ABSTRACT  A study was conducted to determine external microbiology of genetically featherless broiler carcasses after forced cloacal fecal expulsion. Full-fed featherless broilers were placed into coops, transported, unloaded, shackled, stunned, suffocated, weighed, and divided into 3 treatments groups. Carcasses were transferred to a separate shackle line and passed through a machine designed to induce defecation (squeeze) and then remove external feces (wash). Treatments were obtained by turning the squeezing and washing components on or off. Treatments were as follows: S carcasses were squeezed but not washed; W carcasses were not squeezed but were washed; and SW carcasses were squeezed and washed. Concentrations of total aerobic microorganisms (AB), Escherichia coli (EC), coliforms (CF), and Campylobacter (CPY) recovered from whole carcass rinses did not vary with treatment ($P > 0.05$). However, counts of Salmonella (SAL) in rinses of S carcasses were 1.4 log$_{10}$ cfu/mL greater than counts of SAL found in rinses of SW carcasses ($P < 0.05$). The SAL prevalence was similar for S (86% positive), W (90% positive), and SW (83% positive) carcasses ($P > 0.05$). Populations of AB and CF recovered from wash water (water applied in the machine after fecal expulsion) for SW carcasses were significantly higher by 3.1 and 1.5 log$_{10}$ cfu/mL, respectively, than the populations of the same bacteria recovered from wash water for W carcasses ($P < 0.05$). Levels of EC and CPY recovered from wash water did not vary with treatment. There was no difference in CPY and SAL prevalence in water collected after washing W carcasses or SW carcasses ($P > 0.05$). Data from the present study show that controlled cloacal fecal expulsion followed by carcass washing immediately after slaughter can be used to minimize the numbers of carcass Salmonella and can reduce the likelihood of visible carcass fecal contamination or cross-contamination to other carcasses and processing equipment.

Key words: broiler, carcass contamination, carcass microbiology, cloacal defecation
reduce gastrointestinal contents of broilers before processing (Farhat et al., 2002; Northcutt et al., 2003).

In the processing plant, research techniques to decrease carcass fecal contamination have involved cloacal plugging (Musgrove et al., 1997) and vent suturing (Buhr et al., 2003) or gluing (R. J. Buhr, personal communication). Mechanical extraction techniques of cloacal contents have also been used on freshly slaughtered poultry by applying a compression force to the exterior surfaces (Simmons, 1988; Van Der Eerden, 1990; Aandewiel et al., 2004; Clark, 2004) or by applying a pulsating vacuum inside the vent (Harben and Clark, 1989). Older devices were designed to be inserted on commercial processing lines after carcass defeathering, but before evisceration (Simmons, 1988; Harben and Clark, 1989; Van Der Eerden, 1990); however, more recently, equipment manufacturers have focused on expressing fecal material from carcasses immediately after slaughter (Aandewiel et al., 2004; Clark, 2004). Clark (2004) suggested that the application of a compression force to a freshly slaughtered and feathered carcass minimized contamination by preventing direct contact between feces and skin surfaces. Aandewiel et al. (2004) indicated that removal of fecal material from broiler carcasses immediately after slaughter reduced the risk of contamination and cross-contamination during scalding and defeathering. Because it is well documented that bacterial contamination may occur during scalding (Mulder and Dorreresteijn, 1977; Slavik et al., 1995; Cason et al., 1999), defeathering (Acuff et al., 1986; Izat et al., 1988; Musgrove et al., 1997; Berrang et al., 2001; Buhr et al., 2003), and evisceration (May, 1961; Thayer and Walsh, 1993; Russell and Walker, 1997), it stands to reason that controlled cloacal fecal expulsion before scalding would minimize unintentional carcass contamination. The present study was conducted to determine the effects of broiler carcass fecal expulsion before scalding on populations of microorganisms recovered from skin surfaces.

MATERIALS AND METHODS

Broilers, Inoculation, and Processing

Broilers used in this study were genetically featherless from the scaleless lines as described by Buhr et al. (2003). Chicks were hatched and reared to 8 to 10 wk of age in a controlled environment-type house. Forty-eight hours before processing, 27 to 30 broilers were challenged orally and intracloacally with 1 mL of a suspension containing approximately 10^8 cells of Campylobacter jejuni. Twenty-four hours later, the same birds were given 1 mL of a suspension containing 10^8 cells of Salmonella Typhimurium administered both orally and intracloacally. On the day of processing, full-fed broilers were placed into coops and transported approximately 0.4 km to the pilot plant. Birds were unloaded, shackled by their feet, and killed individually by electrical stunning (120 V of alternating current for 8 s head to vent) immediately followed by hypoxia induced by asphyxiation. Carcasses were then weighed and divided into 3 treatment groups.

Treatments

Carcasses from all 3 treatments were transferred to a separate shackle line and passed through a compression apparatus designed to express (squeeze) and remove (wash) external feces (Aandewiel et al., 2004). Treatments were obtained by turning on or off the squeezing and washing components of the apparatus. The apparatus is a 180° machine whereby carcasses suspended from an overhead shackle line pass through a circular presser and proceed through a water/air spray (Aandewiel et al., 2004). The apparatus consists of 16 units. Each unit consumes approximately 6.8 L/min at 276 kPa. For this study, each carcass received 0.5 L. After washing fecal material from the carcass, water exits the apparatus through a tapered funnel where it may be collected. Treatments were as follows: S carcasses were squeezed but not washed; W carcasses were not squeezed but were washed; and SW carcasses were squeezed and washed. After treatment, carcasses were weighed and the feet removed at the hocks.

Microbiology

Carcasses were then subjected to a low volume whole carcass rinse (WCR) procedure (Lillard, 1988). For the WCR, carcasses were placed in a bag with 100 mL of 0.1% of peptone solution and shaken in an automated carcass shaking machine for 1 min. After shaking, carcasses were removed aseptically and the rinse was sampled for microbial recovery.

Rinses were analyzed for total aerobic bacteria (AB), Escherichia coli (EC), coliforms (CF), Campylobacter (CPY), and Salmonella (SAL). Serial dilutions of the rinsate were prepared in 0.1% peptone. The AB populations were enumerated on plate count agar (Becton Dickinson, Sparks, MD), A 0.1-mL sample from a serial dilution of the rinse was plated onto duplicate EC/coliform Petrifilm plates (3M Health Care, St. Paul, MN), and plates were incubated at 35°C for 24 h. After incubation, blue colonies closely associated with entrapped gas were counted as EC, whereas blue and red colonies closely associated with entrapped gas were counted as CF. For Salmonella enrichment (replications 1 and 3), 30 mL of the WCR or 30 mL of the water collected from the apparatus exit (W and SW carcasses only) were incubated overnight at 35°C. One-tenth milliliter of rinse or water was transferred to 10 mL of Rappaport-Vassiliadis (Becton Dickinson) broth, and 0.5 mL of rinse or water was trans-
ferred to tetrathionate (Becton Dickinson) broth and incubated 24 h at 42°C. Each broth was then streaked onto brilliant green sulfa plates containing 200 mg/L of nalidixic acid and 25 mg/L of novobiocin (Sigma-Aldrich, St. Louis, MO). Plates were incubated for 24 h at 35°C and then inspected for typical Salmonella colonies. Confirmed samples were recorded as SAL positive and reported as prevalence data. The SAL enumeration was determined on carcass rinses collected during the last 2 replications by directly plating serial dilutions onto brilliant green sulfa plates containing 200 mg/L of nalidixic acid and 25 mg/L of novobiocin, incubating for 24 h at 35°C, and counting typical Salmonella colonies. The SAL-positive colonies were then confirmed using triple sugar iron and lysine iron agar. Each colony type was further identified using the Microgen Salmonella Latex Agglutination Kit (Microgen, New York, NY). The CPY was enumerated by plating 0.1 mL from the serial dilutions onto Campy Blood agar (Blaser; Difco Laboratories, Detroit, MI) and incubating the plates at 42°C for 36 h in a microaerobic environment (5% O2, 10% CO2, and balance N2). Colony-forming units characteristic of Campylobacter spp. were counted. Each colony type identified as CPY was confirmed for genus by examination of cellular morphology and motility on a wet mount under phase contrast microscopy. Each colony type was further identified as Campylobacter spp. using INDX-Campy (jc) culture confirmation test kit (Integrated Diagnostics, Baltimore, MD).

**RESULTS AND DISCUSSION**

Forty-eight hours before each of the 5 processing dates, live birds were sorted and selected for weight (±200 g) to ensure that similar-sized broilers were used for each replication. Although broiler weight was standardized for each replication, size varied during the study from 1.8 to 6.1 kg and the overall average live weight at slaughter was 2.9 kg. After slaughter and treatment, W carcasses gained an average of 7.3 ± 2.6 g (0- to 38-g range) due to moisture absorption from carcass washing. Application of the compression force resulted in weight lost from fecal expression for S carcasses (6.1 ± 2.0 g), and this loss ranged from 0 to 46 g. When the compression force was combined with carcass washing to remove expressed feces, weight loss was offset by moisture retention and SW carcasses gained an average of 2.1 ± 1.9 g (0 to 28 g range). Several other studies have investigated broiler gastrointestinal contents, carcass fecal contamination, or both during processing (Wabeck, 1972; Veerkamp, 1986; May and Deaton, 1989; Papa, 1991; Northcutt et al., 1997; Buhr et al., 1998; Northcutt et al., 2002; Northcutt et al., 2003). Gastrointestinal contents of full-fed chickens have been reported to vary between 50 and 70 g depending upon bird eating patterns, age at slaughter, and processing conditions (i.e., method of immobilization) that may expel feces (Buhr et al., 1998; Northcutt et al., 2002). These same factors possibly contributed to the variation in carcass weight loss or gain during the present study.

The populations of microorganisms recovered from W, S, and SW carcass rinses are shown in Table 1. There was no significant difference in numbers of AB, EC, CF, or CPY recovered from carcass rinses for the 3 treatments (P > 0.05). During the study, SAL prevalence was determined on carcass rinses collected during the first and the third replications, whereas SAL enumeration was performed in the forth and fifth replications. The SAL prevalence was similar for all 3 treatments with 90% SAL positive (26/29) W carcasses, 86%
Squeezed and washed carcasses treated only with the washing component of the mechanical apparatus turned on; washed and squeezed carcasses treated with both the washing and compression components of the apparatus turned on.

Table 2. Populations of bacteria recovered in the water used to wash carcasses after fecal expulsion by the compression apparatus

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Washed</th>
<th>Squeezed and washed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aerobic</td>
<td>3.0 ± 0.5</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>4.0 ± 0.8</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td>Coliforms</td>
<td>3.6 ± 0.7</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>5.4 ± 1.3</td>
<td>4.6 ± 1.6</td>
</tr>
</tbody>
</table>

*Within bacteria type, significant difference in the level of bacteria recovered between washed or squeezed and washed treatments (P < 0.05).

1Means for log_{10} cfu/mL of rinse ± standard error.

2Treatments administered by turning compression (squeezing) and washing components of a mechanical apparatus on or off.

3Washed carcasses treated only with the washing component of the mechanical apparatus turned on.

SAL positive (25/29) S carcasses, and 83% SAL positive (24/29) SW carcasses (P > 0.05). *Salmonella* was not detected in rinses collected from W carcasses, but levels recovered from S carcasses (4.9 log_{10} cfu/mL) were more than 10 times those recovered from SW carcasses (log_{10} 3.5 cfu/mL). This demonstrates that washing after force evacuation removed some of the SAL that may have been deposited on the carcass skin surface. Previous research by Musgrove et al. (1997) demonstrated that cloacal plugging of broilers before slaughter reduced levels of gram-negative enteric bacteria (0.4 log_{10} lower) as compared with nonplugged control broilers. Moreover, plugging also reduced CPY levels (0.5 log_{10} lower) and CPY prevalence (81 versus 97% positive) in carcass rinses as compared with CPY levels or prevalence in rinses of nonplugged control carcasses (Musgrove et al., 1997). Blankenship et al. (1993) showed that fecal contamination on broiler carcasses could be removed by reprocessing (washed, trimmed, or vacuumed, or a combination of these) off-line without compromising carcass microbiology, and reprocessed carcasses were microbiologically equivalent to inspection-passed carcasses. Similar findings were reported by Fletcher et al. (1997) for on-line reprocessing. Buhr et al. (2003) compared the microbiology of featherless broilers where half were plugged and sutured before scalding to prevent cloacal leakage during defeathering. When cloacal leakage was prevented, microbial recovery was reduced by 0.5, 2.1, 2.3, and 1.1 log_{10} cfu/mL for AB, EC, CF, and CPY, respectively (Buhr et al., 2003). Data from the present study show that forced expression of fecal material may increase carcass SAL numbers, but washing expressed feces off the carcasses will then reduce SAL numbers and possibly reduce subsequent intestinal leakage.

During this study, the compression apparatus was set to apply water to carcasses in the W and SW treatments, and this water was collected at the exit for analyses (Table 2). Populations of AB and CF recovered from water after washing SW carcasses were significantly higher (3 and 1.5 log_{10} cfu/mL higher, respectively) than from water used to wash W carcasses (P < 0.05). No significant difference was found in number of EC or CPY recovered from water samples (P > 0.05). This may be explained by the fact that EC was below the level of detection in 2 out of 5 water samples collected for W carcasses, and CPY was not detected in 3 out of 5 of the water samples collected for W. All of the water samples collected from SW carcasses had detectable levels of CPY, but only 3 out of 5 of the SW water samples had detectable levels of CPY. There was a significant difference in the SAL prevalence for water samples with 0 versus 100% positive for W and SW water samples, respectively (P < 0.05).

The present study demonstrated that forced cloacal evacuation resulted in the removal of least 6 g of fecal material per bird for full-fed broilers. After evacuation and washing (SW), carcasses were microbiologically indistinguishable from those that were not forced to evacuate (W). This is significant because high levels of CPY (10^8) and SAL (10^8) were administered to broilers before processing. After compression, nearly 5.0 log_{10} cfu/mL of SAL was deposited onto carcasses, but this was significantly reduced (by 1.4 log_{10} cfu/mL) when expelled material was washed off. Because the location of the compression apparatus on a commercial processing line would be before scalding, fecal material and bacteria would not be deposited into the scald water. Bolder (1998) suggested that the scalding process exposes carcasses to significant levels of organic debris and bacteria from deposited fecal material. Alternative processing procedures, such as controlled expression and removal of gastrointestinal contents during the early stages of processing, could improve carcass uniformity and prevent subsequent cloacal voiding, carcass contamination, and cross-contamination during scalding, defeathering, and evisceration.

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