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INNOVATIVE APPLICATION OF ANTIMICROBIAL COMPOUNDS DURING HIDE REMOVAL AS A MEANS TO REDUCE CARCASS CONTAMINATION BY PATHOGENIC MICROORGANISMS

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Introduction

Hides are considered an important source of pathogenic organisms during slaughter because of fecal contamination experienced during holding (Castillo, Dickson, Clayton, Lucia, & Acuff, 1998). Prevalence levels of *Salmonella* on the hides of cattle have been determined to be as high as 15.4% pre-slaughter (Bacon, Sofos, Belk, Hyatt, & Smith, 2002). Bacteria present on hides can eventually be transferred to underlying "sterile" carcass tissue during the hide removal process. Contamination can occur when manure on the hide surface that has not been washed away before slaughter is carried onto the underlying carcass tissue (Delazari, Iaria, Riemann, Cliver, & Jothikumar, 1998). Because errors in slaughter and dressing have been implicated as the primary vehicles for contamination of beef carcasses (Bacon, Belk, Sofos, Clayton, Reagan, & Smith, 2000), many processors have incorporated hide washes into their systems to reduce levels of microbial contamination in the final product; however, these systems often increase the solubilization and migration of pathogenic bacteria on hide surfaces. Selective application of antimicrobial compounds to hide opening areas targets the specific site on the hide that is of most concern to potentially minimize pathogen transfer from the hide to carcass tissues during harvest without increasing solubilization.

Objectives

To determine the effectiveness of selected antimicrobial agents in reducing pathogenic microorganisms on hides and to verify the efficacy of selected antimicrobial agents in a commercial facility.

Methodology

Trial I

Fresh beef hides (n = 12; 4 per rep) were cut into 900-cm^2 sections with a minimum of 12 sections removed from each. Half of these sections were blown dry and shaved using Oster ClipMaster® clippers (Sunbeam Products, Inc., Boca Raton, FL), and the other half remained unshaved. The following day, hide sections were stretched over plastic clipboards and inoculated over a 400-cm^2 area with a bovine

fecal slurry (10g bovine feces and 10 mL 0.1% sterile peptone water, Difco Laboratories, Detroit, MI) containing approximately 10^6 CFU/g. Inoculum was allowed a 20 min attachment period before gross fecal material was washed off using a handheld, compressed-air sprayer standardized to deliver approximately 1 L of distilled water over 90 sec.

Microbiological samples were collected from each untreated hide following water wash using a sterile sponge (BioPro Sampling System; BioTrace International, Bothell, WA) to determine pre-treatment counts on hide surfaces. Prior to sampling, a sponge was moistened with 25 mL of sterile 0.1% peptone water, and one sample collection then was achieved by firmly rubbing the damp sponge over a 100-cm^2 area of the hide section. The sponge then was transferred to a plastic bag until analysis. Following pre-treatment sampling, sections were subjected to one of six antimicrobial treatments: distilled water; isopropyl alcohol; 3% hydrogen peroxide (Aaron Industries, Inc., Clinton, SC); 2% L-Lactic acid (Purac®, Rotra International, Wood Dale, IL); 10% Povidone-iodine (Vetadine, Vedco, Inc., St. Joseph, MO); and 1% cetylpyridinium chloride (Zeeland Chemicals, Zeeland, MI) using saturated (50 mL), sterile sponges.

Following treatment, hides sections were again sampled as described previously. Each sponge sample then was hand-massaged inside its plastic bag for 1 min before examination for aerobic plate counts (APCs) and coliform and *E. coli* counts. Coliform and *E. coli* counts were generated by plating appropriate dilutions of the sponge sample onto Petrifilm *E. coli* count plates (3M Microbiology & Products, St. Paul, MN). Samples were incubated for 24 ± 2 h at 35°C before colonies were counted. *E. coli* colonies appeared blue with a gas bubble, while total coliform count was achieved by counting both blue and red colonies with a gas bubble. Aerobic plate counts were determined by plating appropriate dilutions of the sponge sample onto Petrifilm aerobic count plates (3M), incubating at room temperature (25°C) for 48 h, and then counting all colonies.

Trial II

Beef carcasses (n = 18) with hides attached were selected from a small commercial processor for use in Trial II. Following exsanguination, approximately 100-cm^2 of the hide in the brisket area was sampled with a pre-moistened, sterile sponge as described in Trial I to determine pre-treatment counts on hide surfaces. Following sampling, hides were shaved in approximately a 400-cm^2 area in the brisket region of the carcass. Cattle then were assigned to receive one of three antimicrobial treatments (2% L-lactic acid, 3% hydrogen peroxide, and 1% CPC), and treatments were applied using saturated (50 mL), sterile sponges.

After application of the designated treatment, hides were sampled again as described above to determine post-treatment counts. Following hide removal, 100-cm^2 of the carcass surface in the brisket area was sampled using a pre-moistened, sterile sponge as described previously to determine carcass counts. Sponge samples were placed in an insulated shipping container with refrigerant to keep them cool for transport to Texas A&M University's Food Microbiology Laboratory (College Station, TX). The following day, sponge samples were hand-massaged inside their plastic bags for 1 min before examination for APCs and coliform and *E. coli* counts. Data were analyzed using PROC GLM of SAS (SAS Institute, Cary, NC). Least-squares means were generated for each main effect and separated using the PDIFF option when appropriate.

Results & Discussion

Trial I

Least-squares means for the interaction of shaving × antimicrobial agent on APC reduction for hide sections inoculated with 10^6 CFU/g fresh bovine feces are reported in Table 1. Within non-shaved samples, 1% CPC and hydrogen peroxide produced among the greatest reductions, and within shaved samples, 1% CPC, 2% L-lactic acid, and hydrogen peroxide produced among the greatest reductions. Least-squares means for the interaction of shaving × antimicrobial agent on coliform reduction for hide sections inoculated with 10^6 CFU/g fresh bovine feces are reported in Table 2. Within non-shaved samples, 1% CPC produced the greatest reduction at 5.3 $\log_{10}$ CFU/100 cm^2, followed by 2% L-lactic acid, iodine, hydrogen peroxide. Within shaved samples, 1% CPC, 2% L-lactic acid, and hydrogen peroxide produced among the greatest reductions with 4.5, 4.1, and 3.9 $\log_{10}$ CFU/100 cm^2 reported, respectively.

Table 1. Least-squares means for the interaction of shaving × antimicrobial agent on APC reduction

	$\log_{10}$CFU/100 cm^2 reduction[a]	
Antimicrobial	Non-shaved	Shaved
Water	0.9c	0.6c
Alcohol	0.5c	1.8bc
1% CPC	4.1a	4.6a
Iodine	1.3c	1.8bc
2% L-lactic acid	2.7b	4.1a
Hydrogen peroxide	4.5bc	4.4a
SEM	0.48	0.48

LS means lacking common letters (a-c) differ ($P < 0.05$).
[a]Log reduction = ($\log_{10}$CFU/100 cm^2 reduction on untreated hide area) – ($\log_{10}$CFU/100 cm^2 reduction on treated hide area).

Least-squares means for the treatment effects of shaving and antimicrobial agents on *E. coli* reduction on hide sections inoculated with 10^6 CFU/g fresh bovine feces are reported in Table 3. Non-shaved samples had a mean reduction of 2.0 $\log_{10}$ CFU/100 cm^2, and shaved samples had a mean reduction of 2.8 $\log_{10}$ CFU/100 cm^2. Within the antimicrobials tested, 1% CPC produced the greatest reduction, followed by 2% L-lactic acid and hydrogen peroxide.

Across all treatments, shaving appeared more advantageous than not shaving when applying antimicrobial agents to reduce bacterial counts on the hide surface. After completion of Trial I, shaving together with 1% CPC, 2% L-lactic acid, and hydrogen peroxide were determined to be the three most effective shaving/antimicrobial combinations, and were selected for further evaluation in Trial II.

Table 2. Least-squares means for the interaction of shaving × antimicrobial agent on coliform reduction

	log_{10}CFU/100 cm^2 reduction[a]	
Antimicrobial	Non-shaved	Shaved
Water	-0.9d	0.5d
Alcohol	0.2d	1.8c
1% CPC	5.3a	4.5ab
Iodine	2.4c	2.5c
2% L-lactic acid	2.8c	4.1b
Hydrogen peroxide	2.2c	3.9bc
SEM	0.43	0.43

LS means lacking common letters (a-d) differ ($P < 0.05$).
[a]Log reduction = (log_{10}CFU/100 cm^2 reduction on untreated hide area) – (log_{10}CFU/100 cm^2 reduction on treated hide area).

Table 3. Least-squares means for treatment effects of shaving and antimicrobial agents on *E. coli* reduction

Treatment effects	log_{10}CFU/100 cm^2 reduction[a]
Shaving	
Non-shaved	2.0b
Shaved	2.8a
SEM	0.17
Antimicrobial	
Water	0.2d
Alcohol	0.9d
1% CPC	4.5a
Iodine	2.4c
2% L-lactic acid	3.3b
Hydrogen peroxide	2.9bc
SEM	0.30

LS means within treatments lacking common letters (a-d) differ ($P < 0.05$).
[a]Log reduction = (log_{10}CFU/100 cm^2 reduction on untreated hide area) – (log_{10}CFU/100 cm^2 reduction on treated hide area).

Trial II

The average initial hide counts before treatment application were 8.1 log_{10} CFU/100 cm^2 for APC, 4.2 log_{10} CFU/100 cm^2 for coliforms, and 4.5 log_{10} CFU/100 cm^2 for *E. coli*. Least-squares means for APCs, coliform, and *E. coli* counts and log reductions on brisket areas of hides before and after treatment are reported in Table 4. For APCs, 1% CPC produced the greatest reduction on the hide with 3.9 log_{10} CFU/100 cm^2 reported. For coliforms and *E. coli*, there were no ($P > 0.05$) differences among treatments for hide reductions. Though few differences existed between antimicrobial treatments, all three resulted in approximately a 3 log_{10}CFU/100 cm^2 reduction when applied to shaved hide surfaces in the brisket region of the carcass.

Table 4. Least-squares means for APCs, coliform, and *E. coli* counts and log reductions on brisket area of shaved hides before and after treatment with 1% CPC, 2% L-lactic acid, or hydrogen peroxide

		log_{10}CFU/100 cm^2		
Indicator organism	Treatment	Before	After	Reduction[a]
APC	1% CPC	8.2a	4.4c	3.8a
	2% L-lactic acid	7.5b	5.2b	2.3b
	Hydrogen peroxide	8.7a	6.5a	2.2b
	SEM	0.22	0.21	0.28
Coliform	1% CPC	4.6b	1.3b	3.3a
	2% L-lactic acid	3.7c	1.1c	2.6a
	Hydrogen peroxide	5.2a	2.6a	2.6a
	SEM	0.20	0.27	0.29
E. coli	1% CPC	4.3b	1.3a	3.0a
	2% L-lactic acid	3.2c	1.1b	2.1a
	Hydrogen peroxide	5.1a	2.1a	3.0a
	SEM	0.24	0.29	0.33

LS means lacking common letters (a-c) differ ($P < 0.05$).
[a]Log reduction = (log_{10}CFU/100 cm^2 reduction on untreated hide area) – (log_{10}CFU/100 cm^2 reduction on treated hide area).

Conclusions

By shaving and applying an antimicrobial agent directly to the hide opening area in the brisket region, we were able to reduce bacterial counts on hide surfaces. This method targets a specific area on the hide that is very susceptible to fecal contamination, yet very critical when opening up the hide for removal. Selective application of these antimicrobials to shaved hide opening sites can reduce bacterial counts on hide surfaces, and therefore potentially reduce final carcass counts in these areas by decreasing the bacterial load before opening. Further research should be conducted to determine effectiveness along additional areas of the hide surface, and to evaluate the practicality of this process outside of a research setting.

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