PROCESSING, PRODUCTS, AND FOOD SAFETY

Comparison of environmental and egg microbiology associated with conventional and free-range laying hen management

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ABSTRACT Eggs from alternative production practices are a growing niche in the market. Meeting consumer requests for greater diversity in retail egg options has resulted in some unique challenges such as understanding the food safety implications of eggs from alternative production practices. A study was conducted to determine what, if any, differences exist between nest run conventional cage-produced eggs and free range-produced eggs. A sister flock of brown egg layers was maintained in conventional cage and free-range production with egg and environmental sampling every 6 wk from 20 to 79 wk of age. Aerobic, coliform, and yeast and mold populations were monitored. Environmental microbial levels were not always indicative of egg contamination levels. When significant differences ($P < 0.05$ and $P < 0.0001$, dependent on season) were observed among treatments for coliforms, shell contamination levels of free-range nest box eggs and free-range floor eggs were always greater than those of conventional cage eggs, which remained low throughout the study (0.42–0.02 log cfu/mL). Shell yeast and mold levels were significantly greater in free-range floor eggs than in free-range nest box eggs and conventional cage eggs throughout the entire study. Egg contents contamination levels were extremely low for all monitored populations and treatments. Season of the year played a role in both environmental and egg microbial levels. Winter had the lowest levels of all populations monitored for all treatments, except for aerobic free-range floor egg shell emulsions, which were increased (3.6 log cfu/mL). Understanding the differences in microbial populations present on conventional cage-produced and free range-produced eggs can lead to the development of effective cleaning procedures, enhancing food safety.

Key words: egg microbiology, conventional cage, free-range, environmental, shell egg

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INTRODUCTION

Eggs are a significant agricultural commodity in the United States. According to the American Egg Board, approximately 215.7 million cases (77.65 billion eggs) were produced in the United States in 2009 (AEB, 2010). The estimated per capita consumption of shell eggs and egg products in the United States in 2008 was 248.9 (AEB, 2010). Of table eggs (shell eggs) produced in the United States, 9.5% are from free-range, cage-free, organic, and other specialty production methods (IBIS World, 2009). Although the overall percentage of shell eggs from nonconventional production is small, it has continued to grow and represents consumer desires for greater choice in egg buying options.

In the United States, various standards, guidelines, and regulations govern the production and processing of shell eggs. Federal egg grading standards are established by the USDA Agricultural Marketing Service (USDA, 2000a). Each egg processor is required to meet the appropriate state egg laws according to their facility location and distribution practices. Some choose to participate in the USDA voluntary egg surveillance program to produce shell eggs bearing the USDA grade shield (USDA, 2005). In 2009, the Food and Drug Administration published a final rule with the intent of controlling *Salmonella* infection of eggs during production, transportation, and storage (FDA, 2009). Egg producers with more than 50,000 hens on site had to be in compliance by July 9, 2010. Producers with 3,000 to 50,000 hens on site must meet the rule requirements by July 9, 2012. Producers with fewer than 3,000 hens on site do not fall under the jurisdiction of this rule. Some producers and processors choose to participate in the USDA National Organic Program (USDA, 2000b). With all these rules and guidelines, no federal or state standards exist for microbial levels on or in shell eggs.

Today's egg cartons can have a wide variety of verbiage, at times overwhelming consumers. Some of the

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more common terms found on cartons include cage-free, free range, free roaming, vegetarian fed, and antibiotic-free and various nutritional claims. In many cases (with the exception of the National Organic Program), no federal or state standards are associated with these terms, leaving consumers to their own interpretations. A lack of conclusive scientific evidence exists as to the effects of these various production criteria on overall egg microbiology. Most of the research comparing the microbiological implications of various egg production methods has been conducted in Europe, often with conflicting outcomes (De Reu et al., 2005, 2006, 2009; Mallet et al., 2006; Messens et al., 2007; Sulonen et al., 2007; Singh et al., 2009). Furthermore, hen strain, production practice differences, and climate make it more difficult to compare egg microbiological data around the world. This study was undertaken to determine whether environmental and egg microbiological differences exist for the same strain of laying hen between conventional cage and free-range production.

MATERIALS AND METHODS

Hen Management

A flock of Hy-Line Brown hens were hatched at the Piedmont Research Station, North Carolina Department of Agriculture and Consumer Services (Salisbury, NC). The full description of rearing and production management was reported by Anderson (2008). Briefly, all chicks were housed in the same brood–grow pullet house equipped with conventional cages or floor pens. The chicks to be used for conventional caged egg production were raised in a quad-deck system with 13 birds/cage (310 cm^2/bird). The chicks to be used for free-range production were raised in floor pens on litter (929 cm^2/bird) with access to roosts. At 12 wk of age, the floor-raised hens were moved to the range environment to complete the rearing phase.

At 17 wk of age, the conventional cage hens were moved to a quad deck laying house with 6 hens/cage (413 cm^2/hen). For free-range production, 75 hens were housed in each range hut–paddock, equating to 929 cm^2/hen in the range hut, 13 cm roosting space/hen, and 1 nest box/8 hens. The range paddock afforded forage area of 8.04 m^2/hen. All dietary and lighting regimens were equivalent and are detailed in Anderson (2009).

Environmental and Egg Sample Collection

Environmental and egg samples were collected from conventional cages, free-range nest boxes (**FRNS**), and free-range grass (**FRG**) approximately every 6 wk from 20 to 79 wk of hen age (11 sampling periods). Swabs were collected (in triplicate) from the conventional cage wire egg collection area (**CCWS**) and FRNS using a sterile gauze pad (10 × 10 cm) moistened with 20 mL of sterile PBS. After swabbing, each gauze pad was placed in a sterile sample bag and transported to the laboratory on ice. Samples of FRG were aseptically collected using sterile shears to cut a handful of grass 2.5 cm from the ground. The grass samples were placed in sterile sample bags and transported to the laboratory on ice. These sample sites were selected because they were egg contact surfaces. Furthermore, grass from the paddock area provides an indication of hen environmental microbial exposure. All samples were stored at 4°C overnight before analysis.

The following morning, 30 mL of sterile PBS was added to each swab sample and stomacher blended (Stomacher 400 Circulator, Seward Ltd., London, UK) for 1 min at 230 rpm. Grass samples were aseptically cut into small pieces with sterile shears. Grass samples were then weighed and sterile PBS was added to the samples at a 1:10 ratio. Samples were then stomacher blended for 1 min at 230 rpm.

A 30-egg flat of eggs for each treatment [conventional cage (**CC**), free-range nest box (**FRNB**), and free-range floor (**FRF**)] was aseptically collected at the research farm. The CC and FRNB eggs were laid in roll-out style cages and nest boxes, respectively, which allow eggs to roll out into a collection tray. Eggs from each treatment were placed in a clean laboratory bag and transported to the laboratory on ice and stored at 4°C overnight. The following morning, cracked eggs were discarded. For each treatment, 8 pools of 3 eggs each were formed for both shell emulsions and egg contents. Shell emulsion pools were compiled in sterile specimen cups according to the methods of Musgrove et al. (2005b) using 50 mL of 42°C sterile PBS. Egg content pools were formed in sterile laboratory sample bags and stomacher blended 1 min at 230 rpm according to the methods of Jones et al. (2004).

Microbial Assessments

Total aerobic populations were determined by duplicate spread plating 100 uL of appropriate dilutions from shell emulsions and environmental swabs or 250 uL of egg contents onto standard methods agar (Acumedia Manufacturers, Lansing, MI). The plates were incubated at 35°C for 48 h before enumeration. Levels of yeasts and molds were determined by duplicate spread plating 100 uL of appropriate dilutions from shell emulsions and environmental swabs or 250 uL of egg contents onto dichloran rose bengal chloramphenicol agar (Acumedia Manufacturers). Plates were incubated, right side up, for 6 d at 25 to 26°C before enumeration. Coliforms were enumerated by dispensing 1 mL of appropriate dilutions from shell emulsions and environmental swabs or egg contents into violet red bile agar (Acumedia Manufacturers) pour plates with overlay. Duplicate plates per sample were incubated at 37°C for 18 to 20 h before typical colonies were counted.

Statistical Analysis

Before analysis, sampling periods were classified according to the season of the year based on astronomical classification, with the seasons beginning as follows: September 21, fall; December 21, winter; March 21, spring; June 21, summer. According to this classification the number of sampling periods per season was as follows: fall, 5; winter, 2; spring, 2; summer, 2. Treatment and season were the main effects. Microbial counts were subjected to log transformation (SAS Institute, 2002) and analyzed for significance through the general linear model procedure of SAS. Means were separated by the least squares method.

RESULTS

Environmental microbiological results can be seen in Figure 1. The total aerobic population level in FRG was significantly greater than on CCWS for all seasons (Figure 1A; fall, $P < 0.05$; winter, $P < 0.0001$; spring, $P < 0.05$; summer, $P < 0.05$).The FRNS levels were similar to CCWS levels for all seasons except summer, when FRNS were similar to FRG for aerobic contamination. Throughout all seasons, FRG aerobic microbial levels ranged from 5.85 to 6.69 log cfu/mL and CCWS aerobic microbial levels ranged from 3.50 to 4.49 log cfu/mL, representing less than 1 log change in average levels throughout the life of the flock. The FRNS aerobic levels had the greatest variability (3.30–6.18 log cfu/mL) during the course of the study.

The FRG and FRNS coliform levels were significantly greater than CCWS coliform levels throughout the study (Figure 1B; $P < 0.0001$). The FRG coliform counts were 4.31 to 5.36 log cfu/mL, whereas CCWS levels ranged from none detected to 0.64 log cfu/mL. The FRNS coliform levels were similar to FRG coliform levels during all seasons except winter, when the lowest level (1.98 log cfu/mL) was detected for FRNS. The FRG yeast and mold counts (4.70–5.45 log cfu/mL) were significantly ($P < 0.0001$) greater than CCWS yeast and mold counts (0.22–1.02 log cfu/mL) throughout the course of the study (Figure 1C). The FRNS were similar to CCWS during winter and summer and similar to FRG during summer.

Average levels of microbial populations associated with the shell and membranes of eggs from free-range and caged production are seen in Figure 2. The greatest levels of shell emulsion aerobic contamination for all treatments were detected in fall and summer, with no differences found between treatments (Figure 2A). In winter, FRNB shell emulsions had significantly ($P < 0.05$) lower levels of aerobic contamination than FRF and CC shell emulsions (2.19 log cfu/mL vs. 3.60 and 3.30 log cfu/mL, respectively). During spring, FRNB and FRF shell emulsions had significantly ($P < 0.05$) lower aerobic levels than CC shell emulsions (2.79 and 3.06 log cfu/mL vs. 3.87 log cfu/mL, respectively).

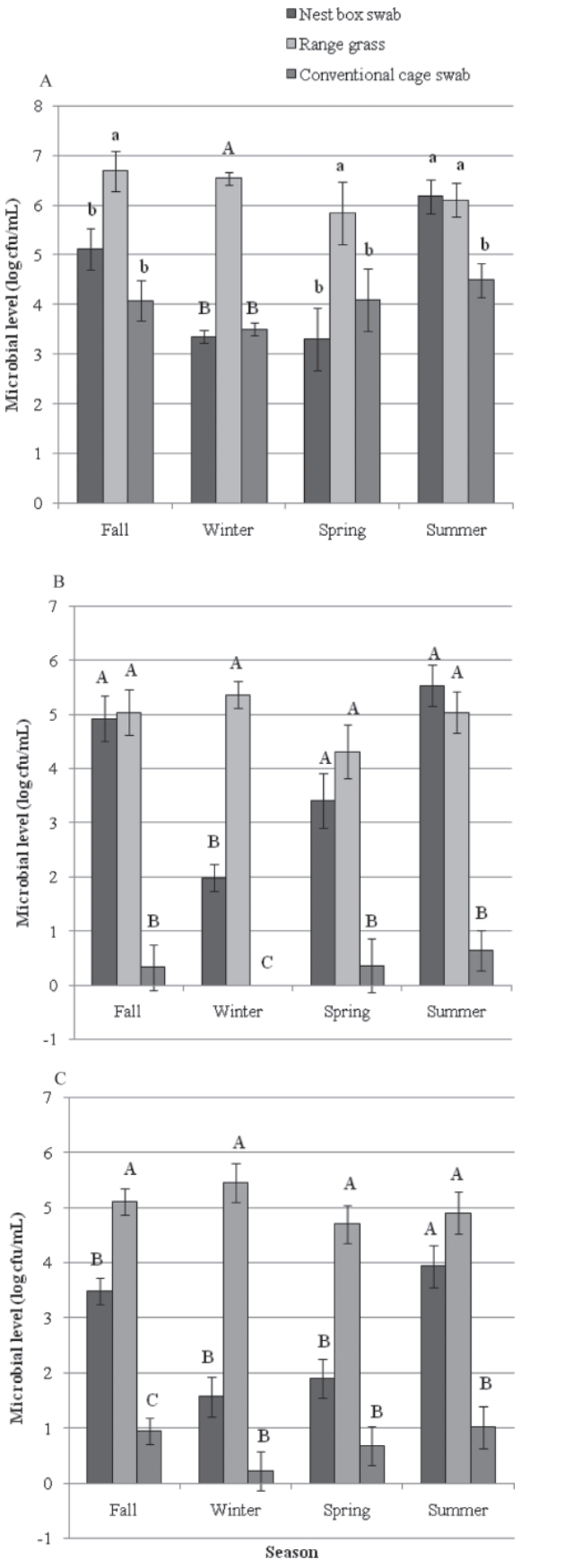

Figure 1. Microbial levels present on nest box swabs, range grass, and conventional cage swabs according to season. A) Total aerobic populations. B) Coliforms. C) Yeasts and molds. Lowercase letters indicate $P < 0.05$ and uppercase letters indicate $P < 0.0001$, separating significant differences within a season.

When significant differences between treatments occurred for levels of coliforms in shell emulsions, FRNB and FRF were different from CC (Figure 2B; fall, $P < 0.0001$; spring, $P < 0.05$; summer, $P < 0.0001$). Winter produced similar coliform levels among all treatments. Winter also had the lowest levels of coliforms present in the shell emulsion for FRNB and FRF. Summer produced the highest levels of shell emulsion coliforms for FRNB and FRF, as well as the lowest counts for CC (2.61 and 2.00 log cfu/mL vs. 0.02 log cfu/mL, respectively). The CC shell emulsion coliform levels were low throughout the study, ranging from 0.42 to 0.02 log cfu/mL. The FRF shell emulsion yeast and mold levels were significantly greater than FRNB and CC shell emulsion yeast and mold levels throughout the entire study (Figure 2C; fall, $P < 0.0001$; winter, $P < 0.0001$; spring, $P < 0.0001$; summer, $P < 0.05$). The CC shell emulsion yeast and mold levels were the lowest each season, with FRNB shell emulsion yeast and mold levels being similar in winter and spring. The lowest level of yeast and mold for all treatments was seen in winter.

Egg content contamination levels were low throughout the life of the flock (Figure 3). During each season, CC had the greatest degree of aerobic contamination among the treatments, with the highest level (Figure 3A; 1.31 log cfu/mL) achieved during summer, which was significantly different from FRNB and FRF degree of aerobic contamination ($P < 0.0001$). Other than the increase exhibited by CC in the summer, detectable levels for all treatments were <0.6 log cfu/mL for aerobic microbial presence in the egg contents. Only a few egg contents pools among all treatments had detectable levels of coliform (Figure 3B). No differences were found among treatments for levels of coliforms present in egg contents. Very low levels of yeast and mold were detected throughout egg production. When detectable levels were present, FRF egg contents always exhibited the greatest degree of yeast and mold contamination (Figure 3C). Although two instances of statistical differences among treatments occurred (fall and summer, $P < 0.05$), the levels detected were of little biological importance because the levels detected were low.

DISCUSSION

Environmental aerobic microbial levels were not indicative of shell contamination for CC eggs in the current study. The CCWS consistently maintained the lowest level of aerobic microorganisms throughout the study, yet CC shell emulsion aerobic levels were some of the highest recorded. Visual inspection of the eggs collected during this study noted the CC eggs were dusty compared with FRNB and FRF eggs. De Reu et al. (2005) found a restrictive positive correlation between aerobic microbial levels in the air and shell contamination. The authors speculate that the dust present on the conventional cage egg shell contributed to the high levels of aerobic populations enumerated from the shell emulsion and egg contents pools. Consequently, FRG

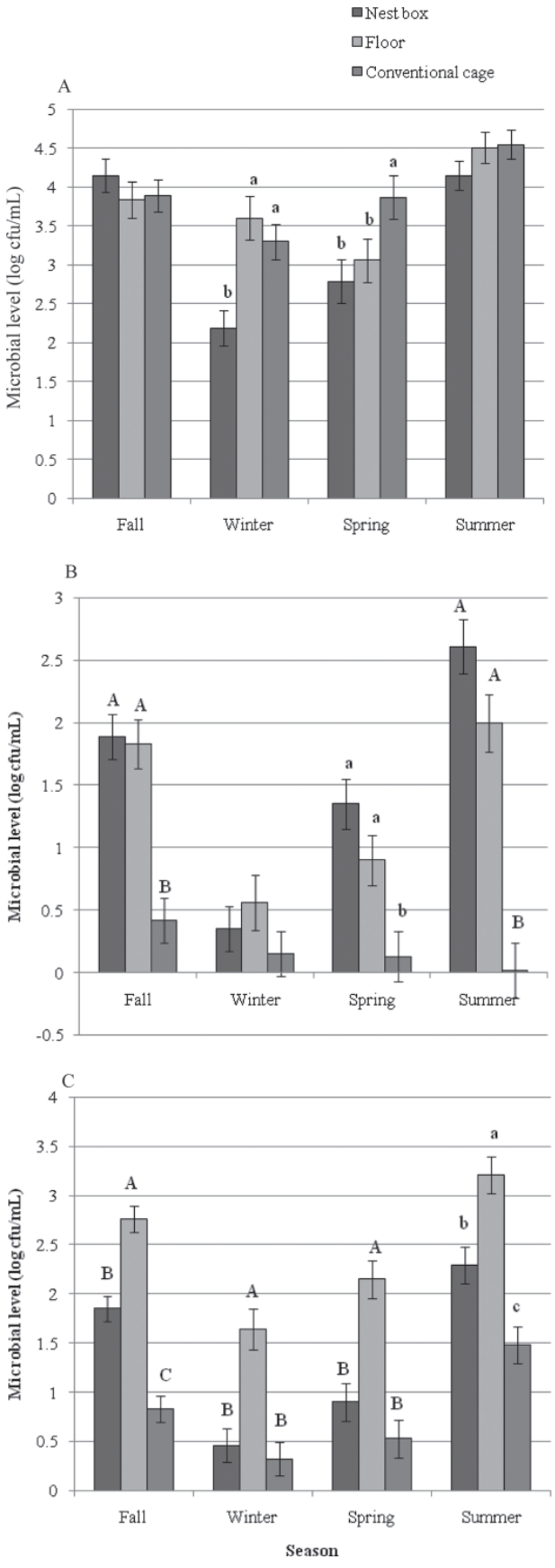

Figure 2. Microbial levels of shell emulsions from free-range nest box, free-range floor, and conventional cage according to season. A) Total aerobic populations. B) Coliforms. C) Yeasts and molds. Lowercase letters indicate $P < 0.05$ and uppercase letters indicate $P < 0.0001$, separating significant differences within a season.

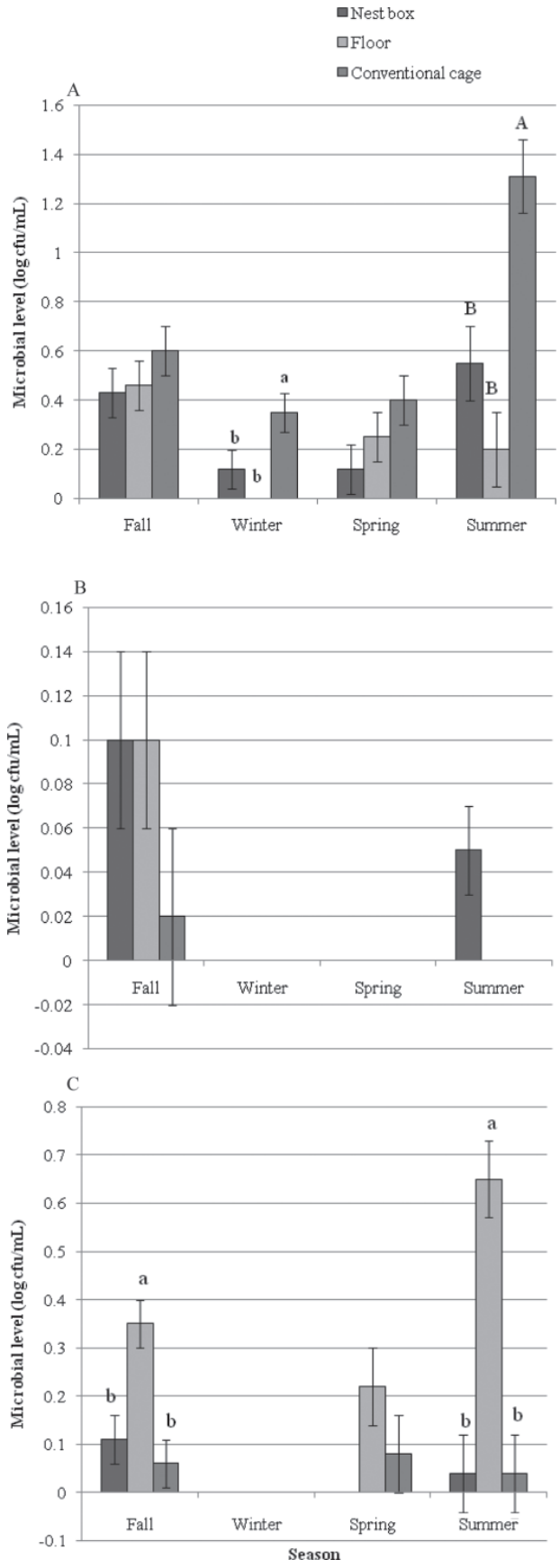

Figure 3. Microbial levels of egg contents from free-range nest box, free-range floor, and conventional cage according to season. A) Total aerobic populations. B) Coliforms. C) Yeasts and molds. Lowercase letters indicate $P < 0.05$ and uppercase letters indicate $P < 0.0001$, separating significant differences within a season.

environmental samples had significantly higher aerobic levels that were not seen in the shell emulsion or egg contents results. Therefore, hen contact with grass in the paddock area did not lead to aerobic microbial transfer in the current study. For all treatments, aerobic shell emulsion level trends mimicked egg contents contamination trends.

Coliform levels detected by CCWS were extremely low. This same phenomenon was seen in CC shell emulsion and egg contents results. Singh et al. (2009) also found conventional cage eggs to have lower levels of *Escherichia coli* and coliforms compared with nest and floor eggs. Conversely, De Reu et al. (2006) reported a lower level of gram-negative organisms on the shells of alternatively produced eggs. In the current study, when FRNS and FRG levels were statistically similar, a corresponding increase in coliform detection was seen in FRNB and FRF shell emulsion pools. Adhering dirt was noted most often on FRF and FRNB eggs. The aligning trends in environmental and shell emulsion samples were not detected in FRNB and FRF egg contents. The FRNB and CC shell emulsion yeast and mold levels mimic the trends of FRNS and CCWS through the study. Yeast and mold levels detected in egg contents pools were so low it is difficult to draw clear correlation conclusions between them and environmental and shell emulsion contamination levels.

Winter resulted in the lowest microbial levels for all treatments and populations with the exception of aerobic microorganisms associated with free-range floor shell emulsions. In 2006, Mallet et al. (2006) also found the lowest levels of microbial populations in winter and the highest levels in summer. Free-range grass samples maintained the highest levels of microbial contamination for all environmental samples monitored throughout the course of the study.

The FRNB and FRF eggs had the greater levels of shell emulsion coliforms, as well as yeast and mold, compared with CC eggs. Current US egg processing guidelines have been found to effectively reduce microbial populations of conventional cage eggs (Moats, 1981; Jones et al., 2004; Musgrove et al., 2005a). De Reu et al. (2006) concluded that in Europe, where commercial egg washing is not a common practice, the high bacterial levels present on floor eggs indicate they should not be consumed. More exploration is needed to understand whether US egg processing guidelines are appropriate to reduce microbial contamination associated with eggs from alternative production practices or whether alterations to the current processing methods are warranted. In the current study, the number of floor eggs laid by the free-range hens decreased as hen age increased.

This study provides useful insight into the seasonal changes in microbial populations associated with conventional and free-range production. De Reu et al. (2009) have surmised that farm practices have a strong influence on the microbial quality of eggs. More research considering the effects of additional housing and

management options, as well as various genetic stocks of laying hens, is necessary for a complete understanding of the microbial implications of alternative egg production practices.

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