Prevalence and Numbers of Bacteria in Broiler Crop and Gizzard Contents

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ABSTRACT Crops or gizzards in broiler carcasses are frequently damaged during processing. The contents from either organ, defined by the USDA Food Safety and Inspection Service as ingesta, may contaminate the carcass. Previous research has shown crop contents are a source of Salmonella contamination on processed carcasses, although less information is available on gizzard contents. The purpose of this study was to determine the prevalence and numbers of total aerobic bacteria, coliforms, Escherichia coli, and Campylobacter in ingesta collected from the crop and gizzard. In each of 3 replicate trials, 10 uneviscerated broiler carcasses were obtained from a processor at the shackle transfer point just prior to evisceration. Liquid crop contents and solid gizzard contents were aseptically collected from each carcass and quantitatively cultured. Total aerobic bacteria, coliforms, E. coli, and Campylobacter were determined for contents from both organs. Crop contents (log cfu/mL), compared with gizzard contents (log cfu/g), contained significantly (P < 0.05) higher numbers of total aerobic bacteria (5.6 vs. 2.9), coliforms (4.2 vs. 2.3), E. coli (3.9 vs. 2.2), and Campylobacter (4.6 vs. 2.2). Escherichia coli prevalence was higher in crop samples (28 of 29) than gizzard samples (19 of 30). Campylobacter prevalence was also higher for crop vs. gizzard samples (29 of 29 vs. 12 of 30). An average of 2.4 g of crop contents and 8.4 g of gizzard contents were recovered. Crop contents contain more bacteria than gizzard contents and contained a higher incidence of E. coli and Campylobacter contamination. However, because of the numbers of bacteria and amount of material in the crop and gizzard, it is unlikely that ingesta contamination would increase overall bacterial counts of prechill broiler carcasses.

Key words: crop, gizzard, ingesta, Escherichia coli, Campylobacter

INTRODUCTION

Ingesta, ingested material in an animal’s body prior to entering the intestinal tract, has been implicated as a source of contamination for broiler carcasses during processing (US Department of Agriculture, Food Safety Inspection Service, 2004). Contents in the crop are a source of ingesta and are prone to breakage during processing. Crops have been shown to contain Salmonella, Campylobacter, and Escherichia coli. Other areas of the upper gastrointestinal tract, including the esophagus, proventriculus, and gizzard, may also contain ingesta.

Pathogenic bacteria have been reported in studies on broiler crops. Hargis et al. (1995) found 52% of broiler crops were positive for Salmonella. Those researchers also reported that crops from broilers previously inoculated with Salmonella enteritidis continued to harbor the bacteria for 7 d (from 3 to 37% incidence, depending on inoculation level). Byrd et al. (1998) observed that 62% of broiler crops, in 7 of 9 flocks, sampled immediately prior to transport to the processing plant, were positive for Campylobacter. Jeffrey et al. (2001) found that 48% of crops from broiler carcasses prior to evisceration were positive for Campylobacter (20 of 22 flocks tested positive). Musgrove et al. (2001) reported 95 to 99% of broiler crops collected in a commercial slaughter plant were positive for Campylobacter; average counts were 3.6 log cfu. Escherichia coli has been recovered at levels of 4.4 log cfu from broiler crops (Berrang et al., 2000).

Approximately 25% of crops observed at a commercial processing plant were observed to have been damaged at the crop removal machine (Hargis et al., 1995). Carcasses that were manually eviscerated were found to have ruptured crops in a range from 22 to 44%, depending on stunning voltage; a 16 to 22% range of rupturing depending on whether heads were removed prior to or during picking; and, an overall average of 22% ruptured crops was calculated for the entire experiment (Buhr and Dickens, 2001). Following that experiment, 41% of crops from male broilers and 31% crops from female broilers ruptured when pulled through the body cavity during manual evisceration (Buhr and Dickens, 2002).

Less information is available for either the microbiology of gizzard contents or the propensity of the gizzard to
rupture during processing. Research has shown an 89% incidence of Campylobacter contamination in fresh chicken gizzards; 52% of the positive samples showed counts of ≤3.0 log cfu/g (Christopher et al., 1982). Khalafalla (1990) reported 28% of chicken gizzards sampled were positive for Campylobacter. Cox et al. (1972) simulated the pH and amount of time that ingesta would be held in various portions of the digestive tract. Salmonella decreased by ≥3 logs when exposed to simulated gizzard conditions at a pH of 2.6 for 90 min. Therefore, it is likely that the influx of acid from the proventriculus may lower bacterial counts and pathogen levels in the gizzard as compared with the crop.

The overall effect of ingesta contamination on the microbiology of postchill carcasses has been studied (Bilgili et al., 2002). Those researchers reported no differences in total aerobes, E. coli, or Campylobacter counts or in Salmonella incidence between carcasses with visible ingesta and those without prior to chilling. The objective of this study was to determine the microbiological profile of ingesta from both crop and gizzard and the amount of contents in each organ to estimate the risk from ingesta contamination on broiler carcasses.

**MATERIALS AND METHODS**

For each of 3 replicate trials, 10 uneviscerated broiler carcasses were collected from a commercial processing plant at the shackler transfer point just prior to evisceration. Carcasses were individually placed into plastic bags and transported to the laboratory. Carcasses were removed from bags, and the neck skin was aseptically cut and pulled away from the carcass to manually isolate the crop from adjacent skin and tissue. Sterile plastic cable ties were used to occlude the esophagus both above and below the crop. A 70% solution of ethanol was applied to the crop surface, and sterile scissors were used to cut a small hole (approximately 1 cm) in the crop. Two milliliters of sterile PBS were pipetted into the crop, which was then manually massaged for 1 min; all recoverable liquid and contents were removed from the crop with a sterile pipette. Typically, only 2 to 3 mL of liquid were recovered. One milliliter was used for 10-fold serial dilutions in PBS and cultured as described subsequently.

The visceral cavity of each carcass was opened to expose the gizzard. Ethanol solution (70%) was applied to the gizzard surface, and sterile scissors were used to cut open the gizzard. Contents were scraped with a sterile spatula into a sterile Whirlpak bag (Nasco, Fort Atkinson, WI). Phosphate-buffered saline was added at 3 mL for each gram of sample, and the bag was manually shaken for 30 s. Ten-fold serial dilutions in PBS were prepared for culture as described subsequently.

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. 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% O2, 10% CO2 and balance N2 (BOC Gases, Chattanooga, TN). 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). Coliform and 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). Petrifilm plates were incubated at 35°C for 18 to 24 h, and the types of colonies characteristic of coliforms and E. coli were counted.

Bacterial numbers were converted to log cfu (mL for crop contents; g for gizzard contents) for statistical analysis. Differences in numbers of bacteria between crop and gizzard content samples were tested by ANOVA using the GLM procedure of SAS (1999), and all significant differences reported were at the P < 0.05 level. Means were pooled across trials as there was no significant trial effect or trial by organ interaction. Crop or gizzard content samples without detectable numbers were treated as missing values in the analysis.

**RESULTS AND DISCUSSION**

Numbers of total aerobic bacteria, coliforms, E. coli, and Campylobacter were significantly (P < 0.05) higher in crop contents compared with gizzard contents (Table 1). Mean numbers ranged from 5.6 log cfu of total aerobes/mL to 3.9 log cfu of E. coli/mL for the crop contents. Mean counts for the gizzard contents ranged from 2.9 log cfu of total aerobes/g to 2.2 log cfu of both E. coli and Campylobacter/g. Prior research has shown that whole crops contained (in log cfu) 6.5 total aerobes, 5.0 coliforms, 4.4 E. coli, and 5.1 Campylobacter (Berrang et al., 2000). Musgrove et al. (2001) reported broiler crops contained 3.6 log cfu of Campylobacter/g. In another study using the most probable number method, chicken gizzards contained Campylobacter in the following distribution: 11% of samples at <0.5 log cfu/g, 6% of samples at 1.3 to 1.7 log cfu/g, 33% of samples from 2.0 to 3.0 log cfu/g, and 48% of samples >log 3.0 cfu/g (Christopher et al., 1982).

There was a lower prevalence of E. coli (19 of 30 vs. 28 of 29, respectively) and Campylobacter (12 of 30 vs. 29 of

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**Table 1. Mean numbers (log cfu) ± SD and prevalence of total aerobic bacteria, coliforms, Escherichia coli, and Campylobacter of contents from broiler chicken crops and gizzards**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Total aerobic bacteria</th>
<th>Coliforms</th>
<th>E. coli</th>
<th>Campylobacter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop, cfu/mL</td>
<td>5.6 ± 0.1 (30/30)</td>
<td>4.2 ± 0.2 (29/30)</td>
<td>3.9 ± 0.2 (28/29)</td>
<td>4.6 ± 0.1 (29/29)</td>
</tr>
<tr>
<td>Gizzard, cfu/g</td>
<td>2.9 ± 0.2 (28/30)</td>
<td>2.3 ± 0.3 (25/30)</td>
<td>2.2 ± 0.3 (19/30)</td>
<td>2.6 ± 0.3 (12/30)</td>
</tr>
</tbody>
</table>

*Means in columns lacking a common superscript differ (P < 0.05).*
29, respectively) in gizzard contents as compared with crop contents (Table 1), which may indicate that the gizzard was a more hostile environment for bacteria than the crop. The limit of detection in this experiment was one cell/3 g or mL of sample for E. coli and one cell/30 g or mL of sample for Campylobacter. Previous researchers have reported the incidence of Campylobacter in broiler crops at 92.2% (Mugrove et al., 2001), 62% (Byrd et al., 1998), and 48% (Jeffrey et al., 2001); the present study reported 100% of crop content samples were positive for Campylobacter. The incidence of gizzard contents found positive for Campylobacter in this study was 40%. This falls between previous research findings, as Christopher et al. (1982) reported an 89% incidence of Campylobacter for chicken gizzards sampled, and Khalafalla (1990) reported a 28% incidence for Campylobacter in chicken gizzards.

Lower mean counts and prevalence of bacteria found in the gizzard as compared with the crop is probably due to a pH effect. Immediately prior to entering the gizzard, ingesta passes through the proventriculus, which secretes HCl for digestive purposes; the approximate pH of this acid secretion is 2.0 (Duke, 1986). Cox et al. (1972) reported that, in conditions similar to those found in chicken gizzards (low pH for 90 min), 3 species of Salmonella decreased from an average of 6.0 log cfu/mL to 2.1 log cfu/mL. The effect of lower pH on reducing Campylobacter has also been observed for the crop. The incidence of Campylobacter-positive crops decreased when live birds were given lactic acid in the water, and even the incidence of postchill Campylobacter-positive carcasses decreased (Byrd et al., 2001).

Crop pH may be negatively affected by the practice of feed withdrawal, conducted to minimize ingesta and fecal contamination in the plant. Crop pH was reported to increase from 5.5 to as high as 6.5 following feed withdrawal (Hinton et al., 2000a). Feed withdrawal has been shown to increase the incidence of Salmonella in broiler crops (Ramirez et al., 1997). Corrier et al. (1999) reported a higher incidence of Salmonella-positive crops in broilers after feed withdrawal, which they attributed to birds ingesting litter contaminated with Salmonella during the withdrawal period. Counts of Salmonella and Enterobacteriaceae decreased with time up to 12 h of feed withdrawal then stayed the same or increased up to 24 h (Hinton et al., 2000a). Those researchers hypothesized that a decrease in lactic acid bacteria and corresponding increase in crop pH was responsible for any increase in Salmonella and Enterobacteriaceae.

To counteract the effect of increased crop pH produced by feed withdrawal, researchers have attempted to reduce crop bacteria of live broilers in the field through fermented liquid feed (Heres et al., 2003), lactose in the drinking water (Barnhart et al., 1999), glucose cocktails in the water (Hinton et al., 2000b), or lactic acid in the water (Byrd et al., 2001). Fermented liquid feed had some effect in reducing Salmonella in young broilers, lactose had no effect, glucose cocktails (7.5%) reduced Salmonella and Enterobacteriaceae, and lactic acid reduced the incidence of both Salmonella and Campylobacter in the crop of pre-transport broilers.

The amount of contents recovered from carcasses in the current study averaged 2.4 g from the crop and 8.4 g from the gizzard. The 2.4 g of crop contents included the 2 mL (or 2 g) of PBS initially pipetted into the crop. A previous report found whole crops (organ with contents included) removed from feed-withdrawn broilers obtained from a commercial processing weighed an average of 5.1 g (Berrang et al., 2000).

To estimate the maximum effect that ingesta could have on carcass contamination, whole carcass counts from prechill, visibly uncontaminated, broiler carcasses were determined from previous studies. These counts were reported from 2 papers within the past 3 yr that used carcasses from the local commercial processing plants (Cason and Berrang, 2002; Berrang et al., 2004). Counts were adjusted for the number of samples, milliliters of rinse used, and halved carcasses to produce the following averages: log cfu per carcass: total aerobes, 6.3; coliforms, 5.3; E. coli, 4.8; and Campylobacter, 5.2. To calculate the amount of increase of whole carcass counts from ingesta contamination, the addition of 1 g of crop contents and 10 g of gizzard contents (based on the maximum amounts found in this experiment) was multiplied by the average bacterial count of each type of content. The total added bacterial load from the 11 g of combined crop and gizzard ingesta would increase whole carcass counts of total aerobes from 6.3 to 6.4 (log cfu). Coliform numbers would remain at 5.3; E. coli would increase from 4.8 to 4.9 log cfu per carcass, and Campylobacter would increase from 5.2 to 5.3 log cfu per carcass. These estimates indicate the maximum effect on carcass counts, assuming all crop and gizzard contents and their associated bacteria remained on the carcass.

Ingesta from the crop has higher counts of bacteria and has a higher incidence of contamination than the gizzard, although the gizzard contains more material. Because of the small amount of crop contents in the carcasses sampled and because of the low numbers and incidence of bacteria found in gizzard contents, ingesta does not appear to be a major contributor to carcass contamination during processing. This finding is also supported by the calculations based on the data collected from the crop and gizzard to estimate the bacterial load that potentially could be added to prechill carcass counts. Results in this study support research published by Bilgili et al. (2002), where contamination of carcasses with visible ingesta did not increase postchill carcass counts.

REFERENCES


