

Bacterial Numbers of Broiler Chickens Affected by Sampling Methods and Location

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Summary

Two experiments were conducted; the first to determine whether numbers of recovered bacteria differed due to sampling method used or due to location on carcass sampled (breast or leg quarters), and the second to determine if numbers of bacteria differed between ventral and dorsal sides of the carcass. In both experiments eviscerated broiler carcasses were obtained from a commercial processing plant just prior to the final inside-outside bird washer. In Experiment 1, carcasses (3 in each of 4 replicate trials) were separated into leg quarters and breast quarters (n=48), and either Rinsed or Ground and stomached for microbiological sampling. In Experiment 2, for three replicate trials of 4 carcasses each, necks, wings, and legs were manually removed, then the remaining trunks were cut through the sides to produce front (ventral) and back (dorsal) halves (n=24), then rinsed. For both experiments coliforms and *Escherichia (E.) coli* were enumerated. In Experiment 1, significantly higher numbers ($P < 0.05$) of coliforms and *E. coli* were recovered from Rinsed than from Ground breast and leg quarters. Leg quarters were found to have higher bacterial numbers than breasts from Ground samples, but no quarter differences were found for Rinsed samples. In Experiment 2, higher ($P < 0.05$) numbers of coliforms and *E. coli* were recovered from the dorsal half compared to the ventral half. Bacterial counts of broiler carcasses are affected by both the sampling method used and by carcass location sampled.

Keywords: Broiler carcass, coliforms, *E. coli*, sample methods

Introduction

Numbers and types of bacteria on processed poultry carcasses vary due to many factors, including recovery methods and carcass location sampled. Previous researchers have used methods such as skin swabbing, skin scraping, or skin excision and rinsing (Patterson and Stewart, 1962). Two review articles have presented other methods for sampling poultry carcasses, including mechanical shaking while rinsing, spraying skin and collecting rinsate, blending skin, direct agar plate-skin contact, filtering of rinsate, and sampling of weep from packaged meat (Baldock, 1974; Patterson and Gibbs, 1975). Numbers of recovered bacteria have been reported to differ due to methods. Fromm (1959) found that direct plating was not effective for sampling broilers or hens but swabbing or excision and homogenizing skin was effective for recovering bacteria. The study also reported that swabbing was more effective than rinsing for recovering aerobic bacteria. Avens and Miller (1970) stated that excision and blending of tissue recovered higher numbers of aerobic bacteria from turkey carcasses than did swabs. A series of experiments reported by McEvoy et al. (2005), using four sampling methods for *E. coli* in turkey carcasses, generally showed a modified carcass rinse procedure and a two-swab method was superior than whole carcass swab or a one-swab method.

Previous reports have also focused on the portion of the carcass sampled, including a review by Patterson and Gibbs (1975). Early reports indicated some areas of the carcass contained larger numbers of bacteria than other portions (Walker and Ayres, 1959; May, 1962). Kotula (1966) found numbers of aerobic bacteria on prechill broiler carcasses were highest on thighs, followed by fewer bacteria on legs and then breasts. Patterson (1972) reported that skin from the neck contained higher numbers of aerobic bacteria than the back; other sites including inside leg, outside leg, vent area and under the wing were mostly equal. Smith et al. (2007) observed higher numbers of coliforms and *E. coli* recovered from the back of pre-chill broilers than from the breast. A few reports have not observed differences within carcasses sampled. Cox et al. (1976) found no difference between thigh and breast areas sampled for aerobic bacteria and *Enterobacteriaceae*. Similarly, Klinger et al. (1981) found no difference in numbers of aerobic bacteria or *Enterobacteriaceae* from five sites sampled on kosher-processed broilers.

Both the method of sampling poultry carcasses to determine bacterial counts and the location of the carcass sampled appears to affect results. Therefore the objective of this study was to determine if two different sampling methods, or different areas of carcasses sampled, affected numbers of coliforms or *E. coli* recovered from prechill broilers.

Materials and methods

Experiment (Exp) 1 was conducted to determine if differences were observable in numbers of recovered coliforms or *E. coli* due to: 1) carcass location (leg quarter vs. breast quarter); and, 2) methods of sample preparation (Rinsed vs. Ground). Three pre-chill broiler carcasses were collected from a commercial processing plant in each of four replicate trials. Carcasses were collected after evisceration and just prior to the final inside-outside bird washer, placed in individual plastic bags, and transported to the laboratory. Carcasses were cut into breast and leg quarters with a clean knife (washed and rinsed in 70% ethanol) and weighed. The breast and leg quarter from one half of each carcass was assigned to either the Rinsed or Ground treatment, and left and right halves were alternated from carcass to carcass. For the Rinsed treatment breast and leg quarters from one carcass half were placed individually into clean plastic bags, then a rinse procedure was conducted with 100 mL 0.1% peptone solution with manual shaking for 1 min. Each breast and leg quarter from the other half of the carcass was placed individually into a food processor (Blixer model RSI BX3, Robot Coupe USA, Inc., Jackson, MS 39236) and ground for 30 s. 25 g of the resulting paste was weighed and added to 225 ml 0.1% peptone in a stomacher bag. The mixture was blended (Stomacher model 400, Seward Limited, West Sussex, United Kingdom BN14 8NL) for 1 min.

Experiment 2 was conducted to determine if there was a difference in numbers of coliforms or *E. coli* between the front (ventral) or back (dorsal) halves of broiler carcass trunks. In each of three replicate trials, four pre-chill broiler carcasses were collected as per Exp 1. Necks, wings and legs were manually cut from each carcass leaving a trunk. Wings and legs display multiple surfaces and are not readily separated into ventral and dorsal halves, therefore they were removed from the carcass and discarded. The trunk was cut into ventral and dorsal halves using a clean knife (washed and rinsed in 70% ethanol) to split the front and back portions by cutting behind the clavicle and downward from the wing insertion through the rib cage. The trunk halves' predominant surface was reflective of the ventral or dorsal side and halves were relatively comparable with regard to weight. Each half was rinsed as described in Experiment 1.

One mL of liquid was removed from each bag (Rinsed or Ground bags in Experiment 1) or 1 mL rinsate (Experiment 2) for serial dilutions in 0.1% peptone, then one mL was plated onto petrifilm *E. coli* / coliform count plates (3M Health Care, St. Paul, MN 55144). Petrifilm plates were incubated at 35⁰ C for 24 h in aerobic conditions, then types of colonies characteristic of coliforms and *E. coli* were counted.

Bacterial numbers were converted to log CFU for statistical analysis; per mL for the Rinsed samples (Exp 1) and all samples in Exp 2; per g for the Ground samples in Exp 1 (based on weight of each quarter, multiplying by a factor determined from dividing the original sample weight in g by 25 g, the portion weight used for stomaching). The initial dilution factor (25 g in 225 ml) was also included for Ground counts. Differences in bacterial counts due to trial (and method of sampling in Exp 1) were tested using analysis of variance with Statistical Analysis Software (SAS) GLM procedures. Means were pooled across trials due to lack of significance or significant interactions ($P < 0.05$). The paired t test in SAS was used to determine differences in bacterial numbers between paired breast and leg quarters numbers in Exp 1 and between paired ventral and dorsal carcass trunks in Exp 2.

Results and Discussion

Numbers of bacteria recovered from either the Rinsed or Ground sample method for breast and leg quarters are shown in Table 1. The Rinsed method recovered significantly ($P < 0.05$) more coliforms from breast quarters than the Ground method (5.5 vs. 4.2 log cfu); the same result was found for leg quarters (5.6 vs 4.7 log cfu). More *Escherichia (E.) coli* were also recovered from the Rinsed than the Ground method for breast quarters (5.3 vs. 4.9 log cfu) and from leg quarters (5.3 vs. 4.6 log cfu).

Various methods utilized by previous researchers have resulted in different numbers of bacteria recovered from poultry carcasses (Barnes and Shrimpton, 1958; Adams et al., 1980; Smith et al, 2007). Some methods utilized were equal to rinsing for recovering bacteria from carcasses (Gill and Badoni, 2005; McEvoy et al., 2005). Although widely used, some researchers recognize that rinsing may not maximize bacterial recovery, and have attempted to improve this method by adding sand to the medium (Patterson, 1972; Hannah et al. 2008).

Table 1. Average numbers (\pm SEM) of coliforms and *Escherichia coli* from broiler carcasses cut into breast and leg quarters and sampled after either rinsing (Rinsed, reported as log cfu/mL) or grinding (Ground, reported as log cfu/g), n=12.

| Organism | Sample Method | Quarter | |
|----------------|--------------------|------------------------------|------------------------------|
| | | Breast | Leg |
| Coliforms | Rinse | 5.5 ^a \pm 0.7 | 5.6 ^a \pm 0.9 |
| | Grind | 4.2 ^{b,x} \pm 0.8 | 4.7 ^{b,y} \pm 1.1 |
| <i>E. coli</i> | Rinse ¹ | 5.3 ^a \pm 0.8 | 5.3 ^a \pm 0.9 |
| | Grind ² | 3.9 ^{b,x} \pm 1.0 | 4.6 ^{b,y} \pm 1.2 |

^{a, b} Means for an organism within a column lacking a common superscript differ significantly ($P < 0.05$).

^{x, y} Means within a row lacking a common superscript differ significantly ($P < 0.05$)

The recovery of fewer bacteria overall from the Ground method was probably due to a dilution effect from the meat associated with each breast or leg quarter. Berrang et al. (2001) found that skin contained more bacteria than meat surfaces of thigh and breast parts taken from prechill broiler carcasses. The muscle below the surface is essentially sterile (Avens and Miller, 1970). The Ground method was designed to be both relatively easy to use (at least comparable to the Rinsed method) and also recover bacteria typically not recovered by various rinsing methods. For example, Lillard (1988) reported that repeated rinsing of the same carcass continued to recover bacteria even after 40 rinse samplings. Problems with bacterial recovery by rinsing and reasons for these difficulties are reported by Thomas et al. (1987). In summary, they state that a liquid film encompasses the carcass surface which protects bacteria, and that bacteria may also adhere to connective tissues by attaching to glucosaminoglycans in collagen. Grinding should liberate these adhesions but the resulting dilution from meat renders this method less sensitive than the Rinsed method for maximizing bacterial recovery.

Differences between breast and leg quarters, by method sampled, are also shown in Table 1. No differences ($P < 0.05$) were found between quarters for the Rinsed samples. For Ground samples, leg quarters were higher for recovered coliforms than breast quarters (4.7 vs. 4.2 log cfu/g). *E. coli* numbers were also higher for leg than breast quarters (4.6 vs 3.9 log cfu/g) for Ground samples.

The significant difference between Ground breast and leg quarters for recovered bacteria (with legs having a higher count than breasts) reflects previous research findings (Kotula, 1966; Bodnaruk et al., 1998). The lack of a difference between breast and leg quarters for recovered bacteria from the Rinsed samples is also supported by previous research. Cox et al. (1976) reported that swabbing produced no difference between breast and thigh surfaces of broilers for aerobic bacteria or Enterobacteriaceae. No differences were found for aerobic bacteria between breast, thighs, and back of broilers when the skin was excised and stomached (Klinger et al., 1981). Therefore the sampling method not only affects the total numbers of bacteria recovered but may also either highlight (per Ground results) or mask (per Rinsed results) differences between areas of the same carcass.

Average numbers of bacteria recovered from the ventral and dorsal carcass trunk portions are shown in Table 2. Significantly higher ($P < 0.05$) numbers of coliforms were recovered from the dorsal portion (3.7 log cfu) than from the ventral portion (3.2 log cfu). Similarly, more *E. coli* were recovered from the dorsal portion (3.4 log cfu) than the ventral portion (2.9 log cfu).

Table 2. Average numbers (\pm SEM) of coliforms and *Escherichia coli* from the front (ventral) or back (dorsal) half of broiler carcass trunks (n=12).

| | Ventral | Dorsal |
|----------------|----------------------------|----------------------------|
| | -----log cfu/mL----- | |
| Coliforms | 3.2 ^b \pm 0.1 | 3.7 ^a \pm 0.1 |
| <i>E. coli</i> | 2.9 ^b \pm 0.1 | 3.4 ^a \pm 0.1 |

^{a, b} Means within a row lacking a common superscript differ significantly ($P < 0.05$).

Prior research has shown different sampling locations on a carcass produce different numbers of recovered bacteria. Broiler carcasses have more bacteria on the back than on the front, and more bacteria on the thigh than the breast (Smith et al., 2007; Kotula, 1966). More *E. coli* was recovered from the back and thighs of post-chill turkey carcasses than from the breast (Bodnaruk et al., 1998). Vaidya et al. (2005) also reported more bacteria on the legs of poultry

carcasses than on other areas sampled. Therefore results from the present study are in general agreement with previous reports.

Breast quarters at 397 g weighed significantly more ($P < 0.05$) than leg quarters at 321 g. The front (ventral) half of the trunk weighed significantly less (521 g) than the back (dorsal) half of the trunk (576 g). Although the difference was significant the percent difference was approximately 10%, showing that efforts to keep the halves similar with regard to weight were successful and comparable to normal variation between whole carcass weights observed during commercial processing.

Results from the experiments showed that rinsing recovered more bacteria than grinding and more bacteria were recovered from the back than the front of the carcass. Therefore, whether reviewing previous publications or designing pending research on broiler carcass microbiology, consideration should be given to sampling methods and locations.

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