Nest use and patterns of egg laying and damage by 4 strains of laying hens in an aviary system[1]

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ABSTRACT Laying hens are strongly motivated to use nests for egg laying, and alternative production systems (e.g., aviaries) provide artificial sites to meet this need and ensure efficient collection of clean, undamaged eggs. However, nests are typically not provided to allow simultaneous use by all hens; therefore, competition or mislaid eggs can result. To understand the influence of strain on laying eggs outside nests and damage to eggs, we compared daily patterns of nests use and egg laying among 4 laying hen strains (Hy-Line Brown (HB), Bovans Brown (BB), DeKalb White (DW), and Hy-Line W36 (W36)). Hens were observed over 3 consecutive days in aviaries with colony nests in the enclosure's top tier (2 nests/unit, 4 aviary units/strain, 144 hens/unit). The number and location of hens in nests and the number, location and condition of eggs throughout aviaries were recorded. Most eggs (90 to 95%) were laid in nests; however, brown hens consistently laid more non-nest eggs and damaged more eggs than white hens ($P \leq 0.05$). Higher nest occupancy by brown hens was correlated with more non-nest and damaged eggs ($P \leq 0.05$). In the morning, brown hens occupied more nest space and laid more nest eggs than white hens (e.g., HB vs. DW: 82.97 and 34.66% of space; 91.35 and 68.73% of nest eggs; $P \leq 0.05$). At midday, white hens occupied more nest space and laid more nest eggs than brown hens (e.g., HB vs. DW: 28.47 and 15.81% of space; 27.39 and 8.29% of nest eggs; $P \leq 0.05$). Brown hens preferred right nest compartments and laid more eggs there, whereas white hens preferred left compartments and W36 laid more eggs there ($P \leq 0.05$). These findings indicate that different strains of hens have different patterns of nest use and laying behavior. In brown hens, heavy morning nest use was related to laying eggs outside nests and more damaged eggs, suggesting insufficient space for oviposition in nests. Specific facility design should be matched to hens' preferences to accommodate behavioral needs of different strains.

Key words: aviary, laying hen, strain, nest, egg laying

2017 Poultry Science 96:3011–3020
http://dx.doi.org/10.3382/ps/pex104

INTRODUCTION

Stimulation of natural species-specific behaviors in domestic laying hens (*Gallus gallus domesticus*) via management of space and resources in alternative production systems compared to the conventional cage is a topical issue. In particular, adequate nest provision is critical to both hen welfare and efficient production. Nests should be designed to allow hens to perform pre-lay behaviors and oviposition in a desirable location with minimal competition to avoid frustration, stress, and possibly retained eggs (Duncan and Kite, 1989; Cooper and Appleby, 1995). Nests should also collect eggs in a manner that maintains clean, intact eggshells for optimal system production.

Artificial nests used in production systems are sometimes considered a "super-stimulus" for eliciting nesting behaviors, as these nests typically offer a more enclosed space with softer flooring that is attractive to hens for performance of both pre-lay behaviors and oviposition compared to other areas in the system (Appleby et al., 1985). Hens also exhibit gregarious nesting; the presence of hens inside a nest further increases its attractiveness, stimulating other hens to

Received December 8, 2016.
Accepted March 26, 2017.

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This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch projects #1002990 and #1010765, and by a grant from the Michigan Alliance for Animal Agriculture (East Lansing, MI).

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also lay eggs in the same location (Appleby and Smith, 1991; Riber 2010; 2012). However, nest site attractiveness and social facilitation coupled with the hens' internal biological rhythm, which compels them to preferentially lay eggs during the morning hours (Boz et al., 2014), can result in overcrowding if nests are not provided in sufficient quantities to allow synchronous nesting by all hens in the system (Abrahamsson and Tauson, 1995; Odén et al., 2002; Freire et al., 2003). Crowding in the nests may increase the risk of heat stress and smothering (Riber, 2010), aggression between hens (Hunniford et al., 2014), or scratches, wounds, and feather loss from birds climbing on top of each other (Appleby and Smith, 1991). Insufficient space for simultaneous use of the nest by all hens may prevent hens from performing pre-lay behavior and oviposition in a preferred location (Appleby and Smith, 1991; Freire et al., 1998; Kruschwitz et al., 2008) and can result in litter or non-nest laid eggs by individuals unable to access the nest (Kruschwitz et al., 2008).

When eggs are laid in nests designed to facilitate egg collection, they are also more likely to remain clean and undamaged. For example, egg damage has been attributed to length of contact time between hens and eggs (Appleby, 1998). When eggs are laid in designated nest areas, the system is designed so that the eggs roll out of the cage onto the egg belt, which both limits hens' access to eggs and carries them away for processing. However, when nests are crowded, the bodies and legs of the hens in the nest may inhibit eggs from rolling away quickly and subject them to jostling, being stepped on or being pecked at. Additionally, eggs laid in the enclosure or litter may remain in proximity to hens for up to 24 h before being manually collected, making them susceptible to damage and microbial contamination (Jones et al., 2015). A high frequency of floor or enclosure eggs results in increased labor to collect eggs, dirtier eggs, and fewer saleable eggs (Appleby, 1984; Singh et al., 2009; Jones et al., 2015).

Current domesticated laying hen strains have been molded by a variety of selection pressures, including greater egg production, egg quality, feed efficiency, longevity, and behavior (Besbes et al., 2002; Wolc et al., 2012). In the modern, intensive production environment in which food, water, lighting, temperature, and humidity are maintained at optimal conditions, egg production may be considered at its maximum potential per strain. However, different strains of birds can vary in behavior including laying location preferences and oviposition time (Tůmová et al., 2007; Singh et al., 2009). Thus, in terms of nest use and production quality, certain strains may be better suited to specific types of laying hen housing. As aviary systems become more common, research is needed to determine optimal space and resource provision for different hen strains to minimize stress to individual birds and maintain efficient production. One such type of alternative system is the aviary, which consists of a tiered enclosure, floor litter area, and nests situated on the top level. The aviary system may provide many advantages such as increased space per bird, a litter area for dust bathing, perches, and secluded nest areas but may also increase competition during high demand periods for specific resources. Previous research using Lohmann white laying hens housed in commercial aviaries of the same design as the current study showed the majority (97%) of eggs were laid in the nest (Jones et al., 2015). However, a recent study comparing 4 strains of laying hens in the same commercial-styled aviary system as the current study showed strains varied in their spatial distribution within the system across the day and night such as in their use of the litter area and upper tiers (Ali et al., 2016).

To date, there has been limited research comparing nest use and laying patterns of various strains of laying hens in current commercial-style aviaries. Therefore, the main objective of the current study was to compare the daily patterns of nest use among 4 different laying hen strains (Hy-Line Brown, Bovans Brown, DeKalb White, and Hy-Line W36) housed in one type of aviary, and to understand whether patterns of nest occupancy were related to higher ratios of eggs laid outside the nest or damaged eggs. It was hypothesized that the different laying hen strains would vary in their location of egg laying as different strains might have different perceptions of nest attractiveness. In addition, strains might be differently influenced by social facilitation or perhaps have differences in the degree to which laying behavior is entrained by an internal circadian rhythm. Variance between strains in turn was expected to result in several outcomes with welfare and production implications such as differences in occasional overcrowding of nests and different ratios of nest to non-nest (enclosure or litter) laid eggs (and consequently different ratios of damaged to intact eggs).

METHODS

Ethics

All research protocols were approved by the Michigan State University Institutional Animal Care and Use Committee prior to the start of data collection (AUF# 01/15-025-00).

Hens, Housing, and System Management

Laying hens at 36 wk of age from 4 genetic strains (n = 576/strain: DeKalb White (**DW**), Hy-Line Brown (**HB**), Bovans Brown (**BB**) and Hy-Line W36 (**W36**)) were used in this study. Strains were chosen based on breeder recommendations as being likely to be used in the United States as alternative housing systems to the conventional cage are adopted. These hens were part of a larger overall study, from which some results have been published regarding differences between strains in distribution throughout the aviary system during day and night (Ali et al., 2016). The description of hens, housing, and system management that follow are therefore largely identical between the current report and the first paper published from this work (Ali et al., 2016).

Prior to placement in the aviary, chicks were reared from hatch in environmentally controlled, windowless houses containing 6 pens per side (n = 12 total pens) at the Michigan State University Poultry Teaching and Research Center (East Lansing, MI). Each pen housed 225 to 250 chicks, with 3 pens per strain (n = 675 to 750 chicks/strain). Chicks were brooded on elevated platforms (122 × 488×46 cm) with plastic flooring and solid sides within these same pens. Feeding space and nipple drinkers were provided as per industry guidelines. From 3 wk of age, chicks were given access to a floor area, and ladders and roosting area were provided at this time.

At 17 wk of age (March 2015), pullets were placed into a commercial-style aviary system (NATURA60, Big Dutchman, Holland, MI) in the Laying Hen Facility at the Michigan State University Poultry Teaching and Research Center. The facility included 4 rooms housing aviary systems with each room containing 4 discrete aviary units (1 unit per strain/room × 4 strains × 4 rooms = 16 units total). Each unit was initially populated with 144 hens. To ensure that each hen-housing unit contained a mix of birds from each rearing room, pullets from each of the 3 rearing pens per strain were randomly allocated to each of the 4 hen housing units per strain. Strains were placed into units within rooms in a balanced fashion to ensure that across the 4 rooms, each strain occupied each of the 4 different unit locations to account for possible effects of units being near the door or at ends versus the center of rows.

As shown in Figure 1, each aviary unit was composed of a 3-level tiered wire-mesh enclosure (each with 61 cm internal ceiling height) and a litter area (divided into an open litter area in front of the tiered enclosure and a litter area underneath the tiered enclosure), for a detailed description of the aviary system, please see Ali et al. (2016).

A colony nest ran the length of each unit in the upper tier, with one central partition creating 2 compartments of equal size. The colony nest was 52 cm wide and each compartment was 122 cm long. In total, this provided 88 cm^2 nesting space per hen at the initial stocking of 144 hens/unit, meeting the United Egg Producer (2016) recommendations of 83.6 cm^2/hen. Nesting areas automatically closed 1 h before lights off (B_1, Figure 2) and re-opened at 03:00 to be available for laying at the start of the day (B_0, Figure 2). Although hens were placed into the aviary at 17 wk of age, litter access was initially restricted in order to train hens to use nest boxes and minimize floor laying. Hens gained access to litter at 26 wk of age when the target of ∼90% of egg production was achieved. After this time, doors on the lower tier of the aviary enclosures automatically opened each morning at 11:30 to allow hens daily access to the litter-covered floor area. The doors closed each evening at 01:00, approximately 5 h after lights off.

Lights were turned on every day at 05:00 and turned off at 20:00 during the observation period (C_0 and C_1 in Figure 2). Eggs were collected daily at 08:00. Feed belts ran at 06:00, 14:00, and 19:30 to deliver feed to hens. At 09:00 and 16:45 the feed belts ran for approximately 10 s to stimulate hens to feed.

Hen Mortality

Hen mortalities were recorded daily for each individual unit. At the end of the study, the total number of hens that had died in each unit from placement to 36 wk was summed, and cumulative mortality calculated as a percentage of the 144 hens originally placed in a unit. Cumulative mortality per each strain was as follows: HB: 1.21%, BB: 1.04%, DW: 1.21 %, W36: 1.39%. Cumulative mortality was used to calculate the exact number of birds inside each unit at 36 wk to accurately estimate egg production for the actual number of hens in each unit.

Observations

Observations were conducted by the same trained observer over 3 consecutive days when hens were 36 wk old immediately preceding routine egg collection by farm personnel. Units were observed in the same order every day in order to be synchronized with the collection routine. Each unit was evaluated for approximately 3 minutes; 1 complete round of observations through all 16 units took ∼45 min. Two rounds of observations were conducted in each 2 h window starting at the beginning of the window to maintain an even time difference between observations of each unit. As shown in Figure 2, observations were conducted in MORNING, MIDDAY, and EVENING. MORNING (Ob_1 and Ob_2), MIDDAY (Ob_3 and Ob_4), and EVENING (Ob_5 and Ob_6) observations included counting hens in each side of the nest; recording the number of eggs laid in each side of the nest, in the litter, and in the tiered enclosure; and recording the number of damaged eggs in those same locations. A "damaged" egg was recorded when there was any visible damage to the outer shell that would deem the egg un-collectible. Such damage occurred in the shape of holes or cracks that would make the yolk or albumin accessible. Eggs that were consumed by hens between observations (as indicated by a declining egg count), were also recorded as damaged. The Ob_0 count (at lights on) served as a baseline count for the morning so that eggs laid the day before were not counted as new.

Data and Statistical Analyses

The number of eggs laid in an observation set (e.g., Ob_1) was calculated by subtracting the number of eggs counted in the preceding period from the count made in the current period. In the case of MORNING Ob_1, the baseline egg count for subtraction was the initial

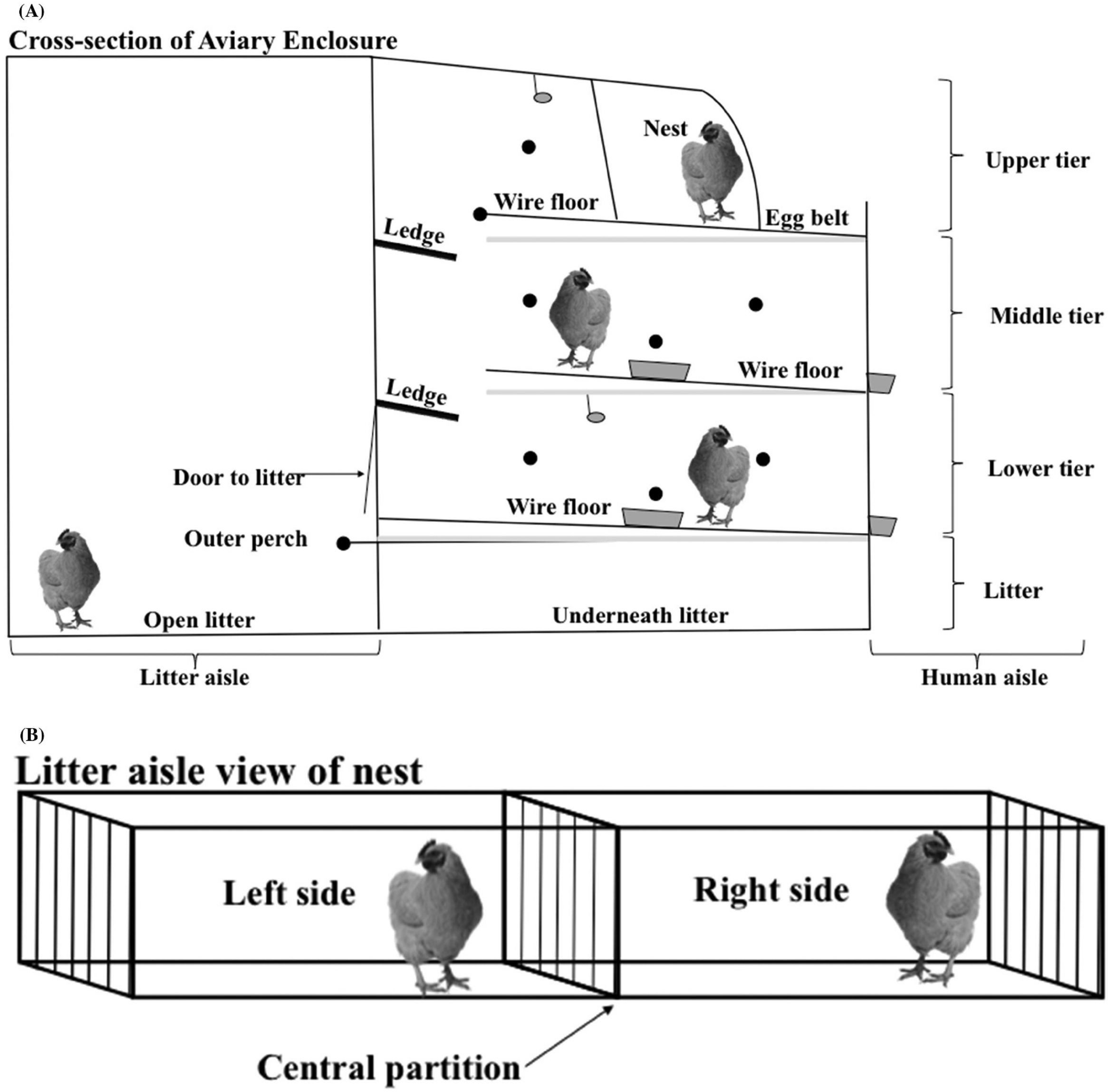

Figure 1. (**A**) An end view of the 3-tiered aviary unit, showing human and litter aisles and locations of litter areas, solid metal ledges, wire floors, the colony nest, manure belts (gray bars), perches (black circles), drinkers (gray ovals), and external and internal feeders (gray boxes). (**B**) A diagram of the nest as viewed from the litter aisle, showing the solid central partition, which creates 2 compartments of equal size (left and right).

egg count made in Ob_0 at 06:00 (Figure 2). The actual number of eggs laid in each time period (e.g., MORNING) was obtained by adding together the number of eggs laid in the 2 observation sets for that period (e.g., Ob_1 and Ob_2).

Total daily egg production was calculated as a percentage for each unit by first dividing the total number of eggs laid in a day in that unit by the actual number of hens in that unit and multiplied by 100 (e.g., if 125 eggs were laid in a unit with 137 hens, then the daily production percentage would be 91.24%). The percentages of eggs laid each day in nests, litter, and the tiered enclosure in a unit were calculated based on the total number of eggs produced that day by hens in that unit (e.g., if 101 eggs were laid in nests out of 125 total eggs, the percentage of nest laid eggs for the unit would be 80.8%). The total percentage of damaged eggs in a unit was calculated by dividing the number of eggs counted as damaged that day by the total number of eggs laid that day.

To examine whether hens were overcrowding nests, nest occupancy was calculated for each unit during the MORNING, MIDDAY, and EVENING time periods and with respect to right and left sides of the nest. Using, the results of Mench and Blatchford's (2014) kinematic analysis of the space needs of W36 hens (318 cm^2/sitting hen), the total number of hens able to simultaneously sit in the nest area of a unit was estimated to be 36 hens (18 hens on either side of the partition, Figure 1B). Thus, at the initial stocking rate of 144 hens/unit 25% of the hens could nest simultaneously. The percentage of nest space occupied was then calculated for each unit by dividing the actual number of hens counted sitting in each side of the nest

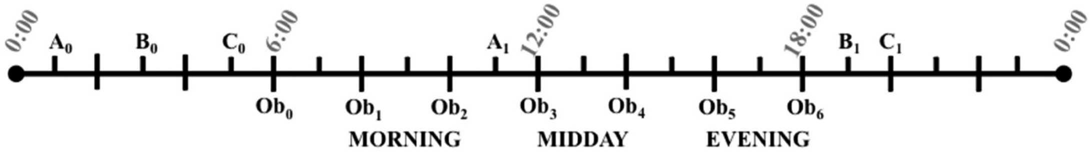

Figure 2. A timeline depicting daily management procedures and data collection events. A_0: Doors on lower tiers of aviary enclosures automatically closed to prevent hens from accessing litter-covered floor areas. A_1: Doors on lower tiers of aviary enclosures automatically opened to allow hens to access litter areas. B_0: Colony nests opened automatically. B_1: Colony nests closed automatically. C_0: Lights turned on 5:00. C_1: Lights turned off at 20:00, beginning with a 30 min period of gradual overhead light dimming followed 15 min later by dimming of a rope light in the middle tier. MORNING (Ob_1 and Ob_2), MIDDAY (Ob_3 and Ob_4), and EVENING (Ob_5 and Ob_6) observations included counting hens in each side of the nest; recording the number of eggs laid in each side of the nest, in the litter and enclosure, and in the tiered enclosure; and recording the number of damaged eggs in those same locations. Ob_0 was a count of eggs only.

by the estimated accommodation capacity of 18 hens per side (e.g., if 21 hens were counted in the right side of the nest, the percent of nest space occupied would be 117%).

Statistical analyses were performed using R software (version 3.3.1), package "stats" (R Core Team, 2013). Descriptive statistics were calculated using the psych package, and data are presented as mean ± standard error of the mean (SEM); $P \leq 0.05$ was considered significant. To describe the influence of different laying hen strains on the pattern of egg laying throughout the day and within the aviary unit, and all possible interactions, generalized linear mixed models (GLMM) were developed with family set to "binomial" (because data were normal and met assumptions of equal variance), with the "logit" link function, using the lme4 package (Bates et al., 2015). Aviary unit and day of observation were included as random effects for all the models. Statistically significant effects in all models were further analyzed with Tukey's honestly significant difference (HSD) multiple comparison procedure using the multcomp package (Hothorn et al., 2008).

The model for total egg production included fixed effects of strain of laying hen (DW, HB, BB, W36), egg-laying location (nest, litter, or enclosure), time of egg laying (06:00, 08:00, 10:00, 12:00, 14:00, 16:00, 18:00), and their interactions. The model for total damaged eggs, included fixed effects of hen strain, location of damaged eggs (nest, litter or enclosure), and their interactions. Finally, the models for nest use (hen occupancy and egg laying in the nest) included fixed effects of strain, time of the day (Morning, Midday, and Evening), side of the nest (Right and Left), and their interactions.

Finally, Pearson's correlation tests were used to examine the relationship between daily nest occupancy and the corresponding prevalence of 1) non-nest laid eggs and 2) damaged eggs. Separate correlations were conducted for each of the 4 strains.

RESULTS

Egg Laying

An interaction between strain, location, and time of day was found for egg laying (Figure 3A and B, Z = 6.69; $P = 0.001$). Brown hens laid about 85% of their daily nest eggs from 6:00 to 10:00, while white hens laid about 55% of their daily nest eggs during those 4 h, and at 8:00, brown hens laid more eggs compared to white hens (Figure 3A: Z = 7.63; $P = 0.001$). From 10:00 to 14:00, white hens laid about 35% of their daily nest eggs while only about 8% of nest eggs were laid by brown hens during this later time period. During 12:00 and 14:00, white hens laid more eggs than brown hens (Figure 3A: Z = 5.15; $P = 0.021$, Z = 2.57; $P = 0.41$, respectively). The largest percentage of non-nest laid eggs for brown hens were laid during the period from 6:00 to 10:00, as they laid more than 80% of their daily non-nest laid eggs in this 4 h period versus only 35% for white hens. At 8:00, more non-nest eggs were laid by brown than white hens (Figure 3B: Z = 6.58;

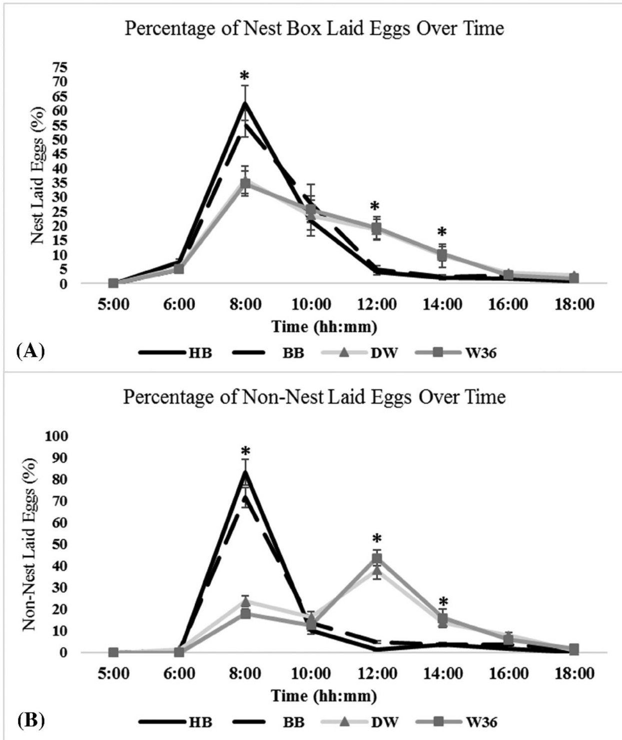

Figure 3. Percentage of nest laid (**A**) and non-nest laid (**B**) eggs over time. HB = Hy-Line Brown, BB = Bovans Brown, DW = DeKalb White, and W36 = Hy-Line W36. All parameters are expressed as mean eggs counts ± SEM. The asterisks (∗) indicate statistically significant differences ($P < 0.05$) between Brown and White hen strains.

Table 1. Total egg production and location of eggs laid by 4 strains of laying hens in an aviary system.

Parameter Strain	Eggs laid daily per hen (%)[1]	Location of eggs (as % of total laid)[2] Nests	Enclosure	Litter
Hy-Line Brown	90.12 ± 0.14[b]	90.53 ± 0.84[b]	5.09 ± 0.26[a]	4.38 ± 0.18[a]
Bovans Brown	90.97 ± 0.15[b]	91.59 ± 0.80[b]	4.63 ± 0.25[a]	3.78 ± 0.21[b]
DeKalb White	92.03 ± 0.16[a]	95.57 ± 0.74[a]	2.12 ± 0.20[b]	2.31 ± 0.16[b]
Hy-Line W36	92.54 ± 0.18[a]	94.93 ± 0.36[a]	2.09 ± 0.16[b]	2.98 ± 0.14[b]
P value	0.022	0.003	0.001	0.031

Parameters are presented as means ± SEM for the aviary units of each strain. [1]Eggs laid daily are expressed as a percentage per hen calculated by using the actual number of hens in each unit. [2]Location of eggs laid each day are expressed as a percentage of the total eggs laid that day (100%) in each unit. [a,b] Means within the same column lacking a common superscript differ significantly ($P < 0.05$).

Table 2. Total damaged eggs and location of eggs damaged by 4 strains of laying hens in an aviary system.

Parameter Strain	Damaged eggs (% of total laid)[1]	Location of damaged eggs (% of total damaged)[2] Nests	Enclosure	Litter
Hy-Line Brown	4.88 ± 0.33[a]	36.87 ± 3.33[a]	48.07 ± 5.32[a]	15.06 ± 1.03[b]
Bovans Brown	5.78 ± 0.46[a]	41.12 ± 4.36[b]	36.29 ± 3.69[b]	18.59 ± 1.36[b]
DeKalb White	1.43 ± 0.12[b]	12.69 ± 1.01[c]	38.62 ± 2.24[b]	48.69 ± 4.12[a]
Hy-Line W36	1.09 ± 0.09[b]	15.73 ± 1.15[c]	32.25 ± 2.75[b]	52.02 ± 4.58[a]
P value	0.023	0.003	0.03	0.004

Parameters are presented as means ± SEM for the aviary units of each strain. [1]Eggs damaged daily are expressed as a percentage of the total eggs laid that day in each unit. [2]Location of damaged eggs each day are expressed as a percentage of the total damaged eggs (100%) found that day in each unit. [a,b] Means within the same column lacking a common superscript differ significantly ($P < 0.05$).

$P = 0.002$). In contrast, white hen laid more than 50% of their daily non-nest eggs during the period from 10:00 to 14:00 versus only 6% for brown hens. In particular, white hens laid more non-nest eggs at 12:00 and 14:00 compared to brown hens (Figure 3B: Z = 4.58; $P = 0.012$, Z = 3.89; $P = 0.036$, respectively).

An interaction was also observed between hen strain and location for egg laying (Table 1; Z = 5.36; $P = 0.002$). HB and BB hens laid more eggs throughout the tiered enclosure compared to DW and W36 hens (Table 1), while DW and W36 hens laid a higher percentage of their eggs inside nests compared to HB and BB hens. HB hens laid a higher percentage of their eggs in the litter compared to the other 3 strains of hens (Table 1). Finally, average daily egg production also differed by strain, as white hen strains DW and W36 laid more eggs than did brown strains HB and BB (Table 1).

Damaged Eggs

For damaged eggs, an interaction was found between hen strain, and location of damaged eggs. First, the percentage of damaged eggs found in the nest was significantly different among the 4 strains (Table 2; Z = 3.86; $P = 0.003$). Pairwise comparisons revealed that BB hens had the greatest number of damaged eggs in the nests compared to hens of the other 3 strains (Table 2), and HB had more damaged eggs in the nests compared to both white strains). On the other hand, more damaged eggs were found in the litter for white hens relative to brown hens (Table 2). Finally, a difference was found in the percent of damaged eggs in the tiered enclosure among the 4 strains (Table 2). Pairwise comparisons revealed more damaged eggs were recorded in tiered enclosures for HB compared with the other 3 strains (Table 2). Again, hen strain affected the proportion of damaged eggs (Table 2) with a higher percentage of brown hens' eggs found to be damaged compared to those of white hens.

Nest Use

An interaction between strain and time of day was found for occupancy of nests (Figure 4A; Z = 10.59; $P = 0.001$). Pairwise comparisons showed that nest occupancy was higher for brown hens in the MORNING compared with nest occupancy by white hens at that time (Figure 4A; HB: $P = 0.002$, 0.003; BB: $P = 0.003$, 0.004, in both cases versus DW and W36 respectively). In 29% of observations during the MORNING, HB nests were overcrowded. However, white hens had higher nest occupancy during MIDDAY observations compared with brown hens (Figure 4A; DW: $P = 0.041$, 0.039; W36: $P = 0.035$, 0.039, in both cases versus HB and BB respectively). No difference was detected in nest occupancy among the 4 strains during EVENING observations.

Corresponding with nest occupancy rates, there was an interaction between hen strain and time of day for nest laid eggs (Figure 4B: Z = 9.69; $P = 0.001$). Pairwise comparisons showed that brown hens laid a higher percentage of their nest eggs in the MORNING compared with white hens (Figure 4B; HB: $P = 0.019$, 0.004; BB: $P = 0.011$, 0.002, in both cases versus DW and W36 respectively). On the other hand, white hens laid more nest eggs at MIDDAY compared with brown hens (Figure 4B; DW: $P = 0.036$, 0.041; W36: $P = 0.029$, 0.036, in both cases versus HB and BB respectively).

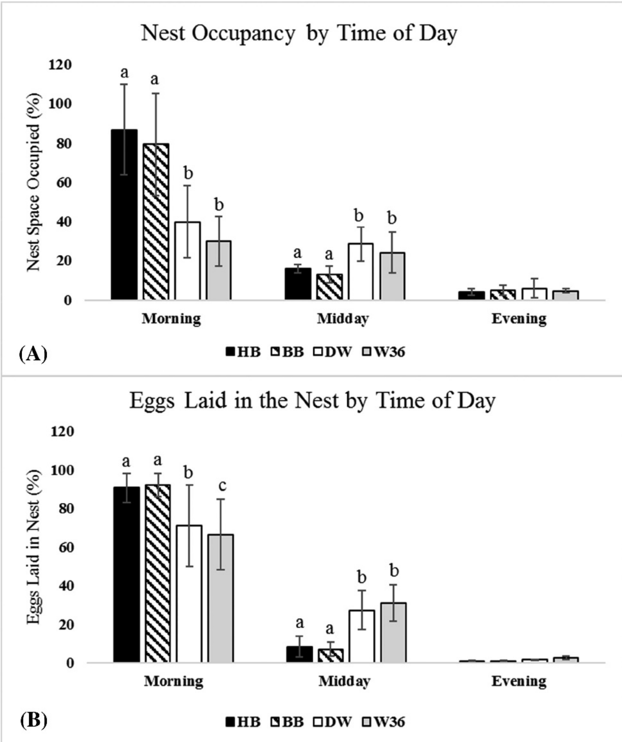

Figure 4. (**A**) Nest space occupied by hens during MORNING, MIDDAY, and EVENING at 36 wk of age expressed as the percentage of the total hens (n = 36) that could fit into the nest area of a unit (based on kinematic of Mench and Blatchford's (2014) estimate of 318 cm^2 per sitting hen). (**B**) Eggs laid in the nest during MORNING, MIDDAY, and EVENING at 36 wk of age expressed as a percentage of the number of eggs laid in a given unit per number of hens in that same unit. HB = Hy-Line Brown, BB = Bovans Brown, DW = DeKalb White, and W36 = Hy-Line W36. All parameters are expressed as mean bird counts ± SEM. Different superscripts indicate statistically significant differences ($P < 0.05$) among different strains at that time of day.

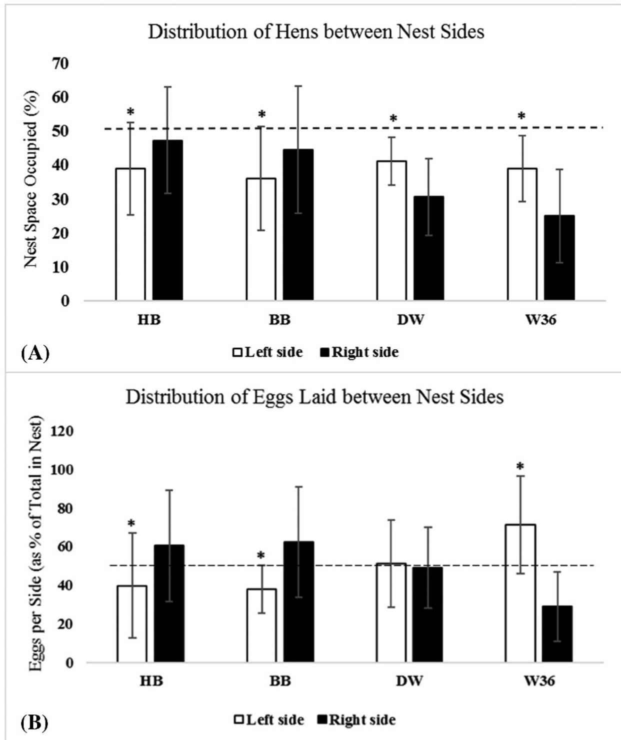

Figure 5. (**A**) Distribution of hens between the left and right sides of the nest expressed as a percent of the total number of hens of each strain observed in the nest. (**B**) The distribution of eggs laid in the left and right sides of the nest expressed as a percent of the total number of eggs laid in the nest by each strain. The dashed horizontal line at 50% represents the percentage of hens (**A**) or eggs (**B**) that would be expected to be in each side of the nest by chance if hens did not have a preference for nest side. HB = Hy-Line Brown, BB = Bovans Brown, DW = DeKalb White, and W36 = Hy-Line W36. All parameters are expressed as mean bird counts ± SEM. The asterisks (∗) indicate statistically significant differences ($P < 0.05$) between occupancy of or egg laying in the 2 sides of the nest by that strain.

However, there was no difference in percent of nest laid eggs among the 4 strains during the EVENING observations.

Finally, interactions were found between hen strain and side of the nest (Right and Left) for both nest laid eggs (Figure 5A: $Z = 7.36$; $P = 0.002$) and nest occupancy (Figure 5B: $Z = 6.89$; $P = 0.009$). Throughout the day, brown hens occupied the right side of the nest at a higher rate than the left side (Figure 5A; HB: $P = 0.002$, BB: $P = 0.0031$) and also laid more eggs on the right side of the nest (Figure 5B; HB: $P = 0.037$, BB: $P = 0.025$). Conversely, white hens occupied the left side of the nest at a higher rate than the right side (Figure 5A; DW: $P = 0.0032$, W36: $P = 0.0029$), and W36 hens also laid more eggs in the left versus the right side of the nest (Figure 5B; W36: $P = 0.025$). DW hens, however, did not differ in egg laying between the left and right side of the nest (Figure 5B).

Positive relationships were found between daily nest occupancy and the number of non-nest laid eggs (i.e., eggs laid in both the litter and the tiered enclosure; Table 3) for both strains of brown hens (HB and BB). Daily nest occupancy was also positively correlated with the number of damaged eggs observed for both strains of brown hens (Table 3).

Table 3. Association between daily nest occupancy and eggs laid outside the nest and damaged eggs for 4 strains of laying hens in an aviary system.

	Parameter	Non-nest laid eggs r_s (P-value)	Damaged eggs r_s (P-value)
Nest occupancy by strain	Hy-Line Brown	0.62 (0.01)	0.53 (0.01)
	Bovans Brown	0.39 (0.03)	0.43 (0.02)
	DeKalb White	0.12 (0.13)	0.09 (0.69)
	Hy-Line W36	0.06 (0.63)	0.25 (0.09)

Parameters are expressed as correlation coefficients with the corresponding P value for the correlation between nest occupancy and 1) non-nest laid eggs and 2) damaged eggs for each strain.

DISCUSSION

Comparative analyses of nest occupancy and location of egg laying among 4 strains of laying hens in an aviary system revealed few differences between strains of the same color i.e., brown BB and HB hens generally showed similar patterns and white DW and W36 were typically similar to each other. However, important differences in laying time, nest use, laying location, and egg damage were present between white and brown strains that could have implications when determining which strains to place into particular aviary designs or in designing or redesigning aviary systems. First, brown hens showed strong circadian rhythms by occupying nests and laying eggs at higher rates in the morning than white hens. The nests in the aviary studied here could accommodate 36 hens per unit at any one time (at a rate of 318 cm^2/hen), allowing approximately 25% of the hens in a unit to nest simultaneously. Given that hens typically lay most of their eggs in the morning, not having enough space for hens to nest synchronously could be expected to result in either adaptation by hens to either lay eggs in nests at other times of day or to lay eggs in other areas of the system. In the face of limited nest space for synchronous nesting, brown hens adapted by laying more eggs outside the nest but maintained a strong circadian rhythm of morning egg laying, both in the nest and in the tiered enclosure. White hens, on the other hand, extended their occupancy of and egg laying in nests through the early afternoon and laid a higher percentage of their eggs in nests. Thus, our findings suggest that the white and brown strains responded differently to limited nest space, with brown hens responding in a way that conflicts with producers' needs for eggs to be laid in desired locations so that eggs are protected, clean and easily collected. In parallel our results, Singh et al., (2009) found that Lohmann brown hens laid a higher proportion of floor eggs in comparison to Lohmann white hens. However, these authors attributed the difference to the brown hens' potentially having lower motivation to use the nest.

The difference in diurnal laying patterns between brown and white strains could also be attributed specifically to genotype, as genetic history influences typical oviposition time (Tůmová et al., 2007; Tůmová and Gous, 2012), including interactions between hen age and genotype (Tůmová and Gous, 2012). Further, time of oviposition within hens of a given strain can change as hens get older (Zakaria et al., 2005; Tůmová and Gous, 2012). Another factor to consider is a hen's need to perform pre-lay behaviors that include searching for and selecting a nesting site and sitting for a period of time prior to oviposition (Cronin et al., 2012a). Pre-lay behavior is influenced by a hen's circadian rhythm to lay eggs in the morning (Channing et al., 2001), by hen age (Riber, 2010), and individual hen tendencies (Cooper and Appleby, 1996) with frustration behaviors exhibited when nest access is removed (Cronin et al., 2012a). Crowding in nests may constrain pre-lay behaviors and consequently delay oviposition as stress in individual hens can cause egg retention within the shell gland (Hughes et al., 1986). Hens provided the opportunity to sit for longer prior to oviposition show lower plasma corticosterone (Cronin et al., 2012b) concentrations post-lay but overall, the corticosterone stress responses following prevention of pre-laying need further investigation (Cronin et al., 2012a). To ensure good welfare in alternative housing systems using group-nests, it is important to take into account all aspects of egg-laying behavior (Ringgenberg et al., 2014). Findings from previous comparative studies involving observation of activity surrounding the nest, aggression, and displacement behavior (Odén et al., 2002), pre-lay behaviors of hens laying outside the nest (Cronin et al., 2005) and tracking of eggs laid by individual hens underscore the importance of examining pre-lay behavior in addition to oviposition itself to understand egg lay pattern discrepancies between strains.

The hens showed nest side preferences with brown hens preferring the right and white hens the left side of the nest, leading to more crowding as well as more eggs (for all but DW) on the preferred nest side. Nest side biases or preferences for end nests (rather than central) have been demonstrated within other hen strains in experimental settings and are stable with age (Riber, 2010; Clausen and Riber, 2012). Here, our colony nest was partitioned into 2 equal halves, with the right side always nearest the door to the room (and potentially with more light) and the left side nearest the back of the room (and potentially darker). Initial nest preferences are potentially exacerbated by social attraction (Riber, 2010), thus overcrowding in nests may be highly influenced by hens' natural tendency to nest gregariously, a possible anti-predator defense mechanism (Appleby, 1984; Riber, 2010; Riber 2012).

Aviary systems with nest boxes or colony nests and roll-away egg catchment largely prevent the problem of egg eating as eggs are inaccessible to hens (Appleby, 1998). But crowding within nests can prevent eggs from rolling away and subject eggs to hen contact, including both deliberate and accidental beak and toe-related damage. Further, non-nest laid eggs rely on manual collection and thus are typically in longer contact time with hens, which might lead to these eggs being damaged (Mohammed et al., 2013). In fact, in the current study higher nest occupancy in MORNING by the brown hen strains was related to a higher proportion of damaged eggs, and it is possible some damaged eggs were never counted if they were consumed or dropped to the litter belts under the tiers before being counted. Thus, in this system, nest crowding within the brown strains affected the quantity of salable eggs.

Hens in the current study were confined to the aviary from 17 to 26 wk of age, in part to train them to use nest boxes rather than lay elsewhere. The temporary exclusion of pullets from the litter area after transfer to the laying facility for this purpose has also been reported to reduce fearfulness and improve plumage condition (Alm et al., 2015). Litter provision after a period of exclusion can impact how hens distribute themselves in the aviary for up to 3 wk as they adapt to using the litter area (Ali et al., 2016). This study was conducted when hens were 36 wk old, after they had been on litter for 10 wk, at which point they may have fully acclimated. However, it is important to determine whether withholding access to a resource hens value does promote nest use and increase the percentage of nest-laid eggs and to what degree such a practice impacts hen welfare.

CONCLUSION

Differences found between circadian patterns of lay, location of egg laying, and amount of damage to eggs found among 4 strains of laying hens in the present study emphasize the need to explicitly consider the responses of genetic strains of laying hens when designing laying hen housing or determining which strain to place in an existing facility. For hens that prefer to lay eggs within a short time frame each day, such as the brown strains studied here, more nest space is needed to accommodate their behavioral synchrony and reduce the numbers of mislaid or damaged eggs. For hens that prefer to lay eggs in a nest but have less diurnal constraints, current nest space allowances may be sufficient. However, future research should also examine whether hens of these white strains experience stress as a result of limited nest space even if they seem to adapt to the limitation by laying at other times of day. Practically, findings from this study suggest that Hy-Line Brown and Bovans Brown hens should be placed in aviaries with more nest space per hen to ensure eggs are laid in nests.

ACKNOWLEDGMENTS

The authors thank Nicholas Newsome and Lorri Jensen for their assistance collecting data, and Sarah Ison for her assistance with the revised statistical analysis (all of Michigan State University, East Lansing, MI). We would also like to thank Angelo Napolitano and the Michigan State University Poultry Teaching and Research Center (East Lansing, MI) personnel for their assistance with and contribution to this research. Research was supported in part by a grant from the Michigan Alliance for Animal Agriculture (East Lansing, MI) and by Hatch funds from the U.S. Department of Agriculture (USDA). Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the USDA.

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