=0.427). Overall, data from all six replicates indicated that 30/36 (83.3%) of untreated carcasses, 25/36 (69.4%) water treated, and 2/36 (5.6%) treated with PoultrypHresh™ were positive with *Campylobacter* (Figure 1). PoultrypHresh™ met FSIS standards for *Campylobacter*, which have a maximum acceptance of 15.7% in broiler carcasses. [4] These data demonstrate that an agitated dip treatment of PoultrypHresh™ significantly lowered the presence of *Campylobacter* on whole bird carcasses compared to either untreated or water treated carcasses (Table 1). Bird rinse samples directly plated with no enrichment demonstrated that 33.3% of untreated and 22.2% water treated carcasses were found to be positive for *Campylobacter*, while one of the 36 carcasses (2.8%) treated with PoultrypHresh™ at pH 1.4 were positive (Figure 2). Once rinsates were enriched using 2X Bolton's broth, 75.0% of non-treated and 60.7% of water treated carcasses were contaminated with *Campylobacter*, while only 2.9% of carcasses treated with PoultrypHresh™ were contaminated (Figure 3).

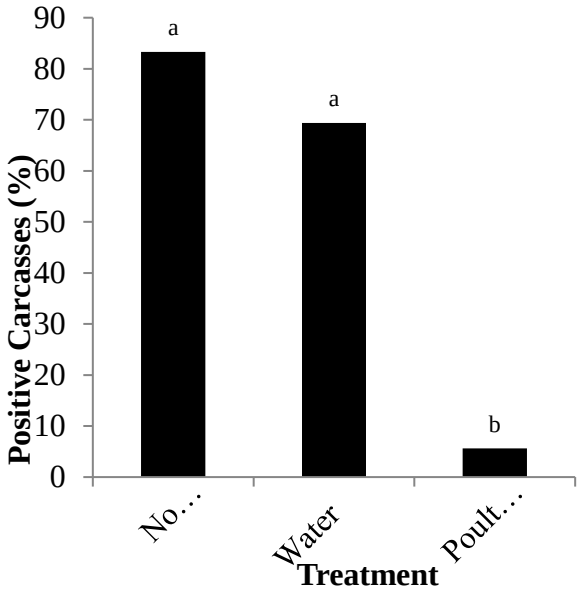
The present study demonstrated that PoultrypHresh™ can significantly ($P \leq 0.05$) reduce the presence of natural *Campylobacter* on broiler carcasses. These data show that an antimicrobial dip can be effective in the elimination of *Campylobacter* and

may be an option for lowering the prevalence in commercial processing facilities. PoultrypHresh™ applied as an agitated dip treatment for whole broiler carcasses at pH 1.4 for 20 s can significantly ($P \leq 0.05$) lower the number of *Campylobacter* positive broiler carcass rinse samples. A single hurdle application treatment of this antimicrobial may reduce the presence of *Campylobacter* on whole carcasses, helping poultry processors to succeed in meeting regulatory requirements. Additional PoultrypHresh™ applications at multiple intervention points throughout processing plants may further increase microbial reductions.

Table 1. Number of direct, enriched, and overall samples positive for *Campylobacter* in untreated, water treated, and PoultrypHresh™ treated at pH 1.4. Samples not positive on direct analysis were enriched and re-evaluated.

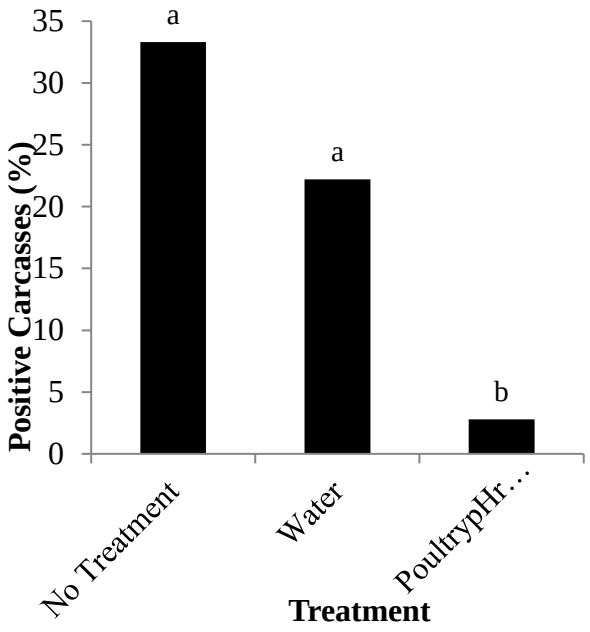
	Direct		Enriched		Overall		Pr > |t|[1]
Treatment	+	-	+	-	+	-	
Control	12	24	18	6	30	6	-
Water	8	28	17	11	25	11	0.222
PoultrypHresh	1	35	1	34	2	34	<0.001

[1] Probability that the treated groups are not different from the untreated control.



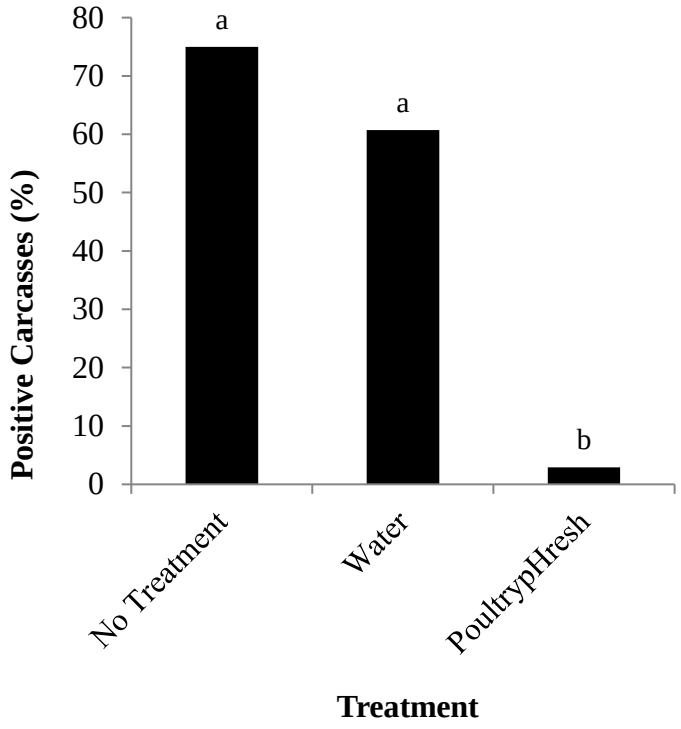
[1] a,b values with different superscripts are significantly different by Chi Square test for independence.

Figure 1. Overall percentage of carcasses positive for *Campylobacter* in untreated, water treated, and PoultrypHresh™ treated at pH 1.4[1]



[2] a,b values with different superscripts are significantly different by Chi Square test for independence.

Figure 2. Percentage of *Campylobacter* in carcass rinses directly plated (no enrichment) of untreated, water treated, and PoultrypHresh™ at pH 1.4 treated carcasses in a 20 s agitated dip[2]



[3] a,b values with different superscripts are significantly different by Chi Square test for independence.

Figure 3. *Campylobacter* presence in carcass rinse samples enriched for 48 h in 2X Bolton's Broth of untreated, water treated, and PoultrypHresh™ treatment at pH 1.4[3]

Conclusion

Stricter performance standards by USDA-FSIS are forcing poultry processors to establish more effective means of treating foodborne microorganisms throughout plants. *Campylobacter* continues to be one of the main pathogens of concern for the poultry industry. The results showed a significant reduction in the presence of *Campylobacter* after carcasses were treated with PoultrypHresh™. This shows the product may be a valuable addition to processing plants and assist processors in meeting regulatory requirements.

References

1. National Institute of Allergy and Infectious Diseases (NIH). Campylobacteriosis. United States Department of Health and Human Services. [Online] 2015 [cited 2018]. Available at: https://www.niaid.nih.gov/topics/campylobacteriosis/Pages/default.aspx

2. Guerrero-Legarreta I. Handbook of Poultry Science and Technology: Secondary Processing. John Wiley & Sons, Inc. 2010; 2:599-600.
3. United States Department of Agriculture (USDA). USDA Finalizes New Food Safety Measures to Reduce *Salmonella* and *Campylobacter* in Poultry [Online] 2016 [cited 2018]. Available at: http://www.usda.gov/wps/portal/usda/usdamediafb?contentid=2016 /02/0032. xml&printable=true&contentidonly=true
4. Almanza AV. Changes to the *Salmonella* and *Campylobacter* Verification Testing Program. Food Safety and Inspection Service 2015; 80(16):3940-3950.
5. Food Safety and Inspection Service (FSIS). New Performance Standards for *Salmonella* and *Campylobacter* in Not-Ready-to-Eat Comminuted Chicken and Turkey Products and Raw Chicken Parts and Changes to Related Agency Verification Procedures. United States Department of Agriculture, Federal Register 2016; 81(28):7285-7300.
6. Dickens JA, Cox NA, Bailey JS, Thomson JE. Automated microbiological sampling of broiler carcasses. Poult Sci 1985; 64:1116-1120.
7. MLG 41.04 United States Department of Agriculture. Laboratory Guidebook. Isolation and Identification of *Campylobacter jejuni/coli/lari* from Poultry Rinse, Sponge, and Raw Product Samples/ [Online] 2016 [cited 2018]. Available at: http://www.fsis.usda. gov/wps/wcm/connect /0273bc3d-2363-45b3-befb-1190c25f3c8b/MLG41.pdf? MOD=AJPERES