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Microbiological contamination of shell eggs produced in conventional and free-range housing systems

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ABSTRACT

The present study was conducted to determine microbiological contamination of free-range and conventional chicken eggs produced under controlled conditions. Eighty-four certified *Salmonella*-free Bovan Brown chicks (age 2 days) were grown in 6 separate floor pens until age 16 weeks, and then moved into 3 conventional battery cages (BC) or 3 free-range (FR) housing systems. Total aerobic microorganisms and Enterobacteriaceae on egg shell surfaces were enumerated weekly when the hens were 20–27 weeks of age ($N = 535$ and $N = 541$ for BC and FR, respectively). Prevalence of *Salmonella* and *Campylobacter* were determined on crushed egg shells ($N = 212$ and $N = 176$, respectively) and in feces ($N = 36$ and $N = 30$, respectively) collected from hens at 24 and 28 weeks of age. Counts of total aerobic microorganisms recovered from BC and FR eggs ranged from 5.0 to 6.0 $\log_{10}$ CFU/mL. Numbers of Enterobacteriaceae averaged 1.0 log CFU/mL higher (90% greater) on FR eggs than on eggs from BC hens. *Salmonella* was not detected on any of the eggs collected from BC hens (0/212), but prevalence on eggs collected from FR hens was 2.36% positive (5/212). Prevalence of *Campylobacter* recovered from eggs collected from FR (26.1% positive or 46 out of 176 positive) was significantly higher ($P \leq 0.0001$) than the prevalence of *Campylobacter* recovered from eggs from BC hens (7.4% positive or 13 out of 176 positive). These data demonstrate that FR eggs, where hens have more contact with eggs after oviposition, have greater microbiological contamination on the egg shell surface than eggs produced in the BC cage systems.

1. Introduction

Consumer emphasis on the humane treatment and environmentally friendly production of eggs has influenced U.S. egg producers to expand into alternative markets, transitioning from conventional battery cage housing systems to free-range production. Moreover, the European Union (EU) issued the Council Directive 1999/74/EC in 1999 stating that member states should not house laying hens in conventional battery cages after January 2012 (European Commission, 1999). Eggs produced in alternative systems are of interest to the U.S. industry because they demand premium pricing and potentially higher profit to egg producers; however, little is known about the microbiological challenges associated with alternative production practices in the U.S.

Previous studies examining differences in microbiological contamination levels between conventional and alternative egg production systems have reported conflicting results (De Reu, Grijspeerdt, Heyndrickx, Uyttendaele, & Herman, 2005a; De Reu et al., 2005b, 2009; Huneau-Salaun, Michel, Huonnic, Balaine, & le Bouquin, 2010; Jones, Anderson, & Guard, 2012; Jones, Anderson, & Musgrove, 2011; Messelhausser et al., 2011). In a controlled experimental setting, De Reu et al. (2005a, 2005b) found that levels of total aerobic microorganisms on eggs collected from an aviary housing system (5.5–6.0 log CFU/mL) averaged approximately 90% higher than numbers of total aerobic microorganisms recovered from eggs produced in conventional or furnished cage systems (3.8–4.6 log CFU/mL). However, when these same researchers conducted a similar experiment in a commercial setting, the number of total aerobic microorganisms recovered from eggs collected from non-caged systems were only slightly higher (4.98 $\log_{10}$ CFU/egg shell) than numbers on surfaces of eggs from furnished cage systems (4.75 $\log_{10}$ CFU/egg shell; De Reu et al., 2009). Huneau-Salaun et al. (2010) found similar but less pronounced differences when they compared numbers of total aerobic microorganisms recovered from eggs laid in 'on-floor' housing systems as compared to counts recovered from eggs laid in

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conventional cage systems (4.82 and 4.40 $\log_{10}$ CFU/egg shell, respectively). Another study conducted on a research farm found the opposite of De Reu et al. (2005b; 2009) and Huneau-Salaun et al. (2010) and reported that total aerobic microorganisms on eggs from free-range nest boxes were 90% lower (2.25 and 2.75 $\log_{10}$ CFU/mL, respectively) than those recovered from eggs laid in conventional cages (3.25 and 3.75 $\log_{10}$ CFU/mL, respectively) during both winter and spring (Jones et al., 2011).

Messelhausser et al. (2011) tested a total of 2710 eggs from retail stores in Germany for prevalence of *Campylobacter* spp. and *Salmonella* spp., and detected *Campylobacter* isolates in 11 (4.1%) of the pooled egg shell samples (4 from eggs sold as free-range and 7 from eggs sold as barn eggs) while *Salmonella Enteriditis* (SE) was detected in 3 (1.1%) of the pooled egg shell samples. Two of the SE-positive eggs came from battery cage housed hens and 1 SE-positive egg came from hens housed in deep litter (Messelhausser et al., 2011). Jones et al. (2012) examined prevalence of coliform, *Salmonella* and *Campylobacter* on eggs produced in conventional cages and free-range housing, and reported no difference in *Salmonella* or *Campylobacter* prevalence among the two housing systems. They reported significantly higher prevalence of *Campylobacter* ($P < 0.0001$) on nest boxes in the free-range environment as compared to prevalence of *Campylobacter* recovered from conventional cages. However, Jones et al. (2012) did not test chicks at hatch or pullets prior to relocation to battery cages or free range-areas for *Salmonella* and *Campylobacter*. Chicks or pullets may have carried pathogens with them to the production environments from either the hatchery (University hatchery) or grow-out pens (Jones et al., 2012).

While results of these studies provide insight into the microbiological risks associated with non-caged housing systems, most of these projects, except those by Jones et al. (2012, 2011) were conducted outside of the U.S. There are differences in egg production and processing practices among European and North American countries including the use of furnished cages in Europe, differences in laying hen breeds, environmental climate and humidity differences across continents, and differences in egg washing and refrigeration practices. Thus, the objective of this study was to characterize and compare the microbiological status of egg surfaces produced in the U.S. in free-range and battery cage housing systems by determining the levels and prevalence of total aerobic microorganism, Enterobacteriaceae, *Salmonella* spp., and *Campylobacter* spp.

2. Materials and methods

2.1. Housing

Eighty-four, 2 day old, certified *Salmonella*-free Bovan Brown chicks were purchased from a commercial hatchery and transferred to the University poultry research center (Institutional Animal Care and Use Protocol Approval Number 2011-008). Chicks were housed under brooders in 6 indoor floor pens on pine shavings in groups of 14 chickens per pen and given *ad libitum* access to feed and water. At 16 weeks of age, pullets from three of the pens were moved to 3 free-range housing systems (FR) and pullets in the remaining three pens were moved into 3 battery cages (BC).

The FR house system shared a common roof, but the inside and outside range areas were separated by fencing. The FR house was partitioned into three separate floor pens (1.5 m by 3.0 m), and each pen had a separate bird doorway leading to a distinct 7.6 m by 13.7 m outdoor range. All 3 of the range areas were surrounded by chain link fencing with outside electric wiring and nylon netting was stretched across the top of the fencing to prevent entrance by predators. To create similar environments for each range, light-weight shading tarps were spread over 1/3 of the netting to supply equal amounts of shade and grass was mowed short prior to pullet introduction into the range. Each indoor section of the range house was equipped with 9 nest boxes containing pine shaving with accompanying perches, waterers, feeders, and pine shavings on the floor. Bedding was changed weekly by farm staff.

For caged layers, two banks of battery cages were maintained in an indoor poultry house. Banks were 3 cages high, 4 cages per level, and 2 cages wide. Each battery cage measured 61 cm by 61 cm by 41 cm (0.15 m^3) of space. Pullets from one floor pen were divided into sets of 3 or 4 pullets per set and one set was placed into an individual battery cage along a single row. A total of three rows staggered among the two banks were used throughout the study and care was taken to avoid cross-over of feed or feces from one group to another during the study. Cages consisted of wire mesh flooring and were equipped with feed troughs and nipple drinkers. Egg collection troughs were attached to the front of the cages, with wiring dividers to preclude eggs from one cage mixing with eggs from another cage. Paper lining was spread under each row of hens for collection of excreta. Paper liners were changed twice per week.

Two weeks after transferring pullets to perspective housing systems, feces from birds were tested for *Salmonella* contamination. Fecal samples were collected from each pen and placed into sterile plastic bags using sterile latex gloves. Six individual fecal droppings were pooled into one bag and three bags were collected from each pen or battery cage. Samples were obtained using a new latex glove changed between each pen and cage. Bags containing feces were secured in a clean Ziploc bag and refrigerated overnight before being transported to the USDA Agricultural Research Service, Russell Research Center in Athens, Georgia in a Styrofoam cooler within 24 h for analysis to determine presence of *Salmonella* as described in Musgrove et al., 2005.

2.2. Diets

All of the diets used in this study are commercially available layer diets. FR diets were plant-based while the BC diets included animal sources of fat and protein. FR diet consisted of 18% protein with 2778 ME/kg, while the BC diet consisted of 19% protein with 2780 ME/kg. Feeds were procured in the total amount needed for the eight week-egg collection period, and were stored together on pallets until used.

2.3. Egg collection and storage

Egg collection began when BC and FR hens reached 20 weeks-of age and continued until hens were 27 weeks-of-age, corresponding to week 0 to week 8. Eggs from both production systems were collected every morning between 9:00 and 10:00 am. Collections were performed using a clean latex glove for each pen and eggs were placed into a new, clean, cardboard egg carton which was sealed before transportation to the lab. Eggs were held in cartons at room temperature for 24 h before shell surface microbiological analyses were performed.

2.4. Egg shell microbiological determination

Microbiological testing was performed on eggs laid during weeks 1, 3, 4, 5, 7, and 8. During the first four weeks of collection, eggs from Saturday through Wednesday were analyzed. Results from the first week of egg collection showed no differences in numbers of microorganisms recovered between eggs collected Saturday compared to bacteria numbers on eggs collected Sunday, Monday, Tuesday, or Wednesday. Thus subsequent weeks involved analyses of eggs collected from Sunday through Wednesday only