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Effect of pre-evisceration, skin-on carcass decontamination sanitation strategies for reducing bacterial contamination of cattle during skinning*

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Abstract: The effectiveness of pre-evisceration, skin-on carcass sanitation on reducing bacterial contamination of beef carcasses was tested using 3 cattle per treatment and 3 cattle as controls at each of 3 abattoirs in southern Wisconsin. The sanitation procedure included stunning, bleeding, tying off the esophagus, sealing the anus, and then sanitizing the hide with: i) 20% trisodium phosphate, ii) 200 ppm iodophor, iii) 75% ethanol, or iv) hot water (ca. 80 °C). Two sets of combined sponge samples (3×100 cm^2) were taken from the hide before and after the sanitation step, as well as from the carcass after the final wash. Our results revealed that average reductions in numbers of total aerobic bacteria on the hide ranged from 0.06 to 3.58 $\log_{10}$ cfu per cm^2 depending on the sanitation method. However, regardless of the various sanitation methods tested, no significant differences were found between test and control groups in the level of total aerobic bacterial contamination on the carcasses after the final wash.

Key words: Pre-evisceration, beef carcass decontamination, sanitation, adhesive, trisodium phosphate (TSP), iodophor, ethanol, hot water

Yüzüm öncesi-derili karkas dekontaminasyon sanitasyon stratejilerinin sığır karkaslarında derinin yüzülmesi esnasında oluşan bakteriyel kontaminasyonun azaltılmasına etkisi

Özet: Yüzüm öncesi-derili karkas sanitasyonunun sığır karkaslarında kontaminasyonun azalması üzerine etkisi Güney Wisconsin'de 3 kesimhanenin her birinde her bir deneme için 3 ve kontrol için 3 sığır kullanılarak test edildi. Sanitasyon işlemi bayıltma, kanın akıtılması, özafagusun bağlanması, anüsün kapatılması, ve derinin i) % 20 trisodyum fosfat, ii) 200 ppm iodofor, iii) % 75 etanol ya da iv) sıcak su (≈ 80 °C) ile sanitasyonu aşamalarından oluştu. Sanitasyon işleminden önce ve sonra deriden ve son yıkama işleminden sonra karkastan kombine sünger-sürme tekniği (3×100 cm^2)

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kullanılarak 2 şer adet örnek alındı. Sonuçlar, deride toplam aerobik bakteri sayısındaki ortalama azalmanın sanitasyon metoduna bağlı olarak 0,06 ile 3,58 log_{10} kob/cm^2 arasında değiştiğini ortaya koydu. Ancak, son yıkama sonrasında alınan örneklerde, kontrol ve test grupları arasında toplam aerobik bakteri sayıları bakımından, önemli bir azalma bulunmadı.

Anahtar sözcükler: Yüzüm-öncesi, karkas dekontaminasyonu, sanitasyon, yapıştırıcı, trisodyum fosfat (TSP), iodofor, etanol, sıcak su

Introduction

The primary goal of effective slaughter is to protect the essentially sterile muscles of the carcass from becoming contaminated by the gastrointestinal (GI) tract and/or hide. Since many pathogenic microbes originate in the GI tract and can be present on the hide (1-4), the manner in which cattle are slaughtered is key to preventing microbial contamination of the associated meat. In recent years, implementation of HACCP to food animal slaughter operations has become mandatory in many countries. In the US, beef, pork, chicken, and turkey slaughter plants are required to randomly sample chilled carcasses for *Escherichia coli* Biotype I for verification of HACCP (5). Therefore, meat plants are continually seeking and/or refining carcass decontamination methods to augment their traditional slaughter procedures to achieve an acceptable level of *E. coli* on carcasses.

Fecal contamination of animal carcasses can be lessened by preventing contamination and/or by subsequent decontamination, both of which can be included in a HACCP system. Prevention of contamination includes employing Good Manufacturing Practices (GMPs) and Sanitation Standard Operating Procedures (SSOPs) in sanitary carcass dressing, and by using interventions with potential to reduce the bacterial load on the hide, thus resulting in a possible reduction in the frequency or level of carcass contamination. Strategies for reducing the bacterial load on chilled carcasses have mainly focused on decontamination of carcasses after skinning and evisceration. A number of chemical and physical interventions, such as organic acid sprays and steam pasteurization, have been evaluated with varying success (6,7). A second strategy that has been studied to a lesser extent is decontamination of highly contaminated hide (4,8-13). If the bacterial load of the hide is reduced, then in principal fewer microbes are available for transfer to the carcass surface during dressing. Among treatments used for sanitizing the hide are ozonated and electrolyzed water, cetylpyridinium chloride, alkaline detergents, organic acids, and quaternary ammonium compounds (4,8-13). For example, Bosilevac et al. (9) applied 1% ozonated water or electrolyzed water to cattle hides and reported ca. 2 to 4 log_{10} cfu/cm^2 reduction in numbers of aerobic colony count and enterobacteria. Similarly, Carlson et al., (12) separately applied 10% acetic acid, 10% lactic acid, 3% NaOH, and 5% sodium metasilicate to hide pieces. These researchers reported reductions of 0.6 to 2.4 log in numbers of *E. coli* O157:H7 when applied before a water wash and reductions of 1.5 to 5.1 log when applied following a water wash. In addition to chemicals, hide clipping was evaluated and reported to lower the total aerobic plate count on the hide (14). However, it was not possible to determine if these reductions would also lead to reductions in the microbial load on the carcass because most studies were performed using hide pieces or on the hide after removal from the carcass. Such studies should be repeated using the total hide under commercial conditions. At present, large scale slaughterhouses spray wash live cattle prior to slaughter to provide physical cleanliness. However, the relationship between hide cleanliness and the microbiological quality of carcasses has not been fully elucidated. McEvoy et al. (2) classified live animals based on the level of dirt on their hide. These researchers concluded that the total bacterial count was lower on carcasses of physically cleaner animals. In contrast Schnell et al. (13) reported no significant differences in microbiological counts on cattle that were de-haired compared to cattle that were not.

In addition to the hide, the GI tract is another major source of microbial contamination. Leakage of ingesta through the esophagus or from the feces through the anus may lead to contamination of the carcass with pathogenic bacteria. This is especially

problematic for small scale slaughter plants that typically dress carcasses on skinning beds rather than by slaughtering on conveyor lines. Interventions to minimize carcass contamination from the GI tract are lacking. As one approach, closing both ends of the GI tract and removing it as a single piece may be sufficient to eliminate contamination from the GI tract. Bung bagging, a procedure for isolating the anus and extra genital organs in female animals in a bag before evisceration, is a common practice in some countries, including the U.S. However, bung bagging is not performed until after the opening of the rear legs and, thus, fecal leakage is not uncommon, especially when the carcasses are held on skinning beds for extended periods of time.

The objective of this study was to explore the efficacy of pre-evisceration, skin-on carcass sanitation with different chemical sanitizing agents on potentially lowering the levels of bacterial contamination on chilled beef carcasses. In an era where the inspection of meat has shifted from visible cleanness to microbial cleanness, this research is intended to help reduce microbial contamination and control pathogenic bacteria such as *E. coli* 0157:H7 during slaughterhouse operations, particularly for small to very small slaughter plants.

Materials and methods

Pre-evisceration, skin-on carcass sanitation:

A complete flow diagram of the experiment is provided in the Figure. Following stunning and bleeding, both ends of the GI tract were closed by covering the neck with a plastic bag and tying off the esophagus after head removal and by sealing the anus, as well as the outer urogenital organs for cows and heifers, using PVC patches and cyanoacrylamide (Super Glue; Pacer Technology, Rancho Cucamonga, CA). The patches were 8 cm in diameter for steers and bulls and 15 × 8 cm for heifers and cows. Next, the hide was sanitized with selected sanitizing agents by brushing after spraying with a thermoinsulated plastic container and an airless paint sprayer (Model 404plus; Wagner Spray Tech. Corp., Minneapolis, MN). Sanitizing treatments were as follows: i) 20% trisodium phosphate (TSP) (Rhone-Poulenc Inc., Cranbury, NJ) at 45 °C, ii) 200 ppm iodophor

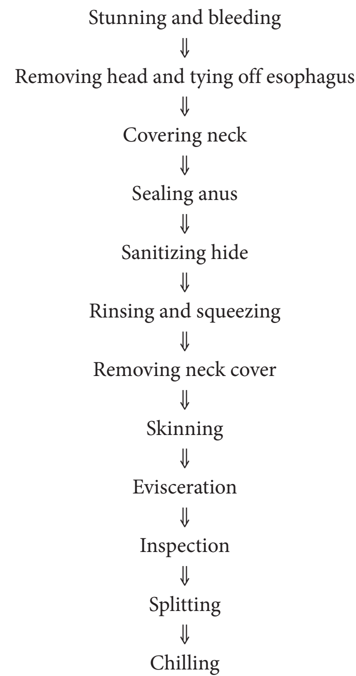

Figure. Flow diagram of pre-evisceration, skin-on carcass sanitation of beef carcasses.

(Microklene; Ecolab Inc., St. Paul, MN) at 45 °C, iii) 75% ethanol (Aaper Alcohol and Chemical Co., Shelbyville, KY) at ambient temperature, and iv) hot water (80 °C).

The sanitation of skin-on carcasses was performed on carcasses positioned horizontally on the skinning bed. Areas most contaminated and/or frequently touched by knives and employees, that being the front and rear legs, peri-anal area, midline, and brisket areas, were the focus of the sanitation efforts. After each spray and wash, carcasses were held for 3 min prior to rinsing and squeezing to remove the excessive fluid by means of a squeegee.

These experiments were conducted at 3 state-inspected meat plants in southern Wisconsin in the presence of a veterinary meat inspector. Three animals for each sanitation treatment and 3 animals for each control (neither hide sanitation nor GI tract sealing) were used at each plant. The animals were selected at random among the cattle to be slaughtered on the day of an experiment for a given plant. Total numbers of the slaughtered cattle used for the present study were 45. Each plant was considered as an independent trial.

Carcass sampling and microbiological analyses:

Two sets of sponge samples (3 × 100 cm^2 each) were taken from the hide before and after sanitation using dry Sponge-Whirlpack bags (International Bioproducts, Inc., Munice, IN, USA) containing 25 mL Butterfield's Phosphate Buffer (International Bioproducts). Each sponge was used to sample 100 cm^2 from each of the peri-anal, midline, and brisket areas. The sponge samples were taken using 100 cm^2 sterile, plastic, disposable 10 × 10 cm templates (International Bioproducts) and sterile gloves following standard procedures (5). The sampling procedure included 10 horizontal scrubbing motions followed by 10 vertical scrubbing motions for each site using sponges previously rehydrated with Butterfield's Phosphate Buffer and then squeezed manually from outside of the sterile bag prior to sampling. Therefore, one sample is composed of sampling a total of 300 cm^2. After evisceration and final wash, another 2 sets of samples (1 sample/side) were taken from each carcass from the round, flank, and brisket areas of the carcass.

The sample bags were transported on ice to the Food Research Institute, University of Wisconsin-Madison, for microbiological testing. Serial dilutions of each sample were made using 0.1% (v/v) peptone (Difco Laboratories, Detroit, MI) following stomaching of sample bags for 2 min at room temperature (Model 400, Tekmar Co., Cincinnati, OH). A 0.1-mL portion of each dilution was surface-plated onto total plate count agar (Difco) in duplicate and incubated at 37 °C for 24 h before colonies were counted. Pathogen levels were converted from cfu/mL to cfu/cm^2 by the following formula where C is mean colony number of duplicate plates, 25 is the amount of buffer, D is the inverse of the dilution factor, and 300 is the total surface area sampled:

$$\text{Cfu} / \text{cm}^2 = (C \times 25 \times D) / 300.$$

Statistical analyses:

Microbiological data were evaluated using a 3 × 5 × 3 × 2 (replicate × treatment × sampling site × subsamples) factorial design. Data were analyzed by analysis of variance for main (fixed) effects using the Statistical Analysis System (SAS) (15). Least squares means were separated using Fisher's least significance difference test (LSD) using the general linear models (GLM) procedure of SAS. A significance level of 0.05 was used for all statistical analyses.

Results

There was appreciable variation in the effectiveness of the sanitizing agents to reduce bacterial numbers on the hide of cattle. Average reductions were 3.38, 1.78, 1.18, and 0.06 $\log_{10}$ cfu/cm^2 for hot water, TSP, ethanol, and iodophor, respectively (Table). Statistical analyses of the data revealed that

Table. Effect of pre-evisceration, skin-on carcass decontamination method on the level of contamination with aerobic bacteria of beef carcasses ($\log_{10}$ cfu/cm^2 ± SD, n = 3).

Samples	Treatments[a]				
	Iodophors[c]	TSP[d]	Ethanol[e]	Hot water[f]	Control
Hide	6.20 ± 0.22	5.64 ± 0. 30	5.86 ± 0.38	6.33 ± 0.39	5.67 ± 0.18
Hide-AS[b]	6.14 ± 0.25	3.86 ± 0.82	4.68 ± 0.50	2.75 ± 0.24	NA[g]
Carcass	2.92 ± 0.21	2.69 ± 0.64	2.65 ± 0.17	2.32 ± 0.20	2.70 ± 0.42

a, all treatments were applied as continuous spray for 3 min followed by holding for 3 min
b, samples taken from hide after sanitation
c, 200 ppm concentration at 45 °C
d, Trisodium Phosphate at 20% (w/v) concentration at 45 °C
e, 75% (v/v) concentration at ambient (ca. 20 °C) temperature.
f, 78-80 °C
g, not applicable

regardless of the sanitation method and associated reduction in microbial load, numbers of total aerobic bacteria on pre-evisceration, skin-on sanitized carcasses were not significantly different between treated and control carcasses ($P > 0.05$).

Discussion

Treatment with the iodophor was relatively ineffective in reducing the microbial load, even when using 200 ppm rather than the 120 ppm suggested by the manufacturer. In general, the chemical sanitizers were less effective than hot water. The use of higher concentrations, larger volumes, or longer contact times may result in better efficacy of the various treatments. More specifically, hides were exposed to chemical sanitizing solutions for 6 min and only 6 L of solution per carcass was used for each chemical treatment. In contrast, the volume sprayed and pressure used for the hot water treatment was appreciably higher since the plant source was used for hot water.

There may be several reasonable explanations for the lack of significant differences between the treatment groups in samples taken after final wash. First, dressing of cattle with wet hides, even in the presence of a low bacterial load, may promote bacterial transfer by employees and equipment from the hide to the carcass. Second, the hide may not be as important as employee practices for skinning and evisceration relative to translocation of bacteria to the surface of a carcass, even if the hide has a very high bacterial load. Finally, it is also noteworthy that in most small-scale meat plants the locations where live animals are handled/killed are very close in proximity to where animals are skinned/eviscerated. The findings from our study are consistent with what Schnell et al. (13) reported about chemical de-hairing, a process wherein sodium sulfide is applied to dissolve the hair on hides in a pressure-controlled cabinet, followed by a subsequent neutralization step with hydrogen peroxide. The authors concluded that de-hairing did not significantly reduce the overall bacterial count on the carcass when compared to a control group, but did enhance the visual cleanness of carcasses. There are many other studies in which various sanitizing agents were applied to cattle hides for decontamination purposes. Reductions of up to 5.0 $\log_{10}$ cfu/cm^2 were reported using hides that were inoculated following removal from the carcass (4,8-13). It remains unknown if greater or lesser reductions would be obtained on the dressed carcasses.

This study was not designed to evaluate the effect of closing the GI tract alone on the subsequent reduction in levels or types of the fecal flora because samples were tested for total aerobic bacteria only. However, a limited number of ethanol treated and hot water washed carcasses were also tested for total coliforms using Violet Red Bile Agar (data not shown). Results indicated no appreciable differences in the numbers of coliform bacteria between control and treated carcasses, despite the fact that feces from the GI tract were prevented from contaminating the carcasses while on the skinning bed. Efforts were not made to vary the holding time, temperature, presence/absence of GI tract closing, and/or other variables because: i) there was no direct evidence to suggest that the lower the bacterial level on the hide, then the lower the bacterial level on the carcass, ii) extending the holding/sanitation time to 6 min would not be deemed economically feasible by cooperative processors, despite the need for longer holding times for increasing the efficiency of chemical sanitizers, and iii) the proposed sanitizing procedure is very labor demanding. That being said, sealing the anus and external genital organs of female cattle using adhesive patches was effective in preventing leakage of feces and other excreta. When such leakage occurs, there is greater opportunity for an employee to experience difficulties to avoid cross contamination.

Results obtained in this study were limited relative to the nature, number, and magnitude of the sanitizing agents used, as well as the microorganisms tested. As a future endeavor, the pre-evisceration, skin-on carcass sanitation approach can be improved upon with more advanced sanitation methods to reduce the bacterial load on the hide to below the detection limit by standard culture methods. In conclusion, strategies for increasing the microbial safety of food animal carcasses should be based on sanitary dressing practices by employees and proper plant designs that are capable of mitigating any untoward effects of poor processing methods.

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