



## Poultry Research

Daytime occupancy of resources and flooring types by 4 laying hen strains in a commercial-style aviary<sup>☆</sup>Ahmed B.A. Ali<sup>1</sup>, Dana L.M. Campbell<sup>2</sup>, Darrin M. Karcher<sup>3</sup>, Janice M. Siegford\*

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## ABSTRACT

In response to consumer demands and legislative measures for improving hens' welfare, many laying hen producers are replacing conventional cages with aviaries. Aviary resources, such as nest boxes, litter areas, ledges, and perches, are intended to increase the display of natural behaviors. However, commercial laying hen strains have been differentially selected for varying traits including egg quality, feed efficiency, and behavior. Therefore, the assumption that laying hens are using the given resources similarly, irrespective of their genetic strain, may be false. This research examined the influence of laying hen strains (brown hens: Hy-Line Brown, Bovans Brown; white hens: DeKalb White and Hy-Line W36) on resource or flooring substrate occupancy (litter areas, nests, elevated wire tiers, ledges, and perches) inside aviaries during peak lay (25 to 28 weeks of age), and whether this occupancy changed in response to litter access. Live observation and video-recording of hens' distribution among different resources were conducted at 3 different times (morning, midday, and evening) for 3 consecutive days, immediately before (PRE), immediately after (IMM), and 3 weeks after (ACC) hens gained access to litter. Observations were conducted in 16 aviary units; 4 units/strain, 144 hens/unit. Data were analyzed in R using generalized linear mixed model with Tukey's post hoc test and  $\alpha$  set at 0.05. More brown hens were in nests than white hens during morning across all periods (PRE;  $P = 0.002$ , IMM;  $P = 0.012$ , and ACC;  $P = 0.015$ ), but more white hens were recorded in nests than brown hens during midday of all periods (PRE;  $P = 0.026$ , IMM;  $P = 0.028$ , and ACC;  $P = 0.024$ ). More white hens were on litter compared with brown ( $P = 0.002$ ), particularly when litter was first accessible. White hens occupied the open litter area in larger numbers than brown hens during midday and evening of IMM and ACC periods ( $P \leq 0.05$ ). Brown hens occupied the underneath litter area in larger numbers than white hens during midday and evening of the ACC period ( $P \leq 0.05$ ). Throughout the day, brown hens occupied wire floors in higher numbers than white hens, whereas the latter occupied both ledges and perches more (all  $P \leq 0.05$ ). These strain differences suggest that specific aviary designs may be best suited to specific hen strains.

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## Introduction

The laying hen industry in North America is transitioning from conventional cage housing systems to noncage systems, including aviaries. In general, noncage systems attempt to meet consumer and legislative demands for improved hen welfare by providing additional space and resources so hens can fulfill their behavioral needs. In aviaries, resources such as elevated structures, perches, nests, and litter-covered floor areas are intended to meet the biologically driven needs of laying hens to perch on elevated sites (EFSA, 2015; Wood-Gush, et al., 1978), lay eggs in nests (Cooper and Appleby, 1995), and dust bathe to groom their feathers (Vestergaard, 1982; Wichman and Keeling, 2008). The complex, multilevel configurations of aviaries also require that hens perform

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more walking and jumping, which can strengthen their musculo-skeletal system (Regmi, et al., 2016).

However, when hens have freedom to move about, there is a risk that they may lay eggs outside of nests or injure themselves as they move within the system (Michel and Huonnic, 2003; Villanueva, et al., 2017). Non-nest eggs increase the amount of labor required for egg collection (Matthews and Sumner, 2014) while simultaneously reducing egg cleanliness (Jones, et al., 2014) and the number of eggs damaged or eaten (Villanueva, et al., 2017). As hens move from level to level within an aviary, they are at risk of falling (Stratmann, et al., 2015b) or of landing poorly if they fly or jump between areas (Campbell, et al., 2016a). Hens may also experience overcrowding or competition for resources if they attempt to use resources simultaneously in response to circadian rhythms or social facilitation (Ali, et al., 2016; Asher, et al., 2013; Campbell, et al., 2016b,c; Collins, et al., 2011). It is therefore important to understand how hens occupy resources, including various types of flooring substrates, in these noncage systems to ensure their provision does in fact improve welfare rather than cause problems.

As we work to understand how hens use the resources and types of flooring, provided to them, a central question is whether different genetic strains of hens have different behavioral needs, perceive resources differently, or have varying abilities to use resources effectively. For example, certain strains of white hens crowded themselves to roost in the highest levels of an aviary at night (Ali, et al., 2019), whereas hens of certain brown strains laid eggs within a short time frame (Villanueva et al., 2017), suggesting these strains should be provided with more high perches and nest space, respectively, to ensure optimal welfare and nest-laid eggs.

Thus, a thorough understanding of how birds occupy and use resources in aviary systems is essential to match genetic strains of laying hen and noncage housing designs (Weeks, et al., 2016). Such knowledge may help avoid welfare concerns such as overcrowding and subsequent aggression, feather pecking, cannibalism, or reduced productivity (Abrahamsson and Tauson, 1995; Freire, et al., 2003; Odén, et al., 2002) while maximizing desired behaviors such as nest use (Ringgenberg, et al., 2015) to ensure aviary systems meet legislative and consumer expectations for improved hen welfare.

Previous studies of this same flock have examined space required by birds to perform key behaviors (Riddle et al., 2018), locations of egg laying (Villanueva et al., 2017), nighttime perch use (Ali et al., 2019), and general patterns of bird distribution (Ali et al., 2016). The main goal of this research was to investigate how laying hens of four strains occupied various specific resources and types of flooring substrates (i.e., nests, perches, two litter areas, wire floors, and metal ledges) in the aviaries throughout the day at peak lay. A secondary objective was to examine whether occupancy of resources or flooring substrates changed in response to the hens having litter access after a period of restriction. The findings will highlight whether there are strain differences that may influence how and what resources and substrate types should be provided in aviaries to meet hen needs.

## Materials and methods

### Housing and system management

A total of 2,304 laying hens of 4 genetic strains ( $n = 576$  each: Hy-Line Brown [HB]; Bovans Brown [BB]; DeKalb White [DW]; Hy-Line W36 [W36]) were used. The hens were part of a larger study, from which some results have been published regarding the distribution of the strains throughout the various tiers of the aviary (Ali et al., 2016). The details of hens, housing, system management,

and mortality are identical to those already reported by Ali et al., (2016). The present study focuses specifically on describing how the different laying hen strains occupy the various types of specific resources (i.e., nests, perches, open litter, and litter under the tiered aviary) and substrates (i.e., wire floors, metal ledges) throughout the light period at peak lay.

Briefly, 16 aviary units (Natura 60, Big Dutchman, Holland, MI, USA) were used to house four different strains of laying hens. The 16 units were divided equally among 4 rooms, and laying hen strains were allocated so all strains were present in each of the rooms (1 unit per strain  $\times$  4 strains  $\times$  4 rooms = 16 units total). Units were stocked at 144 hens/unit following United Egg Producers (2017) recommendations for cage-free egg layers when considering litter and tiered enclosure space.

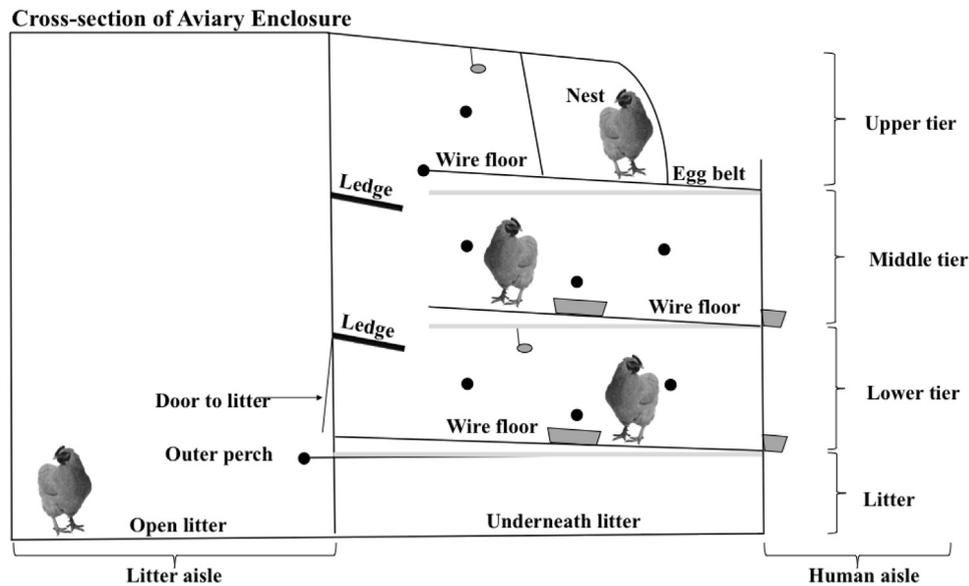
Each aviary unit consisted of a 3-tiered wire enclosure (each tier with a 61-cm internal ceiling height) and a litter area (composed of a litter area underneath the tiered enclosure and an open litter area in front of the tiered enclosure), as shown in Figure 1. Hens were provided with 5-cm feeder space and 88 cm<sup>2</sup> nesting space at the initial stocking of 144 hens/unit. In the present study, each unit had 8 round metal perches extending the full length of the unit (244 cm) that would allow 89% of 144 hens per unit to perch simultaneously (with 15 cm/hen), which exceeded United Egg Producers (2017) guidelines specifying that at least 55% of hens should be able to perch at once. Each hen had 1,132 cm<sup>2</sup> of useable floor area consisting of 551 cm<sup>2</sup>/hen in the tiered enclosure (439 cm<sup>2</sup> on wire floors plus 112 cm<sup>2</sup> solid metal ledge space) and 581 cm<sup>2</sup>/hen in the litter areas. Pin-metered (nipple) drinkers were provided at a rate of 1 per 9 hens. For further details on aviary design and available space per hen, see Ali et al. (2016).

After the 25th wk of age (when the target of ~90% of egg production was achieved), doors on the lower tier of the aviary enclosures opened each morning at 11:30. These doors allowed hens' daily access to litter-covered floor areas after egg-laying. The doors closed again at 01:00, approximately 5 h after complete lights off. For a full description of the lighting program and other details on system management, please see Ali et al. (2016).

### Observations and data collection

Live observations and video-recording of hens' resource use were conducted over 3 consecutive days at each of 3 time periods relative to hens gaining access to litter. Preopening observations (PRE: hens = 25 wk of age) occurred starting 3 d before the first opening of the aviary doors (end of 25 wk) that permitted hens' access to the litter area. Thus, observations during PRE were made of hens inside the tiered enclosures only. Immediate postopening observations (IMM: hens = 26 wk of age) started 1 d after the opening of the aviary door. Observations during IMM captured hens' immediate reaction to gaining access to litter. (As hens were reared on litter, this IMM period was actually a reintroduction of hens to litter after 9 wk without litter access rather than an entirely novel experience.) Acclimated postopening observations (ACC: hens = 28 wk of age) occurred 3 wk after initial aviary door opening. Observations during IMM and ACC periods captured hen distribution throughout the tiered enclosures and in the open and underneath litter areas.

A total of 3 live observation sets per unit were conducted per day (3 sets  $\times$  3 d) during the light period (i.e., when the lights on) for each of the 3 time periods (PRE, IMM, and ACC). Morning observations were conducted 15 min after lights on (morning: at 5:30 for 25 wk and 26 wk and at 5:15 for 28 wk), during the middle of the lights-on period (midday: at 11:30 for 25 wk, at 12:00 for 26 wk, and at 12:15 for 28 wk) and 2 h before lights off



**Figure 1.** An end view of the 3-tiered aviary unit showing wire floors, perches (black circles), solid metal ledges, the colony nest, litter locations, manure belts (gray bars), drinkers (gray ovals), and external and internal feeders (gray boxes).

(evening: 17:30 for 25 wk, 17:45 for 26 wk, and 18:00 for 28 wk). The observations done during midday after the aviary doors opened at 11:30 at 26 wk (IMM) and 28 wk (ACC) began 30 min later than the exact midpoint of the light period to allow hens to distribute throughout the tiers and litter areas of the units. Across the 3 d of each time period, rooms were visited in a different randomized order.

Before the start of data collection, 3 observers were trained for 3 d to establish synchrony within observer pairs and ensure a high level of interobserver reliability. All observations were performed by a pair of observers (composed of 2 of the 3 previously trained observers). One observer was located in the human aisle, whereas the second observer observed from the litter aisle. This placed one observer on each side of the tiered enclosure to allow for simultaneous recording of birds' distribution within a unit from both human and litter aisles (Figure 1). Counts of hens from the litter aisle were done when the observer was positioned at the end of the row or in the preceding unit to the one being observed. Observers used slow and calm movement to avoid bird disturbance as they moved between units. During the observer-training period, hens also become acclimated to the presence of observers in the room performing the data collection routine.

During data collection, each observer counted the number of hens per location throughout the aviary unit, starting from the litter area or bottom tier, depending on whether hens had litter access and working upward. Hens in the upper tier and nest box were recorded only by the observer in the litter aisle as shown in Figure 1. A count of all hens in an aviary unit took approximately 90 sec, and 2 scans (~1 h apart) were made per each observation set in each unit at each time of day. If the feed belt ran during data collection, observations were paused for 5 min to allow hens to regain their normal distribution.

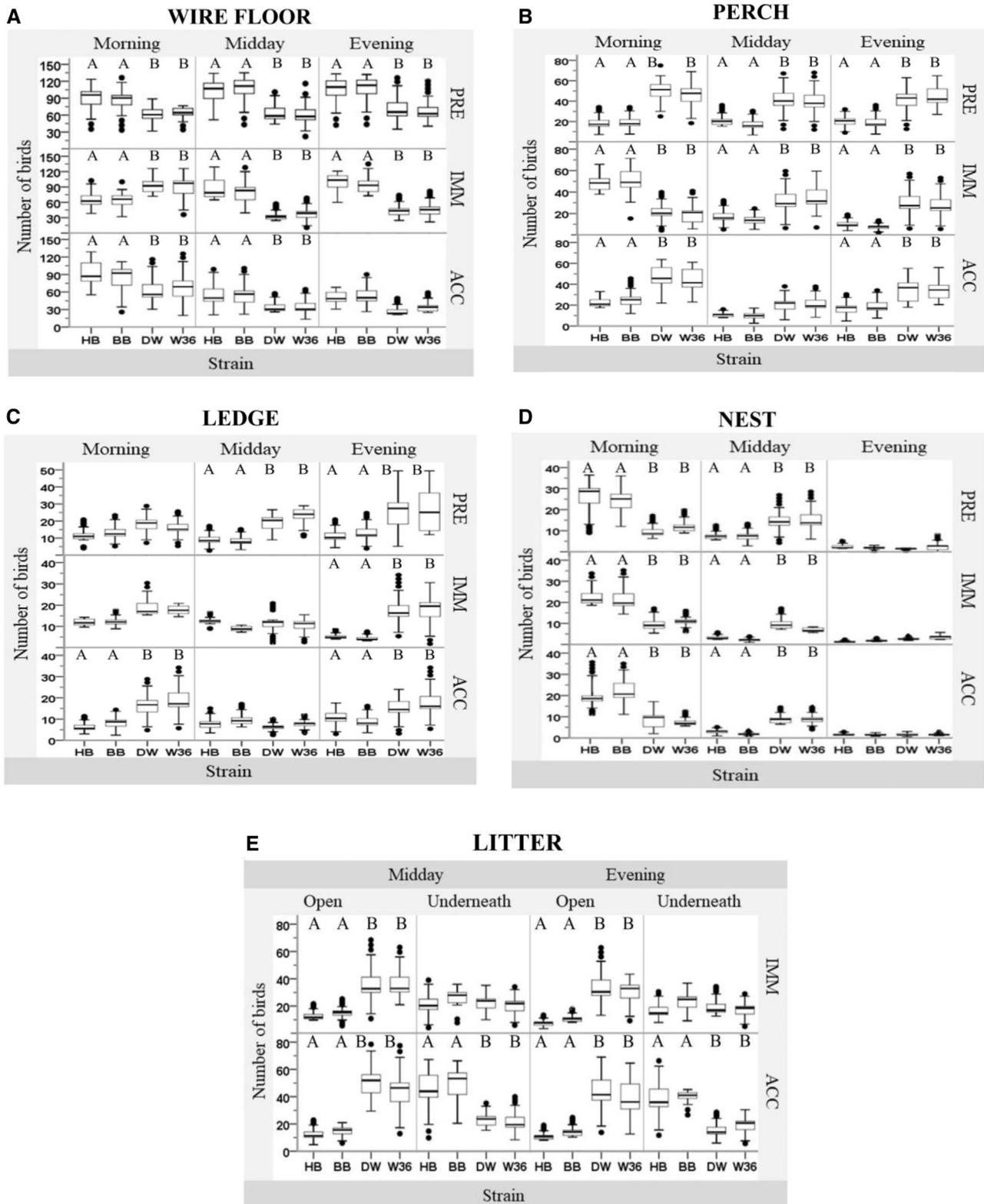
Observations that occurred while aviary doors were closed (i.e., all PRE observations and morning of IMM and ACC observations) were done by counting the numbers of birds within each location as described previously. Observations that occurred when aviary doors were open (midday and evening of IMM and ACC) also included data captured using a combination of video and direct observation for hens in the litter area. Ceiling-

mounted high-resolution digital video cameras (VF450, Clinton Electronics, Loves Park, IL, USA) recorded hens on the open litter area. Hens on litter underneath the enclosure were counted by the observer in the human aisle and simultaneously recorded using a hand-held video camera (VIXIA, HFM41, Canon, Japan). To ensure accurate counts were made of hens underneath the enclosure (as this was difficult because of the narrow opening available for observers to look through), live counts were later confirmed using the video footage.

#### Data and statistical analyses

Aviary unit was the subject of analysis for all statistical tests ( $n = 4$  units/strain). Each observation time point (morning, midday, and evening) included 2 separate counts of hen location within each unit. Statistical analyses were performed using the “stats” package in R (version 3.3.1, R Core Team., 2013). Descriptive statistics were calculated using the “psych” package, and data are presented as mean  $\pm$  standard error of the mean. Following Landis and Koch (1977), interobserver reliability was calculated using Cohen's kappa Agreement coefficient (K), using the “Cohen.Kappa” function in the psych package of R. Interobserver reliability was measured when trainees were observing the same area of the same aviary simultaneously. Interobserver agreement was very high (Kappa = 0.96 [ $P < 0.001$ ], CI [0.90, 0.99]).

Generalized linear mixed models were developed with family set to “Poisson,” with the “log” link function, using the lme4 package (Bates, et al., 2015) to describe the influence of laying hen strain in terms of resource or substrate occupancy within the aviary during different times of day, across different observation periods, and all possible interactions. Aviary unit and day of observation were included as random effects for all models, and  $P \leq 0.05$  was considered significant. Fixed effects in the models included strain of hen (DW, HB, BB, W36), time of day (morning, midday, and evening), observation period (PRE, IMM, and ACC), type of resource or substrate (wire floor, perch, ledge, nest, and litter), and their interactions. Statistically significant effects were further analyzed using Tukey's honestly significant difference multiple comparison procedure using the “multcomp” package



**Figure 2.** Number of birds of each of the 4 strains found occupying (A) wire floors, (B) perches, (C) ledges, (D) nests, and (E) litter areas of the aviary during the PRE period before hens had any access to litter, the IMM period when hens first gained access to litter, and the ACC period after 3 weeks of access to litter. Data in A-D are presented separately for morning, midday, and evening for each of the 3 periods. For the litter areas, data are presented separately for midday and evening for ACC and IMM as there was no litter access in the morning for any period or during the PRE period. Dissimilar letters above boxes indicate significant differences among strains at that time and period.

(Hothorn, et al., 2008). Box plots were generated by R software (version 3.3.1). Lines within boxes represent the mean, whereas the upper and lower boundaries of the box represent the

interquartile range. Whiskers extending from the boxes represent the outermost data point, whereas disconnected points are potential outliers.

## Results

### Wire floor use

Generally, more brown hens were observed occupying wire floors in the tiered enclosure than white hens (Figure 2A), except during the morning of the IMM period when more white hens were on the floor. Specifically, an interaction between strain, time of day, and observational period was found for wire floor occupancy ( $Z = 8.36$ ;  $P = 0.021$ ). More brown hens (HB and BB) than white hens (DW and W36) were observed on wire floors of all tier levels during both PRE and ACC periods and across different times (morning, midday, and evening; Figure 2A,  $Z = 7.63$ ;  $P = 0.012$ ). Contrarily, more white hens occupied wire floors during the morning observation of the IMM period, whereas more brown hens were counted on wire floors during midday and evening of the IMM period ( $Z = 5.69$ ;  $P = 0.023$ ).

### Perch use

More white hens were seen on perches at all times, except during morning of the IMM period, when more brown hens perched than white hens (Figure 2B). Interactions were found among strain, time of day, and observational period for perch use ( $Z = 12.52$ ;  $P = 0.012$ ). More white hens (DW and W36) than brown hens (HB and BB) occupied perches during both PRE and ACC periods and across different times of day (Figure 2B,  $Z = 9.63$ ;  $P = 0.012$ ), except during midday of the ACC period. More white hens again occupied perches than brown hens during midday and evening observations of the IMM period (Figure 2B,  $Z = 8.25$ ;  $P = 0.023$ ); but more brown hens were observed on perches than white hens during morning observations of the IMM period compared with white hens (Figure 2B,  $Z = 7.56$ ;  $P = 0.026$ ).

### Ledge use

Strain differences were less pronounced for ledge occupancy, but more white hens were counted on ledges than brown hens in all cases where differences were observed (Figure 2C). An interaction among strain, time of day, and observational period was found for ledge occupancy ( $Z = 5.63$ ;  $P = 0.027$ ). More white hens (DW and W36) were recorded on ledges than brown hens (HB and BB) during PRE and IMM periods and across all times of day; however, such differences were only significant during midday and evening of the PRE period (Figure 2C,  $Z = 5.25$ ;  $P = 0.029$ ,  $Z = 7.69$ ;  $P = 0.021$ , respectively) and evening of the IMM period (Figure 2C,  $Z = 7.52$ ;  $P = 0.019$ ). During ACC observations, more white hens were again observed occupying ledges than brown hens during the morning and evening (Figure 2C,  $Z = 6.23$ ;  $P = 0.031$ ,  $Z = 5.36$ ;  $P = 0.033$ , respectively).

### Nest use

Nest occupancy was always higher for brown hens than white hens in the morning, although more white hens were counted in nests in the afternoon than brown hens (Figure 2D). An interaction among strain, time of day, and observational period was found for nest use ( $Z = 8.25$ ;  $P = 0.022$ ). Brown hens (HB and BB) occupied nests in larger numbers than white hens (DW and W36) during morning observation across all periods (Figure 2D, PRE;  $Z = 9.63$ ;  $P = 0.002$ , IMM;  $Z = 8.63$ ;  $P = 0.012$ , and ACC;  $Z = 7.99$ ;  $P = 0.015$ ). On the other hand, more white hens were recorded in nests than brown hens during midday observations of all periods (Figure 2D, PRE;  $Z = 5.23$ ;  $P = 0.026$ , IMM;  $Z = 6.60$ ;  $P = 0.028$ , and ACC;  $Z = 5.96$ ;  $P = 0.024$ ). However, there were no differences between

numbers of brown and white hens' nests during the evening for any periods.

### Litter use

More white hens were always observed in the open litter area than brown hens, and more brown hens were counted in the underneath litter area than brown hens in the ACC period (Figure 2E). Specifically, interactions were found among strain, time of day, and observational period for litter use ( $Z = 10.25$ ;  $P = 0.016$ ). White hens (DW and W36) consistently occupied the open litter area in larger numbers than brown hens (HB and BB) when both hens had access to litter (i.e., during midday and evening of IMM and ACC periods; Figure 2E, IMM;  $Z = 9.63$ ;  $P = 0.019$ , ACC;  $Z = 8.63$ ;  $P = 0.021$ , and ACC;  $Z = 7.09$ ;  $P = 0.025$ ). On the other hand, brown hens occupied the underneath litter area in larger numbers than white hens during midday and evening of the ACC period (Figure 2E,  $Z = 7.63$ ;  $P = 0.035$ , and  $Z = 6.36$ ;  $P = 0.026$ , respectively).

## Discussion

Comparative analyses of resource and flooring substrate occupancy and influence of litter provision among 4 strains of laying hens in aviary system revealed almost no differences between strains of the same color. The two white strain (DW and W36) hens were similar to each other, whereas the two brown strains (HB and BB) were similar to each other. However, differences in wire floor, perch, ledge, nest, and litter occupancy were present between white and brown hens that could have implications for determining how to provide resources or flooring types in aviaries. As all birds were raised in the same manner, difference in rearing environments is not a likely explanation for the differences observed in the adult hens that are described below.

### Daytime wire floor, perch, and ledge occupancy

In the present study, distinct differences were detected between brown and white hens in their daytime occupancy of wire floors, perches and, to some extent, solid metal ledges. Specifically, more brown hens were observed on wire floors than white hens across all times of day and periods, except for the morning of the IMM period. The opposite pattern was found for perch occupancy, with more white hens counted on perches than brown hens, except, again, for the morning of the IMM period when fewer white hens perched than brown hens. Although the pattern was not as pronounced, more white hens were also counted on the metal ledges provided to help hens transition between tiers. When the nighttime resource and flooring substrate occupancy patterns of these hens were examined previously (Ali et al., 2019), more white hens were observed on perches and ledges across all periods and more brown hens were again observed on wire floors during PRE and IMM periods. The large number of brown hens observed on wire floors may be attributed to brown hens' tendency to disperse across the system as reported by Channing et al. (2001) and Odén et al. (2002) in their studies of ISA Brown hens in a litter-based system. Taken together with our findings, this suggests that brown hens prioritize maintaining interbird distances rather than crowding to access a specific resource.

These general patterns of more brown hens on wire floors and more white hens on perches and ledges could have several explanations. First, the brown hens in this study were heavier than white hens (Riddle et al., 2018), which could render them less agile or make the perch configurations within the aviary (i.e., diameter, shape, material, height, or location or some combination of factors) less usable by brown hens. However, there may be some innate

differences among the brown and white strains in their preference to use graspable perches during the day. For example, in the single other study to explicitly examine perch occupancy by hens of different strains during the day, Faure and Jones (1982) also reported higher daytime use of perches of all types and heights by white Leghorn hens compared with brown Leghorns. A possible explanation might be white hens use perches or ledges for undisturbed resting (Brendler and Schrader, 2016), and perching may also enhance hens' perception of safety (Newberry et al., 2001). Less perch use by brown hens might then be attributed to the fact that brown hens have been described as less fearful than white hens (De Haas et al., 2013; Fraisse and Cockrem, 2006); hence, brown birds may not seek safety through exhibiting daytime perching as frequently as white hens.

A risk factor analysis of the hens used in this study found that increased use of perches was associated with lower odds of foot problems and increased odds of keel bone damage (Ali et al., 2018). Hens with multiple keel fractures in furnished cages have also been found to perch more and stand and rest on the floor less compared with hens without fractures (Casey-Trott and Widowski, 2016). It is possible that resting on perches is less painful for hens with fractures; however, the impact of perches on hens is not straightforward (Sandilands et al., 2009). Perches have also been linked to increased incidence, prevalence, and severity of keel bone damage and increased footpad dermatitis (Hester et al., 2013; Pickel et al., 2011; Stratmann et al., 2015a; Struelens and Tuytens, 2009), but at the same time, grasping perches has been found to improve leg bone strength (Barnett et al., 1997; Duncan et al., 1992).

#### Nest occupancy

In the present study, more brown hens were counted in nests in the morning, whereas white hens were observed at higher numbers in nests during midday. We investigated patterns of nest use, egg laying, and damage in the same flock in more detail at 36 wk of age and found that this pattern of higher nest occupancy by brown hens in the morning and white hens in midday persisted (Villanueva et al., 2017). Furthermore, in that study, 36-wk-old brown hens laid ~85% of their daily nest eggs during the period from 6:00 to 10:00 (i.e., morning in this study), whereas the white hens laid ~50% of their nest eggs during the same period and continued to lay eggs in the nest well into the afternoon. When examining these same hens' use of various levels in the aviary during the daytime (Ali et al., 2016), we also found several incidences of brown hens overcrowding the third tier (which included the nest) in the morning. Coupled with the knowledge that these hens also damaged more eggs and laid more eggs out of the nest in the morning at 36 weeks of age (Villanueva et al., 2017), brown hens appear to have a strong circadian drive to egg lay in the morning (Channing et al., 2001; Vestergaard, 1982; Yeates, 1963), whereas in the strains of white hens, we examined this compulsion may be more relaxed. Several studies (Tůmová and Gous, 2012; Tůmová et al., 2007) have attributed differences in the diurnal laying pattern between brown and white strains explicitly to genotype variations that might influence their typical oviposition time.

Taken together, brown hens' strong circadian egg-laying rhythm and the projected ability of only ~12% of brown hens in the aviary to nest at once based on their body size (Riddle et al., 2018) may result in some hens being prevented from performing prelay and oviposition behaviors in desired locations, resulting in frustration (Ringgenberg et al., 2014). Limiting hens' ability to exhibit prelay behaviors could also impact their health as it might lead to egg retention inside the shell gland (Hughes et al., 1986). In addition, when hens are unable to access nests, they will end up laying eggs outside the nest area resulting in more dirty, damaged, and lost

eggs (Kruschwitz et al., 2008; Villanueva et al., 2017). Analysis of body size of the same birds as in the present study by Riddle et al. (2018) suggests that 12% (HB) to 15% (DW) of birds should be able to nest simultaneously in these aviary units. At these rates, brown hens almost always overoccupied their nests in the morning (Figure 2D). At 36 weeks of age, brown hens in the same flock as in the present study laid approximately 9.45% of their total eggs outside the nest (Villanueva et al., 2017). The percentage of non-nest-laid eggs peaked in the morning, when both overall eggs laid and hens occupying nests were also highest, suggesting that hens were laying outside the nest as a result of being unable to access nests during preferred laying times (Villanueva et al., 2017). Singh et al. (2009) also found that Lohmann brown hens laid more floor eggs than Lohmann white hens, although they attributed this to less motivation by the brown hens to use the nest. Therefore, it is important to consider how tightly entrained a strain of hens is to laying at a particular time of day to optimize the ratio of nest space per hen to increase the percentage of clean, nest-laid eggs while reducing hen frustration and the number of dirty, damaged, or lost eggs and the labor required to collect non-nest-laid eggs (Kruschwitz et al., 2008; Villanueva et al., 2017).

#### Daytime litter occupancy

In the present study, litter access was restricted when pullets were first transferred to the laying hen facility at 17 weeks of age with hens regaining access to litter at 26 weeks of age, once hens had achieved 90% production. Temporary litter restriction is used by the commercial industry when pullets are first transferred to a noncage production system (Lambton et al., 2010). The practice is intended to help birds to locate feeders and drinkers quickly, adapt to using resources within the tiered enclosure, and enhance nest use and reduce numbers of floor-laid eggs (Alm et al., 2015). However, there is some concern that litter restriction could reduce hen welfare because of reduction in space or by preventing hens from accessing a resource they are strongly motivated to use or both (Bestman et al., 2009; Gunnarsson et al., 2000), and some welfare certification schemes limit litter restriction (e.g., 3 weeks of maximum restriction, Australian RSPCSA (2015)).

Temporary litter exclusion might mean 24 h restriction from litter that lasts for only few days or for several weeks until hens reach peak lay. In other cases, exclusion may be a partial restriction, keeping hens off the litter during morning hours only, when most of the birds are laying their eggs (Villanueva et al., 2016). Partial daily litter restriction is permitted by several industry guidelines in the United States, including the most recent United Egg Producer guidelines (2017) that allow restriction of litter access in the early morning hours to prevent floor laying. This practice is also approved for use in European Union countries (Council Directive 1999/74/EC). However, some countries, such as Sweden, do not permit even temporary litter restriction, as several studies have suggested that litter restriction practice may have negative effects on bird welfare (Bestman et al., 2009; Gunnarsson et al., 2000).

In a previous examination of how hens in this flock distributed themselves across the areas in the aviary, we found that white hens used the entire litter area in larger number than brown hens, particularly when litter was first permitted (Ali et al., 2016). However, the litter area is composed of two areas, with 59% of the litter area underneath the tiered enclosure while the remaining 41% uncovered. Therefore, in the present study, we specifically examined distribution of hens across these two areas to determine if the various strains preferred one type of litter area to another. We observed more brown hens in the underneath litter areas than in the open litter areas, whereas white hens occupied open litter areas in larger numbers than underneath areas. Anecdotally, brown hens

have a reputation for being less fearful (flighty) than white hens, and there is evidence that they do react less fearfully to novel situations (De Haas et al., 2013; Fraisse and Cockrem, 2006). Although the brown hens may appear to be hiding when they use the litter areas underneath the aviary, this may be an anthropomorphic perception. Hens in the underneath area have obstructed views of their surroundings which would hinder them from monitoring the environment to detect predators (Devereux et al., 2005; EFSA, 2015; Metcalfe, 1984). However, the motivation behind the white and brown hens' differential occupancy of the litter areas is purely speculative at this point.

#### Litter restriction/provision

Contrary to the expectation that litter restriction reduces hen welfare, at least one study has found that temporary litter restriction on transferring pullets to the laying facility may improve welfare (Alm et al., 2015). Specifically, the authors reported a reduction in fearfulness, improved feather coverage, and improved eggshell quality (Alm et al., 2015). Confining hens to the aviary for the morning, as was done in the present study, has recently been examined with respect to physical aspects of hen welfare, mortality, body weight, body weight uniformity, and number of hens remaining on litter at night with no differences observed between restricted hens and those with free access to litter (Oliveira et al., 2018). In that study, hens prevented from going to the litter in the morning laid 89% fewer eggs on the litter compared with unrestricted hens ( $1.4 \pm 0.1$  vs.  $12.6 \pm 1.1$  litter laid eggs per hen, respectively, by 76 weeks of age). In the short term, regaining litter access after a period of restriction in the present study resulted in occasional overcrowdings in certain areas of the aviary and was linked to more frequent tier-to-tier hen movement during dark periods, which could have implications for injuries if the movement resulting from slips and falls (Ali et al., 2016). However, hens were found to return to their previous overall and nighttime patterns of resource and space use within 3 weeks of regaining litter access (Ali et al., 2016, 2019). Further research is still required to completely understand the prolonged impacts of initial and partial litter restriction on hens' patterns of distribution and resource use across the lay cycle, as well as the possible impacts on hens' physical measures of welfare and egg production.

#### Conclusion

Distinct differences were found among the 4 strains of laying hens in this study regarding the daytime occupancy of resources and flooring substrates within a commercial-style aviary. Brown hens tended to occupy wire floors at higher numbers than white hens, whereas more white hens occupied elevated perches and ledges. Brown hens occupied nests in larger numbers than white hens during the morning, which resulted in multiple incidences where nests appeared overcrowded. On the other hand, white hens occupied nests in greater number than brown birds during midday, after an extended pattern of nest use across the day. These strain differences were relatively consistent from 25 to 28 weeks of age, although initial access to litter was linked to more white hens occupying wire floors and more hens occupying perches in the morning, indicating a brief disruption in their typical pattern. When hens had access to litter in the IMM and ACC periods, brown hens typically used underneath litter areas in greater numbers than open litter areas, whereas white hens occupied open litter areas in larger numbers.

This study provides further support for considering strain differences when designing noncage housing systems or deciding which strain to use in a particular housing design. As our specific

conclusions can only be applied to the 4 strains and aviary style examined in the present study, further research is still needed.

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#### Ethical considerations

All research protocols were approved by the Michigan State University Institutional Animal Care and Use Committee before the start of data collection.

#### References

- Abrahamsson, P., Tauson, R., 1995. Aviary systems and conventional cages for laying hens: Effects on production, egg quality, health and bird location in three hybrids. *Acta Agric. Scand. A. Anim. Sci.* 45, 191–203.
- Ali, A., Campbell, D., Karcher, D., Siegford, J., 2016. Influence of genetic strain and access to litter on spatial distribution of 4 strains of laying hens in an aviary system. *Poult. Sci.* 95, 2489–2502.
- Ali, A.B.A., Campbell, D.L.M., Karcher, D.M., Siegford, J.M., 2019. Nighttime roosting substrate type and height among 4 strains of laying hens in an aviary system. *Poult. Sci.* 98, 1935–1946.
- Ali, A.B.A., Campbell, D.L.M., Karcher, D.M., Siegford, J.M., 2018. Health, Production, and Resource Use by Laying Hens in an Aviary: A Risk Assessment. Meeting of the *Poult. Sci. Ass.*, San Antonio, TX.
- Alm, M., Wall, H., Holm, L., Wichman, A., Palme, R., Tauson, R., 2015. Welfare and performance in layers following temporary exclusion from the litter area on introduction to the layer facility. *Poult. Sci.* 94, 565–573.
- Asher, L., Collins, L.M., Pfeiffer, D.U., Nicol, C.J., 2013. Flocking for food or flockmates? *Appl. Anim. Behav. Sci.* 147, 94–103.
- Australian RSPSCA, 2015. Layer hens: RSPCA approved farming scheme standards. <https://rspcaapproved.org.au/join#standards>. Accessed April 15, 2019.
- Barnett, J., Glatz, P., Newman, E., Cronin, G., 1997. Effects of modifying layer cages with perches on stress physiology, plumage, pecking and bone strength of hens. *Aust. J. Exp. Agric.* 37, 523–529.
- Bates, D., Maechler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67 (1), 1–48.
- Bestman, M., Koene, P., Wagenaar, J.-P., 2009. Influence of farm factors on the occurrence of feather pecking in organic reared hens and their predictability for feather pecking in the laying period. *Appl. Anim. Behav. Sci.* 121, 120–125.
- Brendler, C., Schrader, L., 2016. Perch use by laying hens in aviary systems. *Appl. Anim. Behav. Sci.* 182, 9–14.
- Campbell, D., Goodwin, S., Makagon, M., Swanson, J., Siegford, J., 2016a. Failed landings after laying hen flight in a commercial aviary over two flock cycles. *Poult. Sci.* 95, 188–197.
- Campbell, D., Makagon, M., Swanson, J., Siegford, J., 2016b. Litter use by laying hens in a commercial aviary: dust bathing and piling. *Poult. Sci.* 95, 164–175.
- Campbell, D., Makagon, M., Swanson, J., Siegford, J., 2016c. Perch use by laying hens in a commercial aviary. *Poult. Sci.* 95, 1736–1742.
- Casey-Trott, T.M., Widowski, T.M., 2016. Behavioral differences of laying hens with fractured keel bones within furnished cages. *Front. Vet. Sci.* 3, 42.
- Channing, C.E., Hughes, B.O., Walker, A.W., 2001. Spatial distribution and behaviour of laying hens housed in an alternative system. *Appl. Anim. Behav. Sci.* 72, 335–345.
- Collins, L.M., Asher, L., Pfeiffer, D.U., Browne, W.J., Nicol, C.J., 2011. Clustering and synchrony in laying hens: The effect of environmental resources on social dynamics. *Appl. Anim. Behav. Sci.* 129, 43–53.
- Cooper, J.J., Appleby, M.C., 1995. Nesting behaviour of hens: effects of experience on motivation. *Appl. Anim. Behav. Sci.* 42, 283–295.
- De Haas, E., Kemp, B., Bolhuis, J., Groothuis, T., Rodenburg, T., 2013. Fear, stress, and feather pecking in commercial white and brown laying hen parent-stock flocks and their relationships with production parameters. *Poult. Sci.* 92, 2259–2269.
- Devereux, C.L., Whittingham, M.J., Fernández-Juricic, E., Vickery, J.A., Krebs, J.R., 2005. Predator detection and avoidance by starlings under differing scenarios of predation risk. *Behav. Ecol.* 17, 303–309.

- Duncan, E., Appleby, M., Hughes, B., 1992. Effect of perches in laying cages on welfare and production of hens. *Br. Poult. Sci.* 33, 25–35.
- EFSA, E.A., 2015. Scientific opinion on welfare aspects of the use of perches for laying hens. *EFSA J.* 197, 1–23.
- Faure, J.M., Jones, R.B., 1982. Effects of sex, strain and type of perch on perching behaviour in the domestic fowl. *Appl. Anim. Ethol.* 8, 281–293.
- Fraisse, F., Cockrem, J., 2006. Corticosterone and fear behaviour in white and brown caged laying hens. *Br. Poult. Sci.* 47, 110–119.
- Freire, R., Wilkins, L., Short, F., Nicol, C., 2003. Behaviour and welfare of individual laying hens in a non-cage system. *Br. Poult. Sci.* 44, 22–29.
- Gunnarsson, S., Matthews, L., Foster, T., Temple, W., 2000. The demand for straw and feathers as litter substrates by laying hens. *Appl. Anim. Behav. Sci.* 65, 321–330.
- Hester, P., Enneking, S., Haley, B., Cheng, H.W., Einstein, M., Rubin, D., 2013. The effect of perch availability during pullet rearing and egg laying on musculoskeletal health of caged White Leghorn hens. *Poult. Sci.* 92, 1972–1980.
- Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. *Biometric. J.* 50 (3), 346–363.
- Hughes, B., Gilbert, A., Brown, M.F., 1986. Categorisation and causes of abnormal egg shells: relationship with stress. *Br. Poult. Sci.* 27, 325–337.
- Jones, D., Cox, N., Guard, J., Fedorka-Cray, P., Buhr, R., Gast, R., Abdo, Z., Rigsby, L., Plumblee, J., Karcher, D., 2014. Microbiological impact of three commercial laying hen housing systems. *Poult. Sci.* 94, 544–551.
- Kruschwitz, A., Zupan, M., Buchwalder, T., Huber-Eicher, B., 2008. Nest preference of laying hens (*Gallus gallus domesticus*) and their motivation to exert themselves to gain nest access. *Appl. Anim. Behav. Sci.* 112, 321–330.
- Lambton, S.L., Knowles, T.G., Yorke, C., Nicol, C.J., 2010. The risk factors affecting the development of gentle and severe feather pecking in loose housed laying hens. *Appl. Anim. Behav. Sci.* 123, 32–42.
- Landis, J.R., Koch, G.G., 1977. The measurement of observer agreement for categorical data. *Biometrics* 33, 159–174.
- Matthews, W., Sumner, D., 2014. Effects of housing system on the costs of commercial egg production. *Poult. Sci.* 94, 552–557.
- Metcalfe, N.B., 1984. *The Flocking Behaviour of Wintering Turnstones Arenaria Interpres and Purple Sandpipers Calidris Maritima*. University of Glasgow, Glasgow, Scotland, UK.
- Michel, V., Huonnic, D., 2003. A comparison of welfare, health and production performance of laying hens reared in cages or in aviaries. *Br. Poult. Sci.* 44, 775–776.
- Newberry, R.C., Estevez, I., Keeling, L.J., 2001. Group size and perching behaviour in young domestic fowl. *Appl. Anim. Behav. Sci.* 73, 117–129.
- Odén, K., Keeling, L., Algers, B., 2002. Behaviour of laying hens in two types of aviary systems on 25 commercial farms in Sweden. *Br. Poult. Sci.* 43, 169–181.
- Oliveira, J.L., Xin, H., Chai, L., Millman, S.T., 2018. Effects of litter floor access and inclusion of experienced hens in aviary housing on floor eggs, litter condition, air quality, and hen welfare. *Poult. Sci.* 98, 1664–1677.
- Pickel, T., Schrader, L., Scholz, B., 2011. Pressure load on keel bone and foot pads in perching laying hens in relation to perch design. *Poult. Sci.* 90, 715–724.
- Regmi, P., Smith, N., Nelson, N., Haut, R., Orth, M., Karcher, D., 2016. Housing conditions alter properties of the tibia and humerus during the laying phase in Lohmann white Leghorn hens. *Poult. Sci.* 95, 198–206.
- Riddle, E.R., Ali, A.B., Campbell, D.L., Siegford, J.M., 2018. Space use by 4 strains of laying hens to perch, wing flap, dust bathe, stand and lie down. *PLoS One* 13, e0190532.
- Ringgenberg, N., Fröhlich, E.K., Harlander-Matauschek, A., Toscano, M.J., Würbel, H., Roth, B.A., 2015. Nest choice in laying hens: effects of nest partitions and social status. *Appl. Anim. Behav. Sci.* 169, 43–50.
- Ringgenberg, N., Fröhlich, E.K., Harlander-Matauschek, A., Würbel, H., Roth, B.A., 2014. Does nest size matter to laying hens? *Appl. Anim. Behav. Sci.* 155, 66–73.
- Sandilands, V., Moinard, C., Sparks, N., 2009. Providing laying hens with perches: fulfilling behavioural needs but causing injury? *Br. Poult. Sci.* 50, 395–406.
- Singh, R., Cheng, K., Silversides, F., 2009. Production performance and egg quality of four strains of laying hens kept in conventional cages and floor pens. *Poult. Sci.* 88, 256–264.
- Stratmann, A., Fröhlich, E.K., Harlander-Matauschek, A., Schrader, L., Toscano, M.J., Würbel, H., Gebhardt-Henrich, S.G., 2015a. Soft perches in an aviary system reduce incidence of keel bone damage in laying hens. *PLoS One* 10, e0122568.
- Stratmann, A., Fröhlich, E.K.F., Gebhardt-Henrich, S.G., Harlander-Matauschek, A., Würbel, H., Toscano, M.J., 2015b. Modification of aviary design reduces incidence of falls, collisions and keel bone damage in laying hens. *Appl. Anim. Behav. Sci.* 165, 112–123.
- Struelens, E., Tuytens, F., 2009. Effects of perch design on behaviour and health of laying hens. *Anim. Welf.* 18, 533–538.
- Tümová, E., Gous, R., 2012. Interaction of hen production type, age, and temperature on laying pattern and egg quality. *Poult. Sci.* 91, 1269–1275.
- Tümová, E., Zita, L., Hubený, M., Skřivan, M., Ledvinka, Z., 2007. The effect of oviposition time and genotype on egg quality characteristics in egg type hens. *Czech. J. Anim. Sci.* 52, 26–30.
- United Egg Producers, 2017. *United Egg Producers Animal Husbandry Guidelines for US Egg Laying Flocks: 2017 Edition*. Retrieved from: <https://uepcertified.com/wp-content/uploads/2015/08/UEP-Animal-Welfare-Guidelines-20141.pdf>. Accessed April 15, 2019.
- Vestergaard, K., 1982. Dust-bathing in the domestic fowl—diurnal rhythm and dust deprivation. *Appl. Anim. Ethol.* 8, 487–495.
- Villanueva, S., Ali, A., Campbell, D., Siegford, J., 2017. Nest use and patterns of egg laying and damage by 4 strains of laying hens in an aviary system. *Poult. Sci.* 96 (9), 3011–3020.
- Weeks, C.A., Lambton, S.L., Williams, A.G., 2016. Implications for welfare, productivity and sustainability of the variation in reported levels of mortality for laying hen flocks kept in different housing systems: a meta-analysis of ten studies. *PLoS One* 11, e0146394.
- Wichman, A., Keeling, L., 2008. Hens are motivated to dustbathe in peat irrespective of being reared with or without a suitable dustbathing substrate. *Anim. Behav.* 75, 1525–1533.
- Wood-Gush, D.G., Duncan, I.J., Savory, C., 1978. Observations on the social behaviour of domestic fowl in the wild. *Biol. Behav.* 3, 193–205.
- Yeates, N.T.M., 1963. The activity pattern in poultry in relation to photoperiod. *Anim. Behav.* 11 (2-3), 287–289.