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Microbiology of Contaminated or Visibly Clean Broiler Carcasses Processed with an Inside-Outside Bird Washer

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Abstract: Processors are washing carcasses with one or more inside-outside bird washers (IOBW) to comply with the zero tolerance for visible feces regulation mandated by the USDA Food Safety Inspection Service. A study was conducted to determine the effect of an IOBW on total aerobic bacteria, *E. coli, Campylobacter*, and *Salmonella* recovered from uncontaminated (control), contaminated, and possibly cross contaminated broiler carcasses at two different IOBW water pressure settings. In each of three trials, 12 commercially processed carcasses, divided into two groups each containing two control carcasses, two carcasses contaminated with 0.1g cecal contents (inoculated with *Campylobacter* and *Salmonella*), and two carcasses uncontaminated and placed adjacent to contaminated birds during washing (to determine cross contamination) were prepared (n=36). Whole carcass rinses were conducted on carcasses before contamination and washing, then again after washing. Carcasses were washed with an in-line commercial IOBW set at 140 birds per minute for a 5 sec dwell time and either 276 or 552 kPa (40 or 80 PSI) water pressure. Counts of total bacteria, *E. coli, Campylobacter*, or *Salmonella* were not significantly affected ($P<0.05$) by contamination with feces, by cross-contamination, or by IOBW pressure. The overall effect of washing was a slight but significant reduction in total aerobic bacteria (4.9 to 4.8) and *E. coli* (3.2 to 3.0) log cfu/ml rinsate. The IOBW decreased the incidence of *Campylobacter* from 22/36 positive carcasses (14 positive incoming carcasses plus 8 inoculated carcasses) to 1/36 positives, while *Salmonella* incidence decreased from 12/36 contaminated (inoculated) carcasses to 3/36 positive carcasses after washing. The IOBW removed carcass contamination to levels equivalent with uncontaminated controls without cross contaminating other carcasses. The incidence of *Campylobacter* was decreased, as was *Salmonella* to a lesser extent. Small reductions of bacterial numbers were noted for total bacteria and *E. coli*.

Key words: Inside-outside bird washer, fecal contamination, *E. coli, Campylobacter, Salmonella*

Introduction

Many poultry processors are now using inside-outside bird washers (IOBWs) as final carcass washers on the evisceration line. The IOBWs assist plants in complying with the government directive mandating zero tolerance for feces on carcasses entering the chiller (USDA, 2005), as washing reduces the incidence of visible feces on carcasses (Fletcher *et al*., 1997). FSIS regulations also require monitoring and reduction of *E. coli* and *Salmonella* on post-chill carcasses (USDA, 1996).

Previous research has been conducted to determine the effect of spray wash cabinets (bird washers), and more specifically IOBWs, on counts and incidence of bacteria on broiler carcasses. Overall, bird washers have been shown to reduce bacterial counts on eviscerated carcasses prior to immersion chilling (Keel and Parmalee, 1968; May, 1974; Izat *et al*., 1988; Bashor *et al*., 2004). The incidence of *Campylobacter* on broiler carcasses was reduced when IOBWs were used in combination with water chilling (Bashor *et al*., 2004). Application of acidified sodium chlorite in an IOBW reduced the incidence of *Salmonella* and *Campylobacter* on broiler carcasses (Kemp *et al*., 2001).

Other research has shown that washers do not always reduce microbiological contamination. Thomson *et al*. (1974) reported no decrease in broiler carcass total bacteria counts after spray washing in water at ambient temperature. Yang *et al*. (1998) found that IOBWs using only tap water did not reduce aerobic plate counts of chicken carcasses. Coliform and *E. coli* carcass counts were not reduced by IOBWs at three different commercial broiler processing plants (Northcutt *et al*., 2003). In a review article, Gill (2004) stated that while carcass washing removes visible contamination, microbiological contamination may remain unaffected.

Processors must remove visible feces from carcasses, yet also hope to reduce bacteria using IOBWs. Washing also has associated operational costs due to increased water usage. Bashor *et al*. (2004) measured water usage in four plants with varying types and configurations of IOBWs and found amounts of water used in IOBWs ranged from 2 to 9 liters per bird. Water

could be conserved by reducing water flow through the IOBW, perhaps by decreasing the incoming pressure. A prior report found no difference in bacterial counts on broiler carcasses when washer water pressures were decreased from 30 to 20 PSI (Thomson *et al.*, 1974). Another study that surveyed a number of plants reported that increasing water to bird washers generally removed more total bacteria and *Enterobacteriaceae* from carcasses, although a few plants that decreased water to bird washers actually lowered bacterial counts on carcasses (Mulder and Bolder, 1981).

IOBWs appear effective at removing visible fecal contamination, and probably reduce most pathogenic bacterial counts and incidence on carcasses. Operation of IOBWs does increase water usage and costs. Therefore, the objective of this study was determining the extent at which IOBWs are effective in reducing bacterial counts and incidence on both visually clean and fecally contaminated carcasses, and whether reducing water pressure would affect reductions in counts or incidence.

Materials and Methods

In each of three replicate trials, 12 eviscerated broiler carcasses were collected from a commercial processing plant shackle line prior to the IOBW. Carcasses were individually placed into plastic bags and transported to the laboratory. Each carcass was subjected to a low volume whole carcass rinse procedure (Cox *et al.*, 1981). Carcasses were rinsed by adding 100 ml sterile phosphate buffered saline (PBS) to each in a bag and shaking with an automated carcass shaking machine for 60 s (Dickens *et al.*, 1985). Rinses were aseptically collected and serial dilutions were prepared for determination of total aerobes, *E. coli*, *Campylobacter*, and *Salmonella* numbers as described below.

Approximately 15 intestinal tracts were also obtained from the evisceration line at the commercial plant and placed together in a clean plastic bag. After transport to the laboratory the ceca were separated from the intestines, and cecal contents were collected and pooled. Cecal contents were stirred manually with a sanitized spatula. On a per gram basis, *Campylobacter* (10^7 CFU), an isolate from broiler chicken feces, and a nalidixic acid-resistant *Salmonella* Typhimurium (10^6 CFU) were added to the cecal contents. The cecal material and cultures were thoroughly combined by manual stirring. Samples of this inoculated cecal mixture were taken and total aerobes, *E. coli*, *Campylobacter*, and *Salmonella* numbers were determined as described below.

The 12 carcasses per trial were divided into two groups of 6 carcasses. In each group, 0.1 g of the inoculated cecal contents was applied to the breast area of two carcasses to simulate fecal contamination. These two contaminated carcasses plus four uncontaminated carcasses were left uncovered at room temperature for 12 min to simulate the maximum time carcasses would typically be exposed in a plant from fecal contamination resulting from evisceration (approximating the time from the beginning of evisceration to final bird washing).

The six carcasses were placed on a shackle line in a pilot plant and washed in an IOBW (model MBW-16, Stork Gamco Inc., Gainesville, GA). Carcasses were placed such that the first two carcasses would enter and exit the IOBW before any other carcasses entered, providing uninoculated controls. The next four carcasses were placed side-by-side, with the first and third carcasses contaminated. The second and fourth carcasses were uncontaminated to determine if cross contamination occurred in the IOBW. The IOBW was operated at an incoming water pressure of 552 kPa (80 PSI) for the first group of six carcasses, then at 276 kPa (40 PSI) for the next group of six carcasses. Between groups of carcasses the IOBW and shackle line was washed and sanitized to eliminate cross contamination from the equipment. The IOBW was operated at a commercial line speed (140 birds per min). However, the pilot plant shackle line was configured on 30.5 cm (12 in) centers per bird rather than the typical commercial setting of 15.3 cm (6 in) centers per bird.

After washing, carcasses were immediately placed into clean plastic bags and rinsed with 100 ml PBS, then rinsate collected for bacterial analysis as described previously. The rinsate, as well as the pre-wash rinsate and cecal samples, were prepared for serial dilution in PBS. Total aerobic bacterial counts were determined by direct plating onto the surface of plate count agar (Becton Dickinson and Co., Sparks, MD). Plates were incubated at 35 C for 24 h. *E. coli* were enumerated by plating 1 mL from a serial dilution of the sample onto duplicate petrifilm *E. coli* / coliform count plates (3M Health Care, St. Paul, MN 55144). Petrifilm plates were incubated at 35 C for 18 to 24 h and the types of colonies characteristic of *E. coli* were counted. *Campylobacter* culture was conducted by direct plating onto the surface of Campy-Cefex agar (Stern *et al.*, 1992) which was incubated at 42°C for 48 h in a sealable plastic bag flushed with microaerobic gas consisting of 5% O_2, 10% CO_2 and balance N_2 (BOC Gases, Chattanooga, TN 37415). Colonies with the characteristic appearance of *Campylobacter* were counted. Each colony type from every sample was confirmed as *Campylobacter* by observation of cellular morphology and motility on a wet mount using phase contrast microscopy. Each colony type was further confirmed by a positive reaction from a serological latex agglutination test kit (Panbio, Inc., Columbia, MD 21046). *Salmonella* (nalidixic acid-resistant) counts were determined by plating onto the surface of BG-Sulfa agar (Becton, Dickinson and Co.,

Table 1: Mean numbers (log cfu/ml) ± SD and incidence of total aerobic bacteria, *Escherichia coli*, *Campylobacter* and *Salmonella* of broiler carcasses before and after the Inside-Outside Bird Washer. Cecal contents (0.1 g) with added *Campylobacter* and nalidixic acid-resistant *Salmonella* cultures, were applied to 12 of the 36 total carcasses prior to washing

	Total aerobic bacteria	*E. coli*	*Campylobacter*	*Salmonella*
Pre-wash	4.9±0.1	3.2 ± 0.1	2.8±0.3	0.0±0.0
	(36/36)	(36/36)	(14/36)	(0/36)
Post-wash	4.8±0.1	3.0 ± 0.1	1.0±0.0	1.4±0.3
	(36/36)	(36/36)	(1/36)	(4/36)
Probability	0.0338	0.0087	-	-

Sparks, MD 21152) with the addition of 200 ppm sodium salt of nalidixic acid (Sigma Chemical Co., St. Louis, MO 63178) (BGS-NAL). BGS-NAL plates were incubated at 35 C for 24 hours and colonies characteristic of *Salmonella* were counted.

Bacterial numbers were converted to log cfu/ml for statistical analysis. Differences in numbers of bacteria due to IOBW pressure or contamination treatment were tested by analysis of variance using the General Linear Models procedure of SAS (SAS, 1999) at the $P<0.05$ level of significance. Means were pooled where no significant differences or interactions were observed. Carcass samples without detectable numbers were treated as missing values in the analysis. The paired t test in SAS (SAS, 1999) was used to determine differences between numbers of bacteria on carcasses pre- and post-IOBW.

Results and Discussion

Numbers of bacteria (log cfu/ml) for pre- and post-washed carcasses are presented in Table 1. Means of contaminated, control, and possible cross-contaminated carcasses, plus the means of carcasses washed at either 276 or 552 kPa (40 or 80 PSI) were combined as there was no significant effect ($P<0.05$) of pressure, contamination treatment, or their interaction on bacteria counts. Total aerobic bacteria decreased slightly but significantly from 4.9 to 4.8 log cfu/ml due to washing, with no change in the incidence of total aerobic bacteria. Similarly, *E. coli* numbers decreased from 3.2 to 3.0 log cfu/ml after washing, and all carcasses remained positive after washing. Although significant per the t test comparison, it is unlikely the pre-and post-wash differences are of practical significance for poultry processors.

Pre-wash *Campylobacter* numbers were 2.8 log cfu/ml, and 1.0 log cfu/ml post-wash. Pre- and post-wash means were not tested for significance as only one carcass was positive for *Campylobacter* after washing. Fourteen carcasses were positive for *Campylobacter* prior to washing, with another eight carcasses receiving *Campylobacter* inoculation from the applied cecal material. The IOBW therefore reduced *Campylobacter* incidence from as many as 22 carcasses to one carcass (100% to 4.5 % positive).

Salmonella, a nalidixic acid-resistant strain, was applied only through cecal material and therefore was absent from all pre-wash carcasses; however, one carcass tested positive for one colony of a nalidixic acid-resistant *Salmonella*. A naturally occurring strain of this type of *Salmonella* could have been present or a cross contamination error occurred during the experiment. After application of the inoculated cecal material to 12 total carcasses, *Salmonella* was found on four post –wash carcasses, for a reduction of positive carcasses from 100% to 33.3%. The mean *Salmonella* count was 1.4 log cfu/ml after washing.

The very slight reduction of total aerobic bacteria (significant only because the t test was used) compares with a report that an IOBW did not reduce aerobic plate counts (Yang *et al.*, 1998). Results may disagree with report from May (1974) where spray washing was found to reduce broiler carcass aerobic counts. Northcutt *et al.* (2003) reported commercial IOBWs lowered total carcass bacteria at one plant but not at two other plants. The very slight reduction in *E. coli* counts agrees with Northcutt *et al.* (2003) who found IOBWs did not reduce *E. coli* counts at three commercial plants. Results from this study show a decrease in *Campylobacter* incidence and counts after the IOBW, which was also reported by Izat *et al.* (1988) and Bashor *et al.* (2004). Yang *et al.* (1998) showed a decrease in *Salmonella* counts (0.4 log cfu/carcass) from inoculated carcasses after the IOBW. Similar data from the current study show a decrease in *Salmonella* incidence due to the IOBW, and a decrease in counts would have been expected.

The inoculated cecal material prepared to simulate fecal contamination on carcasses contained approximately 8.4, 6.9, 7.1, and 4.5 log cfu/g cecal material of total bacteria, *E. coli*, *Campylobacter*, *and Salmonella*, respectively. Applied at 0.1 g per carcass, contaminated carcasses would have had added counts of 7.4, 5.9, 6.1, and 3.5 log cfu/g cecal material of total bacteria *E. coli, Campylobacter*, and *Salmonella*, respectively. The existing bacterial counts of the incoming carcasses (excepting *Salmonella*) prior to inoculation was such that the added bacteria probably would not have appreciably increased the post-wash carcass rinse counts. The results support this idea, as application of contamination did not affect post wash bacterial counts of contaminated carcasses as compared to control

carcasses or uncontaminated carcasses placed beside the contaminated carcasses. The added bacteria were effectively removed without cross contaminating adjacent carcasses in the IOBW. Use of 30.5 cm (12 in) shackle center separation distance (due to pilot facility requirements) instead of the industry standard of 15.3 cm (6 in) shackle center distance could have contributed to the lack of cross contamination.

At a water pressure of 276 kPa, the IOBW used approximately 114 liters/min (30 gal/min). At 552 kPa, the IOBW used approximately 178 liters/min (47 gal/min). As no difference in bacteria counts were observed at different pressures, more water savings would be seen at 276 kPa without compromising the ability of the IOBW to remove bacteria. These findings agree with Thomson *et al.* (1974) where a reduction in water pressure did not result in more aerobic bacteria on broiler carcasses. Mulder and Bolder (1981) also reported that, at a few poultry plants they examined, water usage was decreased with a lowering of bacterial counts on carcasses.

Although slight reductions in bacterial counts were significant, the IOBW did not produce reductions of total aerobic bacteria or *E.coli* that would be of practical significance to poultry processors. The IOBW did reduce incidence of both *Campylobacter* and *Salmonella*, and corresponding counts, with more efficacy shown against *Campylobacter*. Processors may use IOBWs for control of pathogenic bacteria, although *E. coli* counts remain relatively unaffected. Lower water pressure would conserve water without sacrificing bacterial counts or incidence, including pathogenic bacteria, although processors should conduct their own tests before reducing water pressure in their IOBWs. There was no cross contamination observed in this study in the IOBW with carcasses separated by 30.5 cm shackle centers.

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