Microbiological impact of three commercial laying hen housing systems[1]

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ABSTRACT Hen housing for commercial egg production continues to be a societal and regulatory concern. Controlled studies have examined various aspects of egg safety, but a comprehensive assessment of commercial hen housing systems in the US has not been conducted. The current study is part of a holistic, multidisciplinary comparison of the diverse aspects of commercial conventional cage, enriched colony cage, and cage-free aviary housing systems and focuses on environmental and egg microbiology. Environmental swabs and eggshell pools were collected from all housing systems during 4 production periods. Total aerobes and coliforms were enumerated, and the prevalence of *Salmonella* and *Campylobacter* spp. was determined. Environmental aerobic and coliform counts were highest for aviary drag swabs (7.5 and 4.0 log cfu/mL, respectively) and enriched colony cage scratch pad swabs (6.8 and 3.8 log cfu/mL, respectively). Aviary floor and system wire shell pools had the greatest levels of aerobic contamination for all eggshell pools (4.9 and 4.1 log cfu/mL, respectively). Hens from all housing systems were shedding *Salmonella* spp. (89–100% of manure belt scraper blade swabs). The dry belt litter removal processes for all housing systems appear to affect *Campylobacter* spp. detection (0–41% of manure belt scraper blade swabs) considering detection of *Campylobacter* spp. was much higher for other environmental samples. Aviary forage area drag swabs were 100% contaminated with *Campylobacter* spp., whereas enriched colony cage scratch pads had a 93% positive rate. There were no differences in pathogen detection in the shell pools from the 3 housing systems. Results indicate egg safety is enhanced when hens in alternative housing systems use nest boxes. Additionally, current outcomes indicate the use of scratch pads in hen housing systems needs to be more thoroughly investigated for effects on hen health and egg safety.

Key words: hen housing, microbiology, egg, *Salmonella*, *Campylobacter*

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INTRODUCTION

Whereas US consumers have a variety of eggs from various hen housing options available in retail, some states have enacted or are considering laws defining minimum hen confinement standards. In preparation for the European Union transition from conventional cage to alternative housing systems, many studies were conducted to determine the effect of the new law on egg microbiology (De Reu et al., 2005, 2006, 2008, 2009; Mallet et al., 2006; Schwaiger et al., 2008; Huneau-Salaün et al., 2009; Gondek et al., 2013). Due to the dynamic nature of shell egg production, the studies often resulted in conflicting outcomes. In a review of literature concerning hen housing and egg safety, Holt et al. (2011) determined that many confounding factors need to be explored before US housing recommendations can be made based on resulting egg safety. Some of the factors presented include the differences in European and US hen management practices, hen genetics, and climate.

Scientists in the United States have also been investigating the effects of housing systems on egg safety and hen health (Hannah et al., 2011; Jones et al., 2011, 2012; Gast et al., 2013, 2014; Jones and Anderson, 2013). Each study compares different housing systems under controlled research conditions, thus allowing for clear comparisons. To effectively understand the effect of hen housing systems on egg safety,

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controlled research and commercial application studies are both needed. The current study was a national collaboration undertaken to compare commercial conventional cage, enriched colony cage, and cage-free aviary housing systems on environmental impact, food safety, worker safety, bird health and well-being, and food affordability. This paper describes the impact of hen housing system on environmental and egg microbiology.

MATERIALS AND METHODS

Housing Systems and Sample Collection

The experimental protocol was approved by the Michigan State University Institutional Animal Care and Use Committee. Lohmann White laying hens were commercially reared and housed appropriately in conventional cage, enriched colony cage, and cage-free aviary (aviary) systems as described by Jones et al. (2014). Descriptions of the hen management and detailed housing system designs can be found in Jones et al. (2014) and Zhao et al. (2014b), respectively.

Twenty cage (conventional and enriched colony cage) and housing segment (aviary) replicates were identified within each housing system, and all samples were collected from the same replicates for the entire study. Samples were collected during production periods 9, 11, 13, and 15 (hen age 53, 61, 69, and 77 wk). Shell pools were formed from up to 6 shells (no fewer than 3 eggs) by aseptically cracking and rinsing with sterile PBS (to remove most adhering albumen) per replicate for each type of shell pool treatment (Table 1) and placing shells in sterile specimen cups. System wire eggs were defined as eggs laid on the roll-out wire levels of a system and were potentially present in all 3 housing systems. As production progressed, fewer system wire eggs were produced in the enriched colony cage and aviary systems, reducing the total number of shell pools (Table 1). Nest box eggs were defined as eggs laid in the nest boxes of the enriched colony cage and aviary systems. Floor eggs were defined as eggs laid in the forage area of the aviary system.

Table 1. Type and total number of samples collected per housing system[1].

Sample type	Conventional cage	Enriched colony cage	Cage-free aviary
Environmental swabs			
System wire	80	80	80
Nest box		80	80
Scratch pad		80	
Manure scraper[2]	80	80	32
Forage area drag swab			16
Shell pools			
System wire	80	13	63
Nest box		80	80
Floor			77

[1]Samples collected over 4 production periods. Pathogens assessed on all swabs/shell pools. Enumeration of aerobes and coliforms conducted on up to 10 swabs/shell pools for each sample type × housing system combination each collection period.

[2]Only pathogen detection conducted on manure scraper swabs.

Environmental swabs were collected using sterile, premoistened sample sponges (Nasco Whirl-Pak, Fort Atkinson, WI). Table 1 shows the type and number of environmental swabs collected in the 3 housing systems. System wire samples were collected by aseptically swabbing a 30 × 30 cm area of roll-out wire within each of the 20 defined system replicates and were present for all housing systems. Nest box swabs were collected by swabbing a single nest box pad within each system replicate and were present in the enriched colony cage and aviary housing systems. Scratch pad swabs were collected by swabbing the scratch pad (feed was delivered on scratch pads) in each of the identified enriched colony cage replicates. Manure belt scraper swabs were independent of assigned housing system replicates. The manure belt scraper blades at the end of rows were wiped from end to end with a single sponge. In the aviary system, only 8 manure scraper blades were associated with the assigned housing replicates. Drag swabs were collected from the forage area of the aviary system. A prepared sterile 10 × 10 cm drag swab (Solar Biologicals Inc., Ogdensburg, NY) was aseptically held in each hand of the sample collector and dragged for one-half of the length of the row. The 2 swabs were then combined for a single drag swab sample. The process was repeated to completely cover the distance of 2 forage rows each sample period (n = 4 drag swab samples).

Sample Preparation and Laboratory Procedures

All shell pools and environmental swabs were shipped on ice overnight to the USDA Agricultural Research Service laboratories in Athens, Georgia. Upon receipt, 10 mL of 42°C sterile PBS was added to each environmental swab and stomacher blended for 1 min at 230 rpm (Stomacher 400 Circulator, Seward Ltd., London, UK). For shell pools, 10 mL of 42°C sterile PBS was added per shell in the sample pool (30–60 mL, depending on number of shells present) and macerated according to the methods of Musgrove et al. (2005).

Total aerobes and coliforms were enumerated utilizing appropriate dilutions and duplicate plating for half of the assigned replicates of shell pools and environmental samples, excluding manure scraper swabs. The same replicates were used for enumeration each sample period with a possible exception occurring for enriched colony cage system wire shell pools. As enriched colony cage hens began to lay fewer eggs on the system wires, all collected shell pools were used for enumeration. Total aerobe and coliform levels were determined utilizing the methods and media sources described by Jones et al. (2011). The prevalence of *Salmonella* spp. and *Campylobacter* spp. were determined for all samples collected. The methods of Jones et al. (2006) were used to assess the presence of *Salmonella* spp. The prevalence of *Campylobacter* spp. was determined using TECRA as the primary enrichment according to the methods of Richardson et al. (2009) with 2 mL of sample diluent introduced into 18 mL of TECRA.

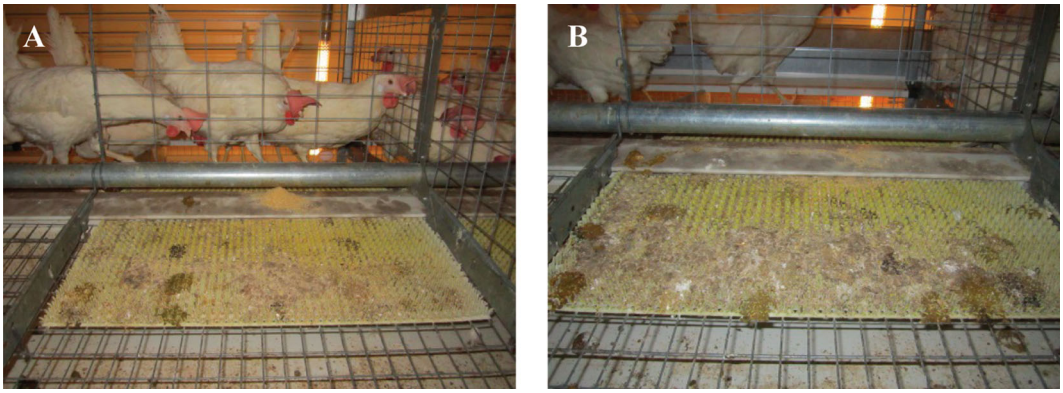

Figure 1. Two examples of enriched colony cage system scratch pads that were scored (A) 3 and (B) 5.

Table 2. Total aerobes, coliforms, *Salmonella* spp., and *Campylobacter* spp. associated with environmental swabs from commercial conventional cage, enriched colony cage, and aviary housing systems[1].

Sample type	Average total aerobes[2] (log cfu/mL)	Average total coliforms[2] (log cfu/mL)	*Salmonella* spp. (%; no. positive/ total no. samples)	*Campylobacter* spp. (%; no. positive/ total no. samples)
Aviary drag swabs	7.5 ± 0.1	4.0 ± 0.3	69 (11/16)	100 (16/16)
Aviary manure scraper[3]			100 (32/32)	41 (13/32)
Aviary nest box	5.5 ± 0.1	1.6 ± 0.2	28 (22/80)	10 (8/80)
Aviary system wire	5.3 ± 0.1	2.1 ± 0.2	18 (14/80)	74 (59/80)
Conventional manure scraper[3]			99 (79/80)	0 (0/80)
Conventional system wire	4.8 ± 0.1	2.3 ± 0.2	25 (20/80)	63 (50/80)
Enriched manure scraper[3]			89 (71/80)	40 (32/80)
Enriched nest box	5.6 ± 0.1	2.7 ± 0.2	16 (13/80)	64 (51/80)
Enriched scratch pad	6.8 ± 0.1	3.8 ± 0.2	23 (18/80)	93 (74/80)
Enriched system wire	4.7 ± 0.1	1.7 ± 0.2	16 (13/80)	65 (52/80)
P-value			0.0002	0.0001

[1]Samples collected over 4 production periods. Pathogens assessed on all swabs. Enumeration of aerobes and coliforms conducted on up to 10 swabs for each sample type, each collection period.

[2]Significant sample type × production period interaction ($P < 0.0001$).

[3]Only pathogen detection conducted on manure scraper swabs.

Nest Box Pad and Scratch Pad Scoring

Nest box (aviary and enriched colony cage) and scratch (enriched colony cage) pads were scored on a 0 to 7 scale with 0: <25% of the pad being visibly dirty, 1: 25 to 50% visibly dirty, 2: 50 to 75% visibly dirty, 3: 75 to 100% visibly dirty, 4: 100% visibly dirty, 5: up to 50% of the pad unusable, 6: 50 to 75% unusable, and 7: 75 to 100% unusable. The unusable area of the pad was defined as a hard pack, typically manure, covering the plastic tines of the pad. See Figure 1 for examples of the scoring system. Nest box pads and scratch pads from 27 cages throughout the enriched colony cage system, from the top and bottom 2 tiers, were scored when hens were 19, 52, and 72 wk of age. The number of eggs laid in the nest box and outside of the nest box was also counted from the same cages. Four nest box pads from each of 24 segments of the aviary systems (n = 96 pads) were assessed for visible cleanliness at 25, 50, and 76 wk of age.

Statistical Analysis

Microbial counts for the duplicate plates were averaged then subjected to log-transformation before analysis. Plate counts with no growth were converted to zero after log-transformation. Significance was determined through the GLM procedure of SAS with housing system and sample period as main effects. Means were separated by the least square method. The prevalence of *Salmonella* and *Campylobacter* spp. were compared among the main effects using the chi-squared operation and goodness-of-fit test. Environmental swabs and shell pools were compared among themselves. Probabilities of $P < 0.05$ were considered significant. All statistical analyses were conducted using SAS software (SAS Institute Inc., 2002).

RESULTS AND DISCUSSION

Environmental Swabs

The average counts for total aerobes and coliforms, as well as the prevalence of *Salmonella* and *Campylobacter* spp. in the environmental swabs collected during 4 periods of sampling are shown in Table 2. The greatest average level of aerobic organisms occurred in aviary drag swabs (7.5 log cfu/mL) followed by enriched colony cage scratch pads (6.8 log cfu/mL). The lowest average aerobic environmental counts were associated

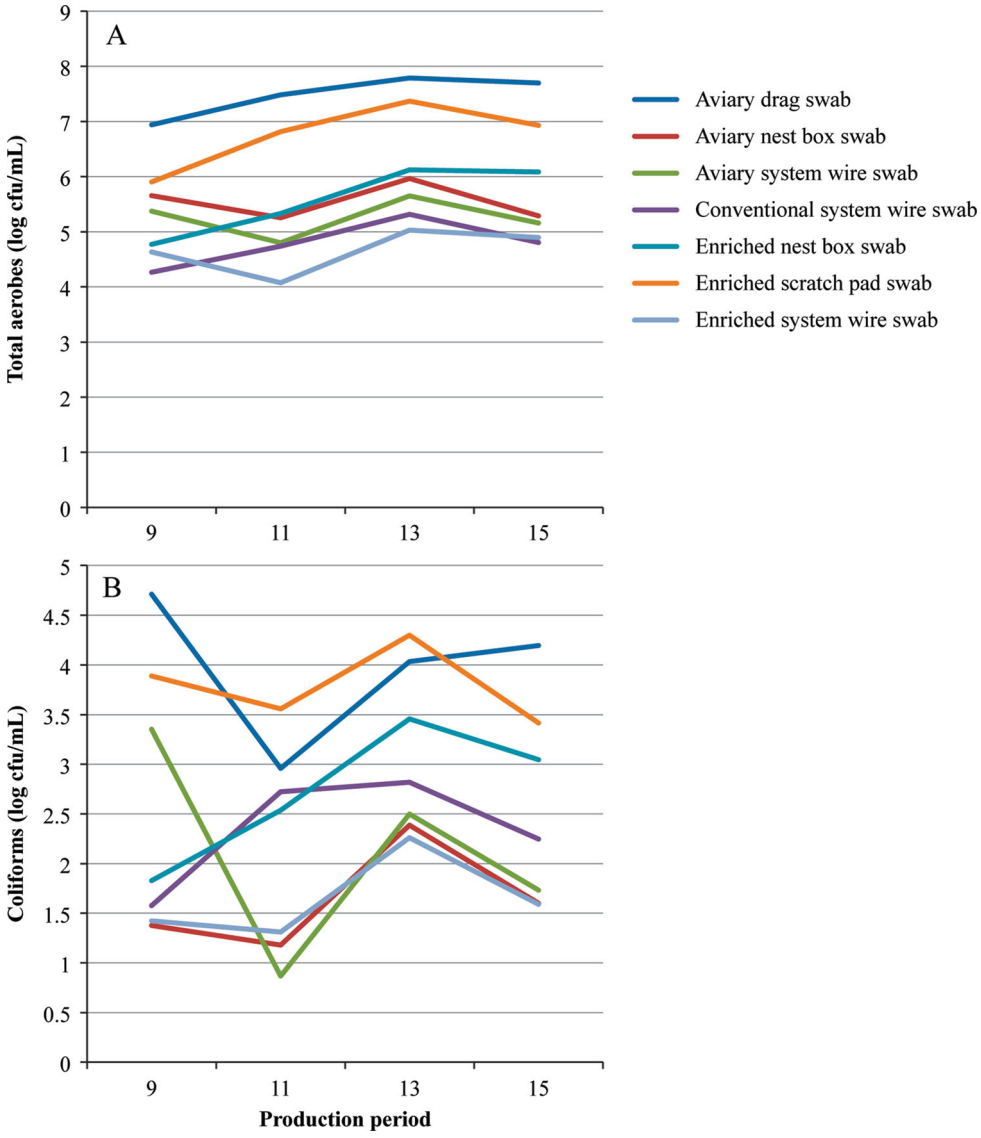

Figure 2. Effect of sample type × production period interaction on levels of A) total aerobes and B) coliforms associated with environmental swabs ($P < 0.001$).

with the system wire swabs (4.7 log cfu/mL, enriched colony cage; 4.8 log cfu/mL, conventional cage; 5.3 log cfu/mL, aviary). There was a significant environmental swab × production period interaction ($P < 0.001$) for total aerobes (Figure 2A). During all 4 collection periods, aviary drag swabs had the greatest aerobic organism contamination followed by enriched colony cage scratch pad swabs. Aerobic organism levels gradually increased in enriched colony cage nest box swabs, then plateaued for the last collection period. Conventional cage and enriched colony cage system wire swabs had the lowest levels of aerobic contamination throughout the study.

Average coliform counts were greatest for aviary drag swabs (4.0 log cfu/mL) and enriched colony cage scratch pads (3.8 log cfu/mL; Table 2). The lowest levels of environmental coliform contamination were found in aviary nest box and enriched colony cage system swabs (1.6 and 1.7 log cfu/mL, respectively). There was a significant environmental swab × production period interaction ($P < 0.001$) for coliforms (Figure 2B). Aviary drag swabs and enriched scratch pad swabs had consistently greater levels of coliform contamination compared with the other environmental samples. Coliform levels gradually increased in enriched colony cage nest box swabs, as was seen in the corresponding total aerobic counts.

The prevalence of *Salmonella* and *Campylobacter* spp. in the environmental swabs is presented in Table 2. Manure scraper swabs had the greatest prevalence of *Salmonella* spp. (100% aviary; 99% conventional cage; 89% enriched colony cage; $P < 0.0002$). Aviary drag swabs also had an elevated level (69%) of *Salmonella* spp. contamination. The lowest levels of *Salmonella* spp. prevalence were found in enriched colony cage system wire (16%) and nest box (16%) swabs. The greatest prevalence of *Campylobacter* spp. ($P < 0.0001$) was detected in aviary drag swabs (100%) and enriched colony

Table 3. Total aerobes, coliforms, *Salmonella* spp., and *Campylobacter* spp. associated with shell emulsion pools from commercial conventional cage, enriched colony cage, and aviary housing systems[1].

Sample type	Average total aerobes[2] (log cfu/mL)	Average total coliforms[2] (log cfu/mL)	*Salmonella* spp. (%; no. positive/ total no. samples)	*Campylobacter* spp. (%; no. positive/ total no. samples)
Aviary floor	4.9 ± 0.1	1.0 ± 0.1	7.8 (6/77)	2.6 (2/77)
Aviary nest box	3.5 ± 0.1	0.2 ± 0.1	1.3 (1/80)	5.0 (4/80)
Aviary system wire	4.1 ± 0.1	0.6 ± 0.1	4.8 (3/63)	4.8 (3/63)
Conventional system wire	2.8 ± 0.1	0.1 ± 0.1	7.5 (6/80)	1.3 (1/80)
Enriched nest box	2.6 ± 0.1	0.2 ± 0.1	7.5 (6/80)	5.0 (4/80)
Enriched system wire[3]	3.5 ± 0.1	0.2 ± 0.1	0 (0/12)	16.7 (2/12)

[1]Samples collected over 4 production periods. Pathogens assessed on all shell pools. Enumeration of aerobes and coliforms conducted on up to 10 shell pools for each sample type, each collection period.

[2]Significant sample type × production period interaction ($P < 0.0001$).

[3]Means represent the first 3 production periods. No enriched system wire shell pools were produced during the final period of collection.

cage scratch pads (93%). None of the conventional cage manure scraper swabs were positive for *Campylobacter* spp., but 41 and 40% of aviary and enriched colony cage manure scraper swabs, respectively, contained *Campylobacter* spp. Nest box swabs from the aviary had low levels (10%) of *Campylobacter* contamination, whereas 64% of enriched colony cage nest box swabs were contaminated. System wire swabs from all housing systems were highly contaminated with *Campylobacter* (74% aviary; 63% conventional cage; 65% enriched colony cage).

Visual inspection and scoring (0–7 scale) of aviary nest pads (D. Campbell, M. Makagon, J. Swanson, and J. Siegford, 2014, personal communication Department of Animal Science, Michigan State University) and enriched colony cage nest and scratch pads provided insight into usage by hens. In the aviary system, a total of 288 nest pads were scored over the 3 observation times. The nest pads were consistently scored 0 (only 7 aviary nest pads received a score of 1 during the study). Median enriched colony cage nest pad scores were 0, 1, and 1 (19, 52, and 72 wk of hen age, respectively). During both the 52- and 72-wk observation periods, 97% of the eggs produced in the observed replicates were laid in the nest box. Median enriched colony cage scratch pad scores (with observation range) were as follows: 19 wk, 1 (1–2); 52 wk, 4 (1–7); and 72 wk, 2 (1–7).

Shell Pools

The highest levels of average total aerobic organisms were associated with aviary floor (4.9 log cfu/mL) and system eggs (4.1 log cfu/mL; Table 3). The lowest levels of total aerobic organisms were found in enriched colony cage nest box and conventional cage system wire shells (2.6 and 2.8 log cfu/mL, respectively). There was a significant housing shell pool × production period interaction ($P < 0.001$) for total aerobic organisms. Aviary floor and system wire eggs maintained the greatest level of aerobic organism contamination throughout the study (Figure 3A). Conventional cage and enriched colony cage nest box eggs consistently maintained the lowest level of aerobic organism contamination.

Average coliform levels for all shell samples were 1 log cfu/mL or less. The greatest average shell pool coliform count was associated with aviary floor eggs (1.0 log cfu/mL). There was a significant housing system shell pool × production period interaction ($P < 0.001$) for coliform levels (Figure 3B). Coliform levels associated with the shells were low for all housing systems/egg types (1–5%), but aviary floor and system wire eggs had the greatest counts.

There were no significant differences between shell sample types for the prevalence of *Salmonella* or *Campylobacter* spp. (Table 3). Very low levels of shell *Salmonella* spp. contamination occurred throughout the study (0–8%). No enriched colony cage system wire shell pools were positive for *Salmonella*, but there were also only 12 pools formed throughout the entire study due to appropriate nest box use in the enriched colony cage system. *Campylobacter* spp. contamination was greatest for the enriched colony cage system wire shell pools, but again, there were only 2 positive pools out of the 12 collected during the study.

A restrictive positive correlation has been found between environmental dust levels and aerobic counts on eggshells (De Reu et al., 2005). Aviary shell pools had the highest total aerobes cultured during the study. In the current study, considerably higher dust concentration was found in the aviary system compared with the conventional and enriched colony cage systems (Zhao et al., 2014a). Furthermore, Huneau-Salaün et al. (2010) and Jones et al. (2011) have attributed dust to high shell aerobic counts.

Aviary forage area and enriched colony cage scratch pads were found to have the highest levels of total aerobic and coliform contamination. Both of these sample sites have high surface area components (litter and feed, respectively). The scratch pads and nest box pad (both aviary and enriched colony cage) were of similar construction and material. The introduction of feed on the scratch pad appears to result in a much higher microbial load and prevalence of *Campylobacter*. Additionally, the visual scoring of the nest and scratch pads showed very little visible dirt on the nest pads, which is assumed to be due to reduced defecation on the nest pads.

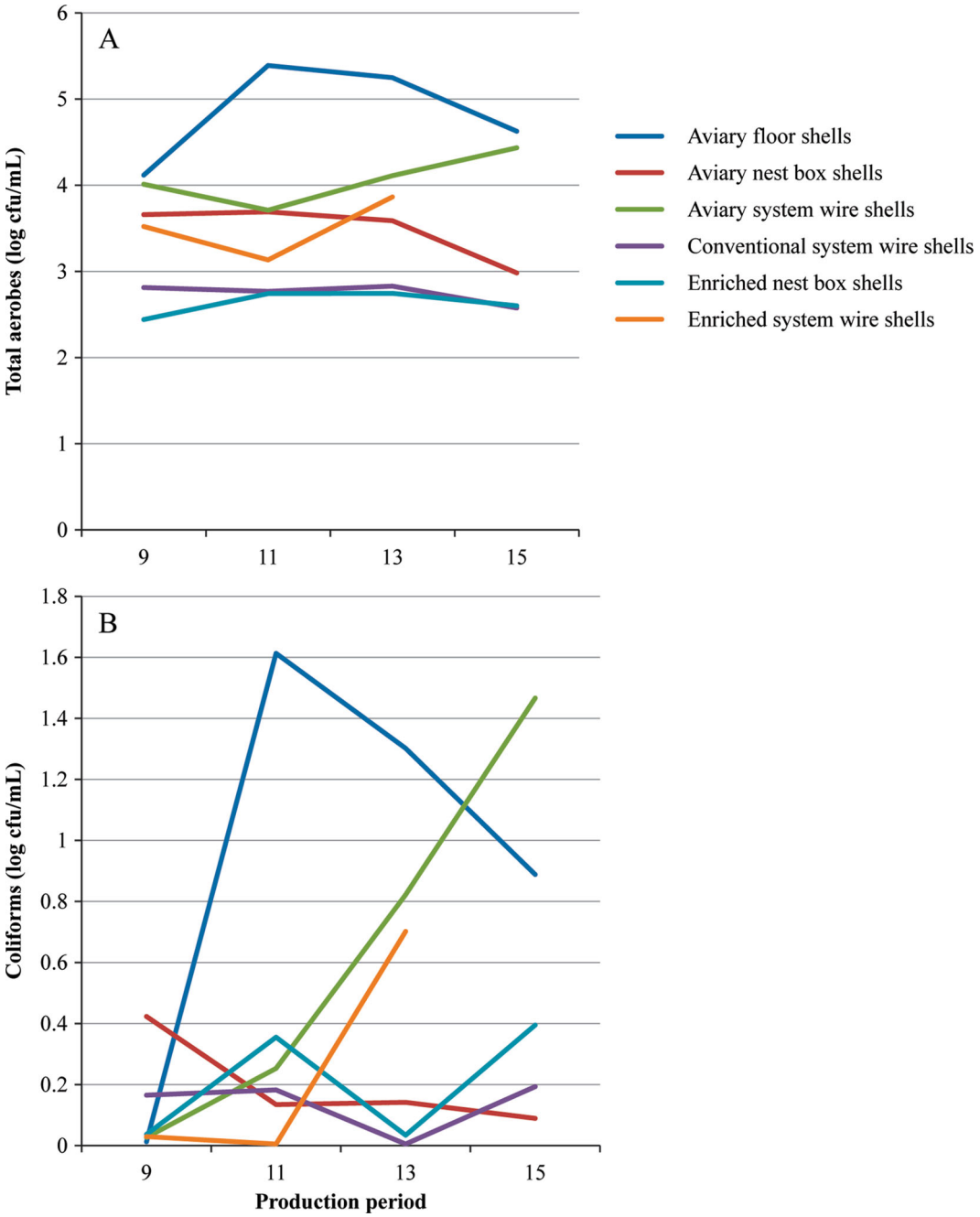

Figure 3. Effect of sample type × production period interaction on levels of A) total aerobes and B) coliforms associated with eggshell pools ($P < 0.001$).

Management practices for alternative systems need to address reducing floor eggs to enhance food safety. Floor eggs from the aviary system consistently had the greatest microbial loads. Jones and Anderson (2013) also report higher levels of *Enterobacteriaceae* associated with floor eggs. De Reu et al. (2006) recommend excluding floor eggs from retail when not washing eggs. Although most eggs in the US are washed before entering retail, some producers may not be required to wash eggs and should be informed of the risk associated with floor eggs.

De Vylder et al. (2009) and Gast et al. (2013) disagree as to the effect of orally challenging hens with *Salmonella* Enteritidis and resulting *Salmonella* detection in feces or tested organs based on housing systems. All 3 housing systems compared in the current study were shedding *Salmonella* spp. at a high rate (89–100% of manure belt scraper blade swabs). The dry manure belt removal system present in all housing systems appears to affect *Campylobacter* spp. detection because 0 to 41% of manure belt scraper swabs were culturally positive yet other environment samples had much higher rates of contamination. Aviary forage drag swabs had high detection levels of *Salmonella* and *Campylobacter* spp. There was no difference in the detection of *Salmonella* and *Campylobacter* spp.

associated with shells from the 3 systems and prevalence was low. Cox et al. (2012) have noted that *Campylobacter* can be present in an injured state resulting in low cultural detection, but given appropriate conditions can still result in illness. The detection rate of *Campylobacter* spp. in the environment of all 3 housing systems indicates *Campylobacter* spp. should be considered in egg safety management decisions. Additionally, *Campylobacter* spp. was the only other human pathogen (besides *Salmonella* spp.) associated with shell eggs in a recent consideration of food sources and disease burden in the United States (Batz et al., 2012). The current study compared the prevalence of *Salmonella* and *Campylobacter* spp. between the 3 housing systems via typical enrichment procedures and did not result in enumerated levels of *Salmonella* and *Campylobacter* spp.

Results of previous egg microbiological comparisons do not agree on housing system effects (De Reu et al., 2005, 2009; Mallet et al., 2006; Sulonen et al., 2007; Huneau-Salaün et al., 2010; Jones et al., 2011; Jones and Anderson, 2013). When reviewing these papers and comparing to current results, it can be concluded that the management of housing systems is important. Environmental dust affects environmental and egg microbiology. Nest box usage in alternative systems influences egg microbiology. Furthermore, floor eggs have the greatest opportunity for exposure to high levels of microorganisms and human pathogens so flocks should be managed to prevent the occurrence of floor eggs. The results of the current study also raise concern about the hen health and food safety implications of scratch pads. Because scratchpads are an important resource for hen welfare, more research is needed (alternative materials, surfaces, substrates, and so on) before clear recommendations can be made, but scratch pads are a point of microbial concern.

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