UNDERSTANDING DYNAMIC HEN BEHAVIORS TO IMPROVE WELFARE IN THE TRANSITION FROM CAGES TO CAGE-FREE EGG PRODUCTION

By

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ABSTRACT

A global trend towards improving farm animal welfare has seen an increase in the use of alternative or cage-free housing systems, and several countries have implemented bans on caged egg production due to their barren nature. Individual states in the U.S. have also established cage-free regulations intending to improve hen welfare by providing more space and resources per hen than are possible in cages. Due to this legislative demand, along with pressure from corporate pledges to source only cage-free eggs within a short timeline, many egg producers are undergoing the costly transition to build, stock, and maintain cage-free production facilities. Hens housed in cage-free systems can move throughout their enclosures and can utilize resources like perches, litter areas, and designated nests. Unfortunately, the addition of these resources, along with increased freedom of movement for hens and interactions among conspecifics within the flock, have also had unintended consequences (including negative hen behaviors such as crowding/crushing one another and cannibalism, and hen behaviors that are undesirable to producers, like laying eggs outside of nest sites). Further, several space and resource guidelines are set on a per-hen basis and may not consider that certain hen behaviors require a varying amount of space, nor do they consider the potential influence of large portions of a flock behaving congruously (in both situations, the amount of space or resources allocated by guidelines may not be the actual amount that is used or needed by hens). As an additional consideration, many of hens' key behaviors are diurnal in nature and it is possible that synchronous flock movement to a certain resource at a certain time of day, coupled with management tactics used to curb undesired behaviors, could prevent hens from performing these behaviors based on their preferred temporal patterns. To better ensure that guidelines on cage-free husbandry and management practices actually improve hen welfare as intended, research should consider the influences of multiple factors on hen behavior. Therefore, the overarching

focus of this project was to examine how laying hens of different genetic strains perform dynamic and space-intensive behaviors in a multi-tiered aviary system. Behaviors chosen for examination were dust bathing and wing flapping, both of which have been deemed important by cage-free legislation. Commercial-style Natura60 aviaries were stocked with 4 genetic strains: Hy-Line Brown [HB], Bovan Brown [BB], DeKalb White [DW] and Hy-Line [W36]. In the first study, we found that white strains had higher rates of litter occupancy and more synchrony in dust bathing behavior compared to brown strains. White-feathered hens also had smaller inter-bird distances while performing a dust bathing bout, whereas hens of the brown strains had larger inter-bird distances and shortened the duration of dust bathing bouts in the presence of more hens on the litter or with less space between nearby hens. During initial placement in the aviaries, we saw a similar behavioral trend based on genetic strain: following a period of complete litter restriction, DW and W36 hens occupied litter in greater numbers and at a faster rate HB and BB hens. When doors to litter opened each day, hens not only gained access to litter but also to unfettered three-dimensional space. Hens of all 4 strains flapped their wings more in the first 85 minutes (11:35am-12:55pm) following doors' opening, suggesting their daily confinement within tiers may have influenced their motivation to wing flap once they had room to do so. While wing flapping, W36 hens required an average of 51.02 ± 4.7 cm of vertical space; however, our hens were cage-reared and housed, and the manner in which they flapped may not be representative of hens with more muscle development and experience with wing flapping. The method we tested should be further utilized on dynamic behaviors on of hens from a variety of strains, ages, and backgrounds. Future work is needed to determine the space requirements of hens for particular resources rather than blanketed guidelines for space as a whole. Differences in social distancing, behavioral synchrony, and the time-of-day around specific resources need to be identified.

This dissertation is dedicated to chickens everywhere.

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LIST OF ABBREVIATIONS

AC	Acclimated time period following hens’ initial litter access in aviaries
BB	Bovans Brown (brown-feathered laying hen strain)
DB	Dustbathing
DW	DeKalb White (white-feathered laying hen strain)
HB	Hy-Line Brown (brown-feathered laying hen strain)
IM	Immediate time period following hens’ initial litter access in aviaries
LO	Litter Occupancy
W36	Hy-Line W36 (white-feathered laying hen strain)
WF	Wing Flapping
WOA	Weeks of Age

CHAPTER 1.

GENERAL INTRODUCTION

Global Improvements to Egg Industry

Commercial egg production has become a successful industry due to decades of innovations in genetics, improved animal health, and the use of consistent management in climate-controlled housing environments (Pelletier et al., 2018; Athrey, 2020, Istiak and Khaliduzzaman, 2022). Small flocks of backyard chickens were the predominant source of eggs for families prior to the 1900s. Throughout the century, egg farmers started to move their hens indoors to protect them from the elements, and contained them within stacked, raised cages that kept hens off of the ground and away from excrement (Kidd and Anderson, 2019). The use of these systems, known as battery or conventional cages, improved hygiene and disease control in flocks, reduced social stressors on hens, and allowed safer and easier work for human laborers (Duncan, 2001). Over time, egg production became consolidated into industrial farms (Leenstra et al., 2016), with large-scale facilities capable of housing hundreds to thousands of highly productive laying hens (Kidd and Anderson, 2019). One of the biggest influences on hen's egg production were the improvements in genome sequencing and selective breeding, which helped accelerate changes to hens' productivity to establish prolific and feed efficient hens. Modern commercial layer strains begin to lay at around 18 weeks of age and hens of some strains average close to 500 eggs in their lifetime (Gautron et al., 2021). Nowadays, the egg sector is still highly industrialized and the majority of the current laying hen population is housed in cages. However, there is a growing cage-free market as well as several alternative housing systems laying hens on commercial farms, including enriched or furnished cages, barns or multi-tiered aviaries, and free-range systems that include access to the outdoors (AVMA, 2012).

Confinement Concern & Changing Industry

Though conventional cages offer some benefit to both human workers and hens they also have several shortcomings, which almost exclusively impact hens (Duncan, 2001; Lay et al., 2011; Hemsworth, 2021). Most notably, hens are unable to perform many of their natural behaviors in caged systems due to lack of space and resources. Considerable growth in the cage-free egg market, coupled with consumer concern for the intense confinement of laying hens, is pushing global egg production away from conventional cages (Rodenburg et al., 2022; Sinclair et al., 2022) in an effort to improve laying hen welfare (Hemsworth, 2021). Initiatives to improve laying hen welfare by reducing the use of extreme confinement systems have arisen globally (Scrinis et al., 2017), and many regulations either outright ban the future construction and use of conventional cages or establish larger minimum space allotments per hen than are currently possible in these cages. In the United States, there are few federal regulations regarding the management and welfare of commercial poultry (Animal Federal Regulations, 2023). Instead, individual states have begun implementing their own regulations for cage-free egg production with the intention of improving animal welfare (Shields et al., 2017; Vogeler, 2021).

Additions in Cage-Free Systems

Cage-free systems allow hens greater freedom of movement compared to caged counterparts (De Mol et al., 2006). In aviary systems, hens can perform ecologically important behaviors, such as scratching and wing flapping, more frequently than hens in caged systems (Tanaka and Hurnik, 1992). With this added freedom of movement, aviary hens can build better bone strength than those hens physically unable to move as often in cages (Regmi et al., 2016). Broken bones are often an issue during moves and depopulation (Budgell and Silversides, 2004), and improving bone strength through increased active behavior is useful in preventing future injury (Leyendecker et al., 2005). In all, aviaries provide the space to allow for more behaviors than are possible in the confinement of a cage—though hen welfare is still reliant on upon good management in these systems (Appleby and Hughes, 1991).

An additional benefit to hen welfare in cage-free systems is the provision of resources like perches and designated nests (Keeling, 1995), both of which are unavailable in a conventional cage. Hens are highly motivated to roost on perches, especially when lights are off, and they often prefer higher perches (Brendler and Schrader, 2016; Campbell et al., 2016a). These behaviors follow a similar roosting pattern to the Red Junglefowl (the extant wild ancestor of the domestic chicken) (Arshad and Zakaria, 2009), suggesting that commercial strains still maintain some innate needs. These needs are more likely to be met in a cage-free system (such as an aviary), preferably with adequate perch space and heights, than they are in a barren conventional cage. Unfortunately, the addition of these resources, along with increased freedom of movement for hens and interactions among conspecifics within the flock, have also had unintended consequences (including negative hen behaviors such as crowding/crushing one another and cannibalism, and hen behaviors that are undesirable to producers, like laying eggs outside of nest sites). To prevent these behaviors, egg producers will sometimes limit the time in which hens can access certain resources, such as keeping hens away from litter until after the morning hours each day to promote oviposition in designated nest sites. Although this temporary restrictive tactic can aid in reducing eggs laid in litter instead (Karcher and Mench, 2018), though restriction may impact welfare (Bestman et al., 2009). This quandary, encouraging positive behaviors while limiting negative behaviors in non-cage systems, is another area in which more research can be done.

Space Use

Caged hens are physically unable to perform many behaviors due to the limited space available, including relatively low cage heights (Hemsworth, 2021). Cage-free systems are intended to accommodate hens' performance of behaviors that are inhibited in cages, and cage-free housing guidelines often stipulate a certain amount of space or resource provision per hen. However, behavior and resource use can vary among hens within a flock, and hens rarely distance themselves evenly in space or utilize resources uniformly (Carmichael et al., 1999; Sibanda et al., 2019). Behavior and space use are affected by space allocation per animal, the group size, and the number of animals in the group within a given area (Appleby, 2004; Widowski et al., 2016). Hens will often aggregate in certain areas at certain times, such as in nests during morning hours when oviposition commonly occurs (Villanueva et al., 2017), or on perches at night to roost (Campbell et al., 2016a). This tendency of hens to crowd certain areas can create pockets of high bird density within an otherwise acceptable amount of space.

Further, hens have individual spacing preferences and may prefer a certain amount of "personal space" in order to successfully perform a behavior (Keeling, 1995). In cage-free systems with large group sizes, hens must space themselves within the context of not just the physical elements of the system but also within a flock of other hens. Space requirements that are based on the movement of a single hen are not sufficient guidelines for a large flock, given that hens are often synchronous in their behaviors (Mench and Blatchford, 2014). For example, if many hens in a group dust bathe concurrently, this can cause crowding in litter areas (Campbell et al., 2016b). Similar to the way one generalized space guideline does not encompass all the variation in space occupied among hens of different strains or used by different behaviors, the behaviors themselves are not done in isolation and may be influenced by the presence of conspecifics (Grebey et al., 2020).

Strain Differences

There are several genetic strains of laying hen used commercially. Strains can be generally be separated into white or brown-feathered hens that predominantly stem from parent flocks of White Leghorn or Rhode Island Red breeds, respectively. Many of these strains have undergone intense selection to improve feed efficiency and egg production, though hens that have undergone more selection show varying behavioral traits compared to those hens that have undergone less selection (Giersberg et al., 2019). Further, brown-feathered and white-feathered hens show different stress and fear responses compared to one another (Nelson et al., 2020). Hens of strains with white feathers are often more social and active or exploratory than brown-feathered strains (Dudde et al., 2018; Hewlett and Nordquist, 2019), as well as more flighty or excitable (Ziemiańska et al., 2020). Hens of different genetic strains show behavioral variation in response to unpredictable or changing environments (Pusch et al., 2018). Additionally, several studies have shown that aviary-housed hens of different strains utilize space and resources differently (Abrahamsson et al., 1996; Ali et al., 2016; Ali et al., 2019). White-feathered and brown-feathered hens show varying levels of physical activity (Kozak et al., 2016), with white-feathered hens generally using resources located in higher tiers more than brown-feathered hens (Garant et al., 2022). These behavioral differences among genetic layer strains may impact their ability to thrive in cage-free systems (Dallimore, 2014). As mentioned, modern commercial egg production has created an extremely efficient bird that was bred to exist in a controlled environment, where, in terms of production and health, they thrive. Certain modern layer strains may not be robust or resilient enough to successfully adapt from the barren cages the strains have been selected to live in to complex cage-free systems (Star et al., 2008).

Many cage-free guidelines for space provision also do not consider size discrepancies among different layer strains. Brown-feathered hens are generally heavier than white-feathered hens and tend to occupy more physical space compared to white-feathered strains (Riddle et al., 2018). However, differences in the skeletal and musculature structure among genetic strains may influence space requirements for dynamic behaviors. Pufall and colleagues (2021) reported heavier pectoralis muscles in white-feathered hens compared to brown-feathered hens, and white-feathered hens are known to occupy more space while wing flapping (Riddle et al., 2018).

Dust Bathing and Litter

Dust bathing is an important behavior for laying hens. In an appropriate resource (such as dry, friable litter), the performance of dust bathing is thought to realign hens' feathers and remove lipids from their skin (Vestergaard, 1982; Olsson and Keeling, 2005). Hens are also highly motivated to dust bathe (Widowski and Duncan, 2000), and it is thought that the performance of a dust bathing bout promotes positive welfare (Colson et al., 2008). In fact, the motivation for dust bathing is so strong that hens will attempt bouts even when litter or another appropriate stimulus is absent, such as in conventional or colony cages (Duncan, 2001; Louton et al., 2016). Aviaries and other cage-systems providing litter are more likely to satisfy the desire to dust bathe compared to wired cages (Hemsworth, 2021). Litter is therefore a critical component of aviaries. Unfortunately, the presence of a litter area may facilitate hens' performance of other behaviors that are undesirable to producers. Although hens are motivated to lay eggs in nest boxes (Cronin et al., 2012), they may still find litter-covered floors appealing for nesting and oviposition, similar to their wild ancestors the red jungle fowl, which nest on forest floors (Dawkins, 1989). However, reducing the amount of available litter in cage-free systems may not be beneficial overall. Hens may not be enticed to utilize litter if the provision of designated space is too little, and displacement from one resource may cause an uneven distribution of hen activity (Gonzalez-Mora et al., 2020). It is therefore important to understand if different genetic strains dust bathe differently under the same conditions in cage-free systems, as there may be differing requirements for management or resource provision among strains to ensure resources are being used as intended and hens maintain good welfare.

Wing Flapping

Chickens are heavy-bodied, predominantly ground-dwelling birds in the Galliformes order. Though domesticated laying hens have high wing loading compared to their large bodies and therefore have poor flight capacity (León et al., 2021), they still utilize their wings for certain behaviors including locomotion (Tran et al., 2022). Hens are also able to flap their wings while standing stationary, which is generally considered to be a comfort behavior (Nicol, 1989; Tanaka and Hurnik, 1992; Albentosa and Cooper, 2004). Hens are likely to flap their wings more following a period of spatial restriction (Nicol, 1987). Little research has assessed the motivations behind wing flapping in laying hens. However, the behavior is included as a guideline for space allocations in cage-free systems, as legislation from several U.S. states requires laying hens be able to fully extend their limbs without touching the sides of their enclosure. It is challenging to implement practical means to accommodate legislative demands when ethologists still do not fully understand the nuances and motivations behind wing flapping as a behavior. Additionally, it is important to know how often and at what times hens prefer to wing flap. Understanding this information will allow legislative demands to better reflect practical improvements to cage-free husbandry that genuinely benefit hens.

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CHAPTER 2.

DUST BATHING IN LAYING HENS: STRAIN, PROXIMITY TO, AND NUMBER OF CONSPECIFICS MATTER

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Abstract

As housing laying hens in aviaries becomes more common, understanding relationships between social context and performance of key behaviors, such as dust bathing, is important. Expression of behaviors may be increased or repressed by the presence of conspecifics, and degree of behavioral synchrony can affect per hen resource allocation. We investigated relationships between number of hens on litter, number of hens simultaneously dust bathing (DB), and inter-bird distances (IBD) on space used to DB and duration of DB bouts across 4 laying hen strains (Hy-Line Brown [HB], Bovan Brown [BB], DeKalb White [DW] and Hy-Line [W36] at 28 weeks of age. Brown hens needed more space to DB than white hens (HB 1125.26; BB 1146.51 vs DW 962.65; W36 943.39 cm^2; *P*<0.01). More white hens occupied litter at once (43 DW, 41 W36 vs 28 HB, 31 BB; *P*<0.01), and more white hens DB simultaneously than brown hens (11 DW, 19 W36 vs 4 HB, 4 BB; *P*<0.01). Brown hens had larger average IBD (HB 13.99, BB 15.11 vs DW 8.39, W36 7.85 cm; *P*<0.01) and larger minimum IBD (HB 6.76, BB 7.35 vs DW 1.63, W36 1.79 cm; *P*<0.01) but shorter DB durations than white hens (HB 7.37, BB 9.00 vs DW 13.91, W36 15.16 min; *P*<0.01). White hens' DB area decreased if number of hens on litter increased (DW 0.85; W36 0.79 cm; *P*<0.05) or minimum IBD decreased (DW 3.66, W36 2.98 cm; *P*<0.01). Brown hens' DB bout duration decreased as number of hens on litter increased (HB 0.87, BB 0.95 min; *P*<0.01), number of other hens DB increased (HB 0.75, BB 0.69 min; *P*≤0.02) or minimum IBD decreased (HB 2.39, BB 2.31 min; *P*<0.01). In response to smaller IBD and more hens on litter simultaneously, DW and W36 hens minimize DB area while BB and HB hens shorten DB bouts, potentially terminating bouts before fulfilling their needs. Variations in DB behavior among strains should be considered when planning and stocking laying hen aviaries.

Introduction

The U.S. laying hen industry has begun to move from conventional cages to non-cage systems, including open-concept aviaries. As this conversion occurs, it is important to understand how the birds utilize the space and resources provided in these cage-free environments and large social groups. Most aviary systems offer perches, nests, and litter areas to allow hens to perform highly motivated behaviors, making the additional space more valuable to the hens than would be gained by simply adding area (Keeling, 1995). However, birds tend to distribute themselves unevenly within these systems (Channing et al., 2001), clustering around certain resources (Collins et al., 2011), which may lead to crowding at some resources while others appear underused (Appleby, 2004; Ali et al., 2016). Thus, it is important to understand hens' behavior as affected by the social context in aviaries to identify birds' preferred distributions and behavior patterns when housed in groups to inform better aviary designs or more ideal stocking rates to facilitate optimal use of resources.

One of the important behaviors that aviaries promote is dust bathing. This behavior generally occurs on litter, where hens may spend up to 23% of their time each day (Carmichael et al., 1999). Dust bathing is a functionally important behavior, as it realigns feather structure and removes lipids from the skin of birds (Vestergaard, 1982; van Liere and Bokma, 1987). Hens are highly motivated to dust bathe and will work to gain access to litter (Widowski and Duncan, 2000). They will even dust bathe in the absence of an appropriate litter source, such as on wire flooring in conventional cages, which may not improve feather quality (Hughes and Duncan, 1988; Lingberg and Nicol, 1997). As an acknowledgment of the importance of dust bathing to hens, various standards, laws, and welfare accreditation schemes require that hens have access to areas containing substrates suitable for dust bathing. Thus, many aviary designs provide an open floor space that can be covered with litter to facilitate dust bathing by laying hens.

Dust bathing dynamic, composed of many active behaviors and hen movement around a litter area. As hens typically dust bathe in the afternoon, multiple birds in a group are likely to want to dust bathe at the same time (Vestergaard, 1982). There is also evidence for behavioral synchrony in dust bathing among hens housed together (Hoppit et al., 2007). If many hens in a group perform dust bathing concurrently, this may cause crowding on the litter areas (Campbell et al., 2016). It is not fully known how the synchrony of the behavior affects hens who are attempting to dust bathe, and hens of some strains may have a tendency to view a resource as crowded more than others (Mench and Blatchford, 2014; Keeling, 1995). Thus, stocking density and nearby conspecifics may influence hens' ability and desire to perform dust bathing in an unconstrained manner at the time of day a hen would prefer to dust bathe. For example, as bird density increases on litter areas, dust bathing decreases slightly (Carmichael et al., 1999).

Hens also express preferred inter-bird distances between themselves and surrounding hens. Inter-bird distances vary depending on the behavior hens are engaging in, with more dynamic behaviors typically associated with larger distances and socially-facilitated behaviors with smaller distances (Keeling, 1995). Space guidelines do not account for inter-bird distances that are preferred during performance of behaviors (Riddle, et al., 2018).

Further, as there are multiple genetic strains of laying hen used by the egg industry, variation among these strains in weight and size leads to occupancy of different amounts of physical space when hens perform behaviors such as dust bathing or perching (Riddle et al., 2018; Giersberg et al., 2019). Previous work in our lab found significant variations in space used by DeKalb White, Hy-Line White, Hy-Line Brown, and Bovans Brown hens during performance of key behaviors, including dust bathing (Riddle et al., 2018). In addition to differences in the amount of space physically occupied by the body of a hen when performing a behavior, selective breeding for certain traits may have caused behavioral divergences among strains (Albentosa et al., 2003), which may include their preferred inter-bird distances, circadian rhythms, or desire to perform behaviors in synchrony. For example, previous studies have found distinctive distribution patterns, circadian rhythms, as well as different preferences for resources, in 4 of the more common genetic strains (Ali et al., 2019a; Ali et al., 2019b; Villanueva et al., 2017).

The goal of this study was to explore differences in dust bathing behavior among 4 genetic strains of laying hens in an aviary. Building upon previous work from our lab regarding areas used by hens of these same 4 strains while dust bathing (Riddle et al., 2018), we investigated whether strain affected the degree to which hens would dust bathe synchronously, and how the presence of conspecifics and their distance from a hen would affect her performance of a dust bathing bout.

Materials and methods

Ethics

The methods used in this study were approved by the Michigan State University Institutional Animal Care and Use Committee before data were collected or animals were placed in the facilities (Animal use #01/15-025-00).

Hens and Housing

The subjects of this study were 2,304 hens from 4 commonly used genetic strains of laying hens in the U.S. egg industry: Hy-Line Brown (**HB**), Bovans Brown (**BB**), Hy-Line W36 (**W36**), and DeKalb White (**DW**) (n = 576 of each strain). The birds were reared in the pullet house at the Michigan State University (MSU) Poultry Teaching and Research Center in East Lansing, MI. The pullet house was climate-controlled and contained 12 pens, each able to hold

225-250 chicks. Chicks were separated into pens based on strain (3 pens/strain; n = 657-750 chicks per strain). Pens were bedded with pine shavings (~7-10 cm deep), and a roosting area was added at 3 WOA.

At 17 WOA the pullets were moved to a commercial-style aviary system (NATURA60, Big Dutchman, Holland, MI) in the MSU Laying Hen Facility. Pullets were divided by strain across 4 rooms, with each room containing 4 separate aviary units with 1 unit/strain/room for 16 total units (4 units/strain). Each unit was initially populated with 144 pullets, as per the manufacturer's recommended stocking density. The 4 strains were placed into units in a balanced fashion within each room so that each strain was housed in a different unit location within each room to avoid location bias. Each aviary unit consisted of a wire-mesh enclosure with 3 tiers and an external litter area on the floor level. The litter area consisted of an open area in front of the tiered enclosure and an area that extended under the enclosure (see **Figure 2.1**). Hens were provided with 305 cm^2 of open litter area per bird. The stocking densities for each of the 4 strains based on the average live weights of adult hens (kg/m^2 as per Thaxton et al., 2006) were: HB = 65.57 kg/m^2, BB = 61.64 kg/m^2, DW = 56.5 kg/m^2, and W36 = 50.16 kg/m^2. We also calculated the number of hens that could fit into the litter area based on their body size while standing or DB. In both cases, fewer brown hens would be expected to physically fit into the open litter area than white hens (Standing: DW = 77.4 hens, W36 = 76.1 hens, BB = 67.7 hens, and HB = 65.5 hens; DB: DW = 42.7 hens, W36 = 43.8 hens, BB = 37.2 hens, and HB = 36.9 hens).

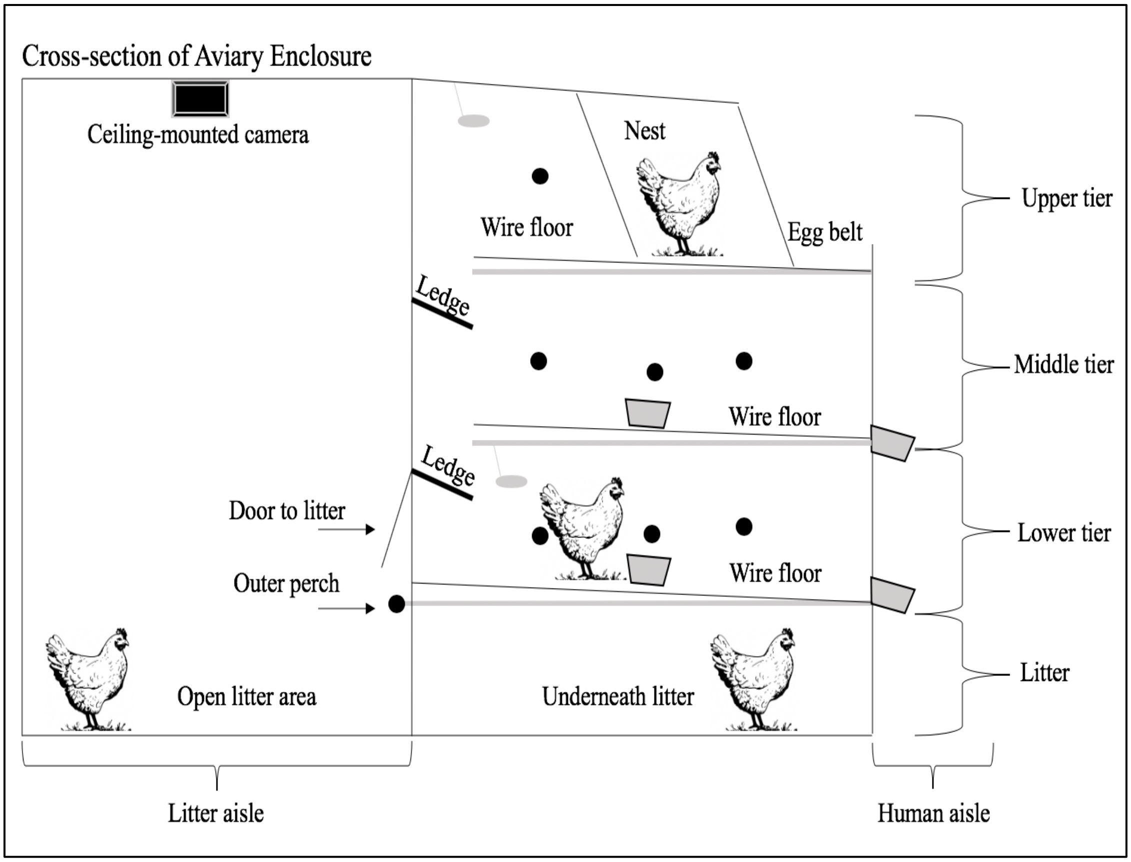

Figure 2.1. Cross-sectional diagram of the tiered aviary enclosure, showing the open and underneath litter areas, human and litter aisles, manure belts (light gray bars), and locations of the nest, drinkers (gray ovals), feeders (gray rectangles), perches (black circles), and ledges.

At the start of the laying cycle, the litter area was bedded with pine shavings (~3-5 cm deep) similar to those provided during rearing. A round metal perch ran along the front of each enclosure to help the hens move between the floor litter area and the tiered enclosure. Light was provided by dimmable LEDs in the ceiling (Agrishift PL 12 watt, ONCE, Inc., Plymouth, MN). For more details on the aviary design, food and water access, and space allotment per hen, see (Ali et al., 2016). The hens remained enclosed in the tiered aviary until 26 WOA, when they were given access to the litter area. This delay allowed the hens to reach ~90% egg production before allowing access to the litter in an attempt to train hens to use the nests and prevent egg-laying in the litter. Starting at 26 WOA, the doors on the lower tier of the enclosure began opening automatically at 11:30 each day to allow hens daily access to the litter area. The doors closed again at 01:00 once hens had returned to the aviary enclosure to roost. The lights turned on automatically each day at 05:00 and dimmed for 30-minutes before shutting off completely at 20:00. Hens had two weeks to acclimate to the litter area before data were collected. For more details on feeding, cleaning, and lighting, see Ali and colleagues (2016).

Video Recording

Prior to the birds' placement in the aviary units, high-resolution digital video cameras (VF450: Clinton Electronics, Loves Park, IL) were affixed to the ceiling, centered above the open litter area of each unit, to record hen behaviors on litter during the day. All cameras were placed at the same distance from the litter. During the study, data were collected from approximately 11:30 to 15:00 as hens follow a circadian rhythm and most often dust bathe in the afternoon (Mishra et al., 2005).

Behavior Definitions

The behaviors examined in the current study are the key elements within the dust bathing (**DB**) behavior sequence, as described by Kruijt (1964). These elements are: bill raking, head rubbing, scratching with one leg, scratching with two legs, side-lying, ventral lying, and vertical wing shaking. A full ethogram describing these behaviors is provided in **Table 2.1**. The start of a bout was recorded when a hen's body touched the litter and she performed any of the key elements of DB (Larsen, et al., 1999; Van Rooijen, 2005). When the hen stood up and did not resume any elements of DB behavior within 10 seconds, the bout was considered to have ended.

Behavior	Abbreviation	Description
Bill Raking	BR	The bill is first moved downward and after touching the litter, it is quickly moved backward and then upward; in this way the litter is raked closer to the bird
Head Rubbing	HR	The side of the head is rubbed on the ground with one quick sweep
Scratching with One Leg	S1L	One leg is moved backward at a time manipulating the litter while the bird has its body in contact with the litter
Scratching with Two Legs	S2L	Two legs are moved backward at a time manipulating the litter while the bird has its body in contact with the litter
Side Lying**	SL	One side of the bird remains flat against the litter while the bird remains still; occasionally wing- and leg-stretching are present
Ventral Lying**	VL	The ventral side of the bird remains flat against the litter while the bird remains still
Vertical Wing Shaking	VWS	Nearly closed wings are held at a distance from the body and are moved vertically, both at the same time in the same direction, to sweep litter into the plumage

Table 2.1. Ethogram of behaviors. Adapted from Kruijt (1964). **created specifically for this project – no definition was present in the paper.

Data Collection

To balance collection of images across the 4 units per strain, we collected DB information from 8 hens per unit for a total of 32 hens per strain and 128 hens in total (8 hens/unit x 4 units/room x 4 rooms = 128). All data were collected by the same trained individual.

Naturally occurring hen activity in the open litter area was recorded on video over the course of 3 days. Selection of DB bouts for analysis was done via convenience sampling using 2 criteria. First, a hen had to be demonstrating a key element of DB behavior as described in the ethogram (**Table 2.1**). Second, the hen needed to be roughly in the center of the open litter area (i.e., not touching any walls or gates or be fully or partially out of sight under the enclosure). Once a DB bout was identified, the duration of the bout was recorded and images were captured from the beginning, middle, and end of that DB bout using the Snipping Tool (Microsoft

Windows, 10.0.15063.13 tool kit). Images were then labeled by hen number (1-8), unit, time, and date, and saved. We observed 32 focal hens DB per strain (8 hens per unit) and gathered measurements from each hen in the beginning, middle, and end of her DB bout. Therefore, we had a total of 96 observations for each strain of laying hen (32 hens/strain x 3 still images per hen). From each selected image, the area each hen occupied while DB was calculated by drawing a line from the hen's most distal anterior point to the most distal posterior point (length); a second line was drawn across the widest part the hen's body (width), including potentially outstretched wings and legs. Inter-bird distances (**IBD**) were measured between the focal hen and the nearest 5-7 surrounding hens in each captured image. The average IBD was calculated using the distances measured between each of these surrounding hens and the focal bird. The minimum IBD was the distance between the focal hen and the closest of these surrounding hens. The surrounding hens were labeled within the captured images to ensure accurate measurements and to avoid re-measuring the same birds (see **Figures 2.2 and 2.3**). The number of surrounding hens for which IBD was recorded varied between images, even sometimes from images recorded at different times within the same dust bathing bout because the focal hen would occasionally relocate herself to an area with more or less conspecifics, or because conspecifics would move away from the focal hen during the DB bout.

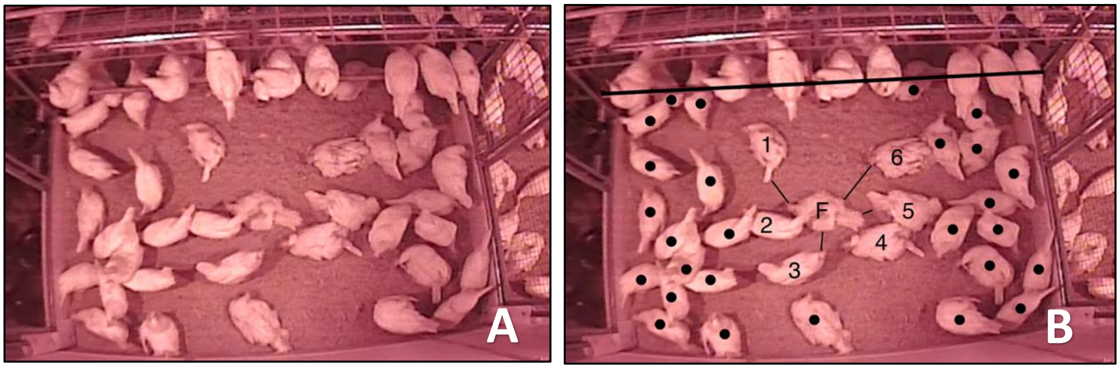

Figure 2.2. **A**). Example of screen capture from video footage of the open litter area showing several hens performing DB. **B**). F indicates the focal hen. Dots indicate hens counted on the open litter area (i.e., at least one-third of the hen's body was past the outer perch as indicated by the black line). Surrounding conspecifics analyzed for IBD are marked with a number (1-6), and lines indicate the closest part of their bodies to that of the focal hen.

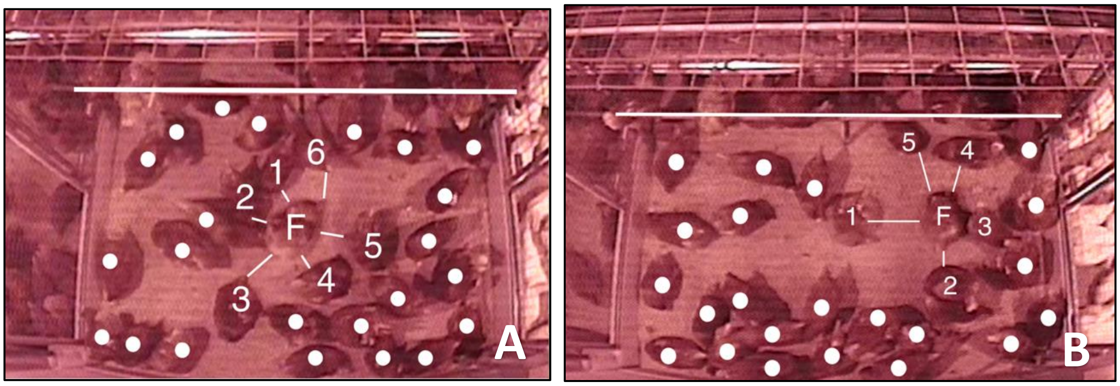

Figure 2.3. Examples of screen captures that show the beginning (**A**) and end (**B**) of a focal hen's DB bout. The focal hen is indicated by F. The hen moved throughout the DB bout as were other hens on litter, resulting in different numbers of hens being assessed for IBD at the beginning, middle and end points of the DB bout. These images also illustrate that it was difficult to distinguish the outline of brown hens from their shadows against the litter.

All measurements were recorded from captured images using ImageJ 1.50i software (Wayne Rasband, National Institute of Health, USA). We first set a scale in ImageJ by measuring a reference object within the open litter area of a known length; in this case the outer perch (244 cm). In each captured image, the scale was set to approximately the same value (595-600 pixels; each pixel then measured 2.4-2.5 cm) along the length of the outer perch to ensure each measurement produced an accurate length in cm within the pictures. All images used in this study captured the open litter area and the outer perch.

Finally, the total number of birds DB (including the focal hen) was counted within each image, and the total number of birds present on the open litter area was counted. Synchrony can be defined as multiple animals in a group performing a behavior simultaneously (Keeling *et al.*, 2017). For this study, we looked at hens DB at the same time on the open litter. In an attempt to record the most accurate number of hens using the litter at once, a hen was considered to be on the litter when her body was at least 1/3 of the way past the outer perch in the open litter area.

Statistical Analysis

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). Counts of hens using litter area and DB, space used for DB by a focal hen, duration of DB, and average and minimum IBD between birds during dust bathing were compared across the 4 strains of laying hens (HB, BB, DW, and W36). Comparisons among strains were performed using one-way ANOVAs using the “car” package, at the level of the individual hen, controlling for repeated measures from each hen, with strain as the main effect and unit (i.e., pen) as a random effect. $P \leq 0.05$ was considered significant. Though we gleaned information from individual hens, the behavior of each hen was likely influenced by conspecifics and therefore pen was considered to be the experimental unit. Statistically significant effects were further analyzed using Tukey’s honestly significant difference multiple comparison procedure using the “multcomp” package (Hothorn et al., 2008).

To explore influences of litter occupancy and IBD on the average duration and space occupied by individual hens while DB, mixed effect regression models were conducted using the “lme4” package (Bates et al., 2015). Aviary unit (i.e., pen) was included as a random effect in all regression models. The first regression model was generated to identify the relationships between number of hens on litter, number of hens DB, as well as the average and minimum inter-bird distances and the average duration of dust bathing bouts. A second regression model was generated to identify the relationships between those same variables and the space occupied by individual hens while DB across the 4 strains of laying hens. Finally, coefficient estimates were transformed and presented as odd ratios (OR).

Results

The Intraclass Correlation Coefficient for measuring agreement (ICC) was used (Shrout and Fleiss, 1979) to measure intra-observer reliability using 10% of measurements for each behavior per strain using "ICC {cran}" package. Intra-observer reliability was calculated during the training period with the observer re-measuring the same birds twice in a random order, and a strong ICC of 0.98 (CI = 0.898) was found.

Area

Hens of both brown strains occupied more space while DB than the hens of both the white strains (*P*<0.01 for all comparisons). BB hens used the greatest area while performing a DB bout (1146.51 ± 240.55 cm), followed by HB hens (1125.26 ± 222.94 cm), and the areas used to DB by hens of these 2 brown strains were not different from each other (*P*=0.6). Hens of both white strains occupied a similar amount of space to each other while DB (DW: 962.65 ± 165.13 cm; W36: 943.39 ± 152.90 cm; *P*=0.99).

Relationships between IBD and DB Area and Bout Duration

The W36 and DW hens spent an average of approximately 15 and 13 minutes per DB bout, respectively; while the BB and HB hens had average DB bout durations of approximately 9 and 7 minutes, respectively (**Table 2.2**). When looking at the average IBD between DB focal hens and nearby conspecifics on the litter, we found that, on average, DW and W36 hens DB with a smaller minimum IBD than BB and HB hens (**Table 2.2**). The DW and W36 hens were also more likely to show synchronous DB behavior, have more hens on the open litter area simultaneously, and have longer DB bout durations compared to the brown hens (**Table 2.2**).

Strain	Number of hens on litter[1]	Number of hens dust bathing birds simultaneously[2]	Average duration (min)[3]	Minimum IBD (cm)[4]	Average IBD (cm)[5]
Hy-Line Brown	28.30 ± 8.47^a	3.82 ± 3.27^a	7.37 ± 6.98^a	6.76 ± 3.67^a	13.99 ± 4.65^a
Bovans Brown	30.97 ± 8.46^a	4.04 ± 3.92^a	9.00 ± 5.11^a	7.35 ± 3.89^a	15.12 ± 7.34^a
DeKalb White	42.8 ± 8.6^b	11.26 ± 3.86^b	13.92 ± 8.60^b	1.63 ± 2.73^b	8.39 ± 3.83^b
Hy-Line W36	41.5 ± 6.59^b	10.21 ± 3.53^b	15.16 ± 8.58^b	1.79 ± 1.74^b	7.85 ± 3.65^b

Table 2.2. Synchronous DB and litter occupancy, DB duration, and average and minimum IBD of focal hens among 4 laying hen strains. Data are presented as means ± SEM. Different superscripts indicate statistical significance ($P<0.05$). [1]Indicates the total number of hens present on the open litter area (i.e., at least one-third of the hen's body is past the outer perch on the open area). [2]Indicates the total number of hens DB on the open litter area. (Note: the focal hen is included in both counts of both total number of hens on litter and the total number of hens DB.) [3]Total duration of the focal hen's DB bout in minutes. [4, 5] Minimum and average IBD in centimeters for each strain.

Hens of the 2 white strains in the present study also responded differently to decreasing IBD than hens of the 2 brown strains. As the number of hens occupying the litter area increased, DW and W36 hens were more likely to reduce the amount of physical space they occupied during a DB bout compared to BB hens (**Table 2.3**). As the minimum IBD decreased (i.e., the "buffer zone") between the focal hen and nearby conspecifics decreased, DW and W36 hens were more likely to reduce the area they used to DB compared to BB hens.

In contrast, as the minimum IBD decreased between a focal DB hen and other hens on the litter, HB and BB hens were more likely to reduce the duration of DB bouts compared to DW hens (**Table 2.3**). As increasing numbers of hens occupied the litter area, HB and BB hens were more likely to decrease the duration of their DB bouts compared to DW hens. Finally, HB and BB hens were also more likely to decrease their DB duration compared to DW hens when the number of hens DB on the litter increased.

Parameter		Odds ratio	Z	95% CI	P-value
Area of focal hen while DB					
Intercept [BB]		1.09	8.96	-0.37-0.75	0.00
Strain x Litter Occupancy					
DW		0.85	-4.25	0.24-1.43	0.004
W36		0.79	-6.96	0.15-2.34	0.003
Strain x Minimum IBD					
DW		3.66	3.99	1.58-4.48	0.00
W36		2.98	2.23	1.30-3.74	0.00
Duration of focal hen DB bout					
Intercept [DW]		1.14	15.52	-0.41-0.72	0.00
Strain x Litter Occupancy					
HB		0.87	-3.36	0.42-1.43	0.002
BB		0.95	-6.96	0.73-1.26	0.004
Strain x Number of Birds DB					
HB		0.75	-2.6	0.29-1.99	0.02
BB		0.69	-5.9	0.27-1.75	0.01
Strain x Minimum IBD					
HB		2.39	4.98	0.55-14.73	0.001
BB		2.31	4.52	0.68-10.59	0.003

Table 2.3. Strain differences in area occupied by DB hens and duration of DB bouts. Results are presented as odds ratios and 95% confidence intervals. The first strain listed is tested against the baseline of the second strain (i.e., DW hens compared to BB hens).

Discussion

Area

Previous research, using the same flock of birds and aviary system, looked at the space used by laying hens of these 4 strains when performing certain behaviors, including DB (Riddle et al., 2018). In the current study, we re-calculated the area used by hens of the 4 strains when DB to verify that previous area estimates remained accurate. To do so, we used the same video footage used by Riddle and colleagues, but took different still images and measurements from more (32 hens/strain versus 16 hens/strain) and different individual birds (i.e., we used the same flock, but not the same hens). We found that the average areas occupied by hens of each of the 4 genetic strains during a DB bout were comparable to the results reported previously by Riddle and colleagues (2018). In addition, hens from both brown strains were again found to use more space to DB than did hens of both white strains (Riddle et al., 2018). BB hens, which occupied the largest average area while DB, used approximately 203.12 cm^2 more space than the smallest strain, the DW hens. This is likely due to the fact that brown hens are generally physically larger than white hens and may, therefore, require more space as they perform dynamic behaviors (Appleby, 2004). Hens of both white strains in the present study decreased the amount of physical space they occupied while DB with closer proximity of conspecifics. The hens of the two brown strains did not significantly reduce the area they used to DB in response to closeness of conspecifics but instead shortened their DB bout duration.

Litter Occupancy

Hens from the two white strains examined in this study occupied the open litter area simultaneously to a greater extent than hens of the two brown strains. The number of hens that can fit on the open litter floor area will, of course, vary depending on what the birds are doing, but in general, more white hens would be expected to fit onto the litter than brown hens due to their smaller body sizes (Riddle et al., 2018). For example, 77.4 DW hens could fit onto the litter area while standing compared to 65.5 HB hens, and when DB 43.8 W36 hens could fit into the open litter area compared to 36.9 HB hens. Brown hens, therefore, would be expected to perceive the litter area as crowded at lower numbers of hens than white hens. In the current study, we found 40 white hens on average on the litter at any one time, compared to an average of 30 brown hens, which suggests this to be the case.

Previously, hens from the same white strains had been found to often occupy the litter concurrently at higher numbers throughout the day compared to brown hens (Ali et al., 2016; Ali et al., 2019b). It should be pointed out, however, that Ali and colleagues (2019b) counted more brown hens in the litter area under the tiered enclosure as opposed to the open litter area, which was the location examined in the current study. Thus, observing only the open litter area may not give a full picture of litter occupancy, although it does provide evidence of strain differences in hen distribution.

Synchronous DB Behavior

Domestic fowl have previously been described to DB together, which might indicate that they feel safety in numbers when performing a vulnerable behavior like DB (Duncan, 1980; Keeling, 1995). Alternatively, their synchrony may arise as a result of social facilitation, which leads hens to DB more readily in the presence of other hens already executing DB bouts (Vestergaard et al., 1993). Rebound effect could be another possible explanation for DB synchrony in this system, as the hens did not have 24-hour access to the litter and were kept enclosed in the aviary tiers from 01:00-11:30. Because the hens had delayed access to the litter, they may have an increased propensity to DB synchronously as soon as the doors opened

(Dawkins, 1988; Hughes and Duncan, 1988).

Hens of the white strains were more likely to DB together with more conspecifics than hens of the brown strains—i.e., roughly 10 white birds would DB at the same time compared to 4 brown birds. At present, it is unclear what underlying motivation causes the different degrees of DB synchronicity among the strains. Hens of the white strains in this study generally began to DB immediately upon the opening of the aviary doors, whereas the hens of the brown strains appeared less likely to DB right away. Hens of another white genetic strain, Lohmann Whites, have also been found to DB together at a higher rate during the morning hours (i.e., 11:00-13:00) than in the afternoon (Campbell et al., 2016). Specifically, the hens in that study would DB more often in the morning during peak lay, at approximately 27 WOA. The hens in our study were very close in age (28 WOA) to those hens during data collection. The hens of our two white strains would often DB as soon as the doors opened at 11:30, so it could be theorized that they DB in at a similar time to Lohmann Whites. Therefore, hens of these white strains may be more susceptible to effects of litter restriction than hens of brown strains.

However, Campbell and colleagues (2016) also used the same aviary system that closed from 05:30-11:00, so we cannot be sure if the white hens' inclination to DB early is due to rebound effect or to differences in circadian rhythm. These 4 strains (HB, BB, DW, and W36) also show variability in the time of oviposition. For example, hens of these white strains lay 55% of their daily nest eggs between 6:00-10:00 compared with 85% of nest eggs laid by the hens of the brown strains during this same period (Villanueva et al., 2017). Future studies should focus specifically on the circadian rhythm of DB among different strains of laying hen to parse out any distinctions in behavior due to genetics.

Duration

The W36 and DW hens in this study DB for an average duration of approximately 15 and 13 minutes, respectively; while the BB and HB hens had bout durations of around 9 and 7 minutes, respectively. Durations of DB bouts have previously been reported to last between 20-35 minutes in White Leghorn hens housed in deep-litter floor pens (Vestergaard, 1982). However, as the duration of a DB bout might vary depending on circumstances as well as strain of hen, it may be hard to determine whether reports from the literature represent an ideal bout length (van Rooijen, 2005). Hens housed in fairly unconstrained systems, such as those with larger available areas to use for DB, may show longer DB bout durations that are closer to those previously reported in the literature. For example, hens of another brown strain (Warrens), had a median DB duration of 16.8 minutes when provided with a larger DB area (1,200-1,800 cm2/bird; van Liere et al., 1991). In contrast, other studies using smaller litter areas and varying litter quality, have also found reduced DB bout durations, similar to those in the current study. ISA Brown pullets housed in battery cages affixed to boxes containing sand, providing 259 cm2/bird in each box, performed DB bouts lasting an average of 5 to 10 minutes (Smith et al., 1993). A second study using ISA Brown pullets in cages, again affixed to boxes containing 375 cm2/bird of usable litter found median DB durations of 4 to 7 minutes (Appleby et al., 1993). Finally, Lohmann Brown and Lohmann Selected Leghorn hens, a brown and white strain, respectively, housed in compartments containing litter trays providing 1,000 cm2/hen, DB for 2 to 15 minutes, with bout length influenced by diet or litter substrate (Scholz et al., 2011).

In the current study, hens of all 4 strains were provided 1,132 cm2/bird, including the total litter area and tiered enclosure, with 305 cm2/bird of this space in the open litter area. All hens were raised the same way, fed the same diet, and housed in the same environments (see Ali et al., 2016 for more information). Thus, the differences in DB durations among hens of BB, HB, DW, and W36 strains are likely due to genetic variation in behavior or in their sensitivity to disruption or proximity of conspecifics. For example, the shorter DB duration of brown hens may reflect greater sensitivity to social disruptions or the need for more room to DB successfully. Keeling (1995) suggested that a particular stocking density may be viewed as "crowded" for certain individuals but may be fine for others. In this case, the hens of the white strains in the current study appeared to DB together more often overall and to tolerate the presence of conspecifics while DB to a greater extent than hens of the brown strains. Given these results, we agree with the conclusion that a standard space allowance broadly applied to all strains of laying hens is not be ideal for facilitating key behaviors by hens (Appleby, 2004).

Alternatively, the strains of brown hens in the current study may also simply have shorter DB bouts than the hens of our white strains. It is also possible that the brown hens continued DB bouts under the tiered enclosure or performed several short DB bouts throughout the day, whereas the white hens may have been more inclined to start a DB bout and complete it to the best of their ability in one sitting—thus increasing their duration. In future, DB by hens of various strains should be examined under more optimal conditions (e.g., fewer total hens, more space per individual hen) to see if there are still differences in how hens of different strains perform DB behavior when less constrained.

Inter-Bird Distances

DW and W36 white hens in the present study had the smallest average and minimum IBD—meaning that white focal birds were generally closer to conspecifics while DB. Conversely, BB and HB hens had the largest average and minimum IBD—indicating that focal birds had a larger buffer between themselves and others while DB. Hens have a tendency to cluster together instead of distributing themselves evenly over an area, but the degree of clustering can vary depending on the behavior being performed (Keeling, 1994; 1995). In the case of DB, conspecifics may be inclined to join a focal hen that is DB until large numbers of hens are synchronous in their behavior (Campbell et al., 2019). Conversely, hens may be motivated to perform DB behavior but cannot physically do so due to limited space or due to a conspecific entering their individual space. Because DB is a social behavior (Duncan, 1980; Hoppit et al., 2007), it is possible that hens wanted to DB together but could not fit within the litter area while maintaining their preferred IBD.

Regardless of the underlying cause, our results indicate that hens of the white strains were generally more likely to tolerate smaller IBD than hens of the brown strains. Even if the white hens in our study were unable to move their bodies in the most optimal manner during a DB bout, they still continued the behavior while crowded, whereas the brown hens terminated their DB bouts when they appeared to find IBD to be smaller than tolerable.

Limitations

We were not able to view the entire litter area and could not tell if hens were dust bathing in the space under the tiered enclosure. Additionally, as litter access was restricted throughout the night and for part of the morning, our ability to observe differences in strain-based circadian rhythms in dust bathing behavior was limited. More research should be done in aviaries with 24-hour litter access tracking individual focal hens to further understand if differences we observed in dust bathing bouts are due to distinctive circadian rhythms among the strains. There may also be sampling bias in our results, as we assessed only “ideal” DB bouts where the focal hen was centered on the open litter area. Thus, we may have neglected to analyze certain naturally occurring bouts throughout the day in our attempt to choose clear, higher-quality bouts for measurement. However, the data from all strains were likely equally biased. In future, all dust bathing bouts that occur should be sampled for a more accurate picture of hen behavior and space use within an aviary system.

Conclusion

There are strain differences in how laying hens perform dust bathing in an aviary system with respect to bout duration, varying inter-bird distances, synchrony of behaviors, and, potentially, circadian rhythms. Hens of the white strains used in this study (i.e., DeKalb White, Hy-line W36) showed higher rates of litter occupancy and more synchrony in their dust bathing behavior compared to hens of the brown strains (i.e., Hy-Line Brown, Bovans Brown). The white hens had smaller inter-bird distances while performing a dust bathing bout, whereas hens of the brown strains had larger inter-bird distances and shortened the duration of DB bouts in the presence of more hens on the litter or with less space between nearby hens. This indicates spatial-social differences among strains of laying hen while performing key behaviors such as dust bathing. These findings continue to support the growing number of studies indicating behavioral differences among strains of laying hens. Producers may want to use different strains depending upon the housing system they are stocking.

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CHAPTER 3.

VARIATION IN LITTER OCCUPANCY AND DUST BATHING PATTERNS AMONG LAYER STRAINS FOLLOWING PERIODS OF LITTER RESTRICTION

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Abstract

Producers are moving towards cage-free systems to house laying hens. These include aviary styles with multi-level wire enclosures and litter areas on the floor. In some aviaries with doors, hens can be confined within the tiered enclosure, which can be done to promote oviposition in nests and prevent hens from laying eggs in litter. However, there are multiple genetic strains of laying hen used in the egg industry, and some show different temporal patterns for key behaviors that could affect when they want to be on litter. For example, though dust bathing by laying hens is typically considered to peak in early afternoon, there may be variation in timing of motivation to dust bathe among strains. Differences in hens' temporal patterns coupled with aviary configurations or management practices, may restrict birds' ability to perform important behaviors, such as dust bathing (DB), when they would most prefer to do them. Our objective was to determine if there were strain differences in the temporal pattern of DB. We examined the timing of DB in 4 strains of laying hen (Hy-Line Brown [HB], Bovans Brown [BB], DeKalb White [DW] and Hy-Line W36 [W36]) housed in aviaries using 144 hens of each strain per aviary unit (4 units/strain). We video recorded the number of hens DB and on litter using instantaneous scan sampling every 5 minutes collected at 26 and 28 weeks of age beginning at 11:35 (when litter access began each day) to 20:00 (lights off). Brown strains acclimated to litter access more slowly than white strains. Hens of all strains DB most often soon after gaining access to litter, and counts of white hens DB were overall higher than counts of brown hens DB. Further examination of diurnal rhythm of behaviors, such as dust bathing, under unconstrained conditions by a range of genetic strains of laying hens is needed to design management practices and aviary styles that best meet hens' needs.

Introduction

A pervasive concern for farm animal welfare prompted global initiatives to prohibit the use of intensive confinement systems in commercial farm facilities (Buller et al., 2018), including battery cages to house laying hens. While caged systems offer several benefits for human workers and hens, they also hold significant shortcomings (i.e., a lack of space and behavioral resources) that disproportionately affect hens (Duncan, 2001; Lay et al., 2011). The use of battery cages is negatively perceived by many consumers (Rondoni et al., 2020), including those in the United States (Widmar et al., 2020). Several countries, such as those in the European Union (EU) and Australia, along with some individual U.S. states, have implemented legislation to ban the use of caged systems in commercial egg production, instead requiring producers to house hens in cage-free systems (Scrinis et al., 2017).

Many cage-free initiatives offer guidelines for producers with recommendations on space and resource provision, including designated nest areas and perches, to accommodate the behavioral needs of laying hens raised on commercial farms (Hemsworth, 2021). Litter is also an important addition to many cage-free systems, including in rearing systems where it acts as an early, appropriate pecking stimulus for pullets as they develop (Campbell et al., 2019). In adult laying systems, the presence of a designated litter area should afford hens both the space and substrate necessary to perform important behaviors like foraging and dust bathing. Australian cage-free housing standards require that litter cover enough floor area in a system to simultaneously accommodate one-third of the flock (AU Poultry Standards and Guidelines, 2022). In the EU and Canada, egg producers are asked to provide a litter area that encompasses at least 33% of the usable floor space within housing systems (EU Council Directive 1999/74/EC; CA Code of Practice). Most U.S. cage-free state laws ask producers to follow spacing and husbandry guidelines set forth by the United Egg Producers (UEP). In 2017, the UEP recommended that cage-free systems have litter covering at least 15% of the usable floor space (UEP, 2017); as of 2024, those guidelines state each hen must have at least 21.6 square inches of scratch area (UEP, 2024). Little research has assessed the amount of floor space within cage-free systems that should be dedicated to litter to accommodate behavioral needs and promote good welfare. However, recent findings suggest a reduction in litter area may discourage hens from accessing and utilizing the litter area at all, and displacement from one resource may cause an uneven distribution of hen activity within the rest of the housing system (Gonzalez-Mora et al., 2021).

Unfortunately, hens are also attracted to litter for unintended behaviors, like laying eggs, which are undesirable for producers. To encourage oviposition in designated nests within cage-free systems, producers may regulate when hens can access litter throughout a lay cycle. Some multi-tiered aviaries have doors between the litter area and tiered enclosure that can open or close at predetermined times. It is customary practice for producers using these closable aviaries to keep doors fully closed for the first few weeks after first transferring pullets from rearing facilities, usually only opening doors for litter access once a flock has reached peak egg production and learned to nest in designated areas. This exclusion from litter prior to peak lay is useful in reducing floor laying across a flock cycle (Oliveira et al., 2019). Temporary or short-term seclusion within tiers at the start of lay may also provide some benefits to hen welfare, such as reduced fearfulness and improved feather coverage (Alm et al., 2015). Producers may also continue to regulate litter access to maintain hens' nesting in designated locations, only opening aviary doors a few hours after lights in the systems turn on each morning. Therefore, it is also important to consider long-term effects of continuing litter restriction throughout a flock's lay cycle. Wire flooring in aviary tiers can cause pressure to hens' feet, and consistent confinement to tiers and away from litter may exacerbate footpad lesions over time (Ali et al., 2020). Further, the inability to access a desired resource may negatively affect hens. Some behavioral evidence suggests hens experience frustration when resources are suddenly inaccessible (Olsson and Keeling, 2000); however, it is also possible that hens habituate to management schedules and can predict when they will gain litter access or can experience pleasure as they anticipate litter access (as suggested by Taylor et al., 2020's work on anticipation in hens).

As an additional consideration, many of hens' key behaviors are diurnal in nature and restrictive management tactics could prevent hens from performing these behaviors based on their preferred temporal patterns. Limiting hens' access to desirable resources may also lead to crowding within housing systems, especially if many hens concurrently attempt to access the same area as soon as they are granted access to it. There is also evidence that different genetic strains perform behaviors at different times of day; for example, brown-feathered hens tend to lay eggs earlier in the day compared to white-feathered hens (Tumova et al., 2017; Villanueva et al., 2017). It is possible that restricting access to one resource, such as litter, to control where eggs are laid will also influence performance of other behaviors with diurnal patterns, such as dust bathing. Dust bathing involves hens distributing dry, friable litter through their feathers in a series of dynamic movements that serve to maintain good plumage condition and remove excess feather lipids (Olsson and Keeling, 2005). Hens tend to dust bathe during light hours (Duncan et al., 1998) and have been previously documented to show a diurnal rhythm of dust bathing every two days (Vestergaard, 1982). Aviaries and other cage-free systems that limit litter access in some way may inadvertently inhibit hens' ability to dust bathe at their desired times, which may indicate these systems are not providing ideal conditions for important hen behaviors or improving hen welfare as intended (Oden et al., 2002).

The purpose of this study was to investigate differences in dust bathing patterns and litter occupancy among 4 different aviary-housed laying hen strains (2 white-feathered strains and 2 brown-feathered strains) in response to complete litter restriction until peak lay, and daily restriction from litter each morning through the rest of the lay cycle. Based on prior research examining hens' acclimation to resources, including initial access to litter in aviaries, we hypothesized that hens of all strains would access litter in greater numbers over time as they acclimated to management schedules (Ali et al., 2016). Among strains, however, we expected different patterns of litter occupancy (Ali et al., 2016) and, subsequently, different patterns for dust bathing. Specifically, based upon prior findings that hens of white strains dust bathe in greater numbers at any one time compared to hens of brown strains (Grebey et al., 2020), we predicted that white hens would occupy the litter and dust bathe in larger numbers overall and would acclimate to litter access more quickly than the brown hens.

Methods

Subjects and Housing

We used 4 strains of laying hen: Hy-Line Brown [**HB**] and Bovans Brown [**BB**] (both strains are brown-feathered birds), and Hy-Line W36 [**W36**] and Dekalb White [**DW**] (both strains are white-feathered birds). Hens were reared and housed at the Michigan State University Poultry Teaching and Research Center in East Lansing, Michigan. During both rearing and adulthood, hens were housed separately by strain. Rearing pens contained a brooding platform raised above a floor area, which was bedded with 7-10 cm of pine shavings. Chicks remained on platforms until they were 3 weeks of age [**WOA**], at which point they gained access to litter (3-5 cm pine shavings) on the floor of each pen and were provided perches. Prior to the start of lay, pullets were moved into NATURA60 aviary systems (Big Dutchman). A total of 16 aviary units were used, each stocked with 144 pullets, for a total of 2,304 birds when all units were filled (n = 576 birds per strain). All units were identical and were comprised of a three-tiered wire enclosure and a floor level. The three tiers provided access to food and water, perches, and ledges to assist with transitioning among levels. Nesting areas were located on the top tier. The floor level, acting as the designated litter area, extended in front of each tiered component (the "open litter area," 244 x 180 cm/unit), as well as directly underneath them (the "underneath litter area," 244 x 163 cm/unit). Prior to stocking the aviaries, the litter area in each unit was bedded with approximately 3-5 cm of pine shavings, identical to the rearing pens. Doors on the lowest tier in each unit could open and close at predetermined times to allow hens access to litter. An external perch ran the length of the lowest tier in each unit to assist with jumping to and from the tier. See Ali et al. (2016) for additional details on the aviary configuration.

The 16 aviary units were separated into 4 rooms (within the same facility), with each room containing 4 separate aviary units. Each strain was placed in 1 unit within each of the 4 rooms, so that each room contained all 4 strains, and each strain was placed in a different unit across the rooms to avoid any location bias. Prior to hen placement, study personnel affixed digital video cameras (VF450: Clinton Electronics) to the ceiling in all 16 aviary units (see **Figure 3.1** for visualization of camera and strain placement within aviaries). Each camera was centered so that footage showed the entire open litter area, including the external perch hens use to transition between the bottom tier and the floor. The cameras were unable to capture footage from the underneath litter area, as the tiers obstructed view.

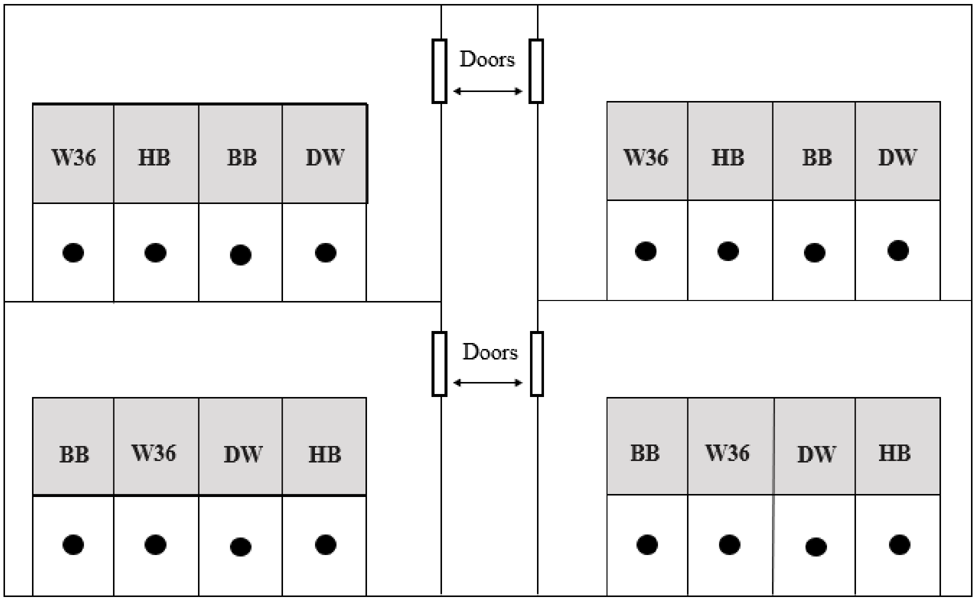

Figure 3.1. Top-down illustration of study facility showing locations of each strain (HB = Hy-Line Brown; BB = Bovans Brown; W36 = Hy-Line W36; DW = Dekalb White) in 16 aviary units split among 4 rooms. Each aviary unit was composed of a multi-tiered enclosure (grey areas in each unit), suspended above the open litter areas (white areas in each unit); litter in each unit ran underneath the tiers. Black dots signify ceiling-mounted video cameras centered above the open litter in each unit; cameras could not capture footage of hen behavior in the litter area underneath the aviary unit. Figure is not drawn to scale and depicts only the 4 rooms used in the study, which are contained within a larger facility containing a total of 12 rooms.

Study Design

Hens did not have access to litter from 17 WOA, when they were first transferred into the aviaries, until they reached peak lay at 26 WOA. This resulted in a 9-week period of complete restriction from litter, followed by daily access to litter for the rest of the lay cycle.

The ceiling-mounted video cameras in each unit captured hen behavior for three consecutive days at two time periods: (**IM**) immediately after hens gained daily access to the litter area in the aviaries for the first time, and (**AC**) 2 weeks later, when hens were acclimated to daily litter access. The 3 days of footage within each period are denoted as IM1, IM2, IM3, and AC1, AC2, AC3, respectively. See **Figure 3.2** for a timeline of hen movement from rearing to adult facilities, periods of litter restriction, and IM and AC.

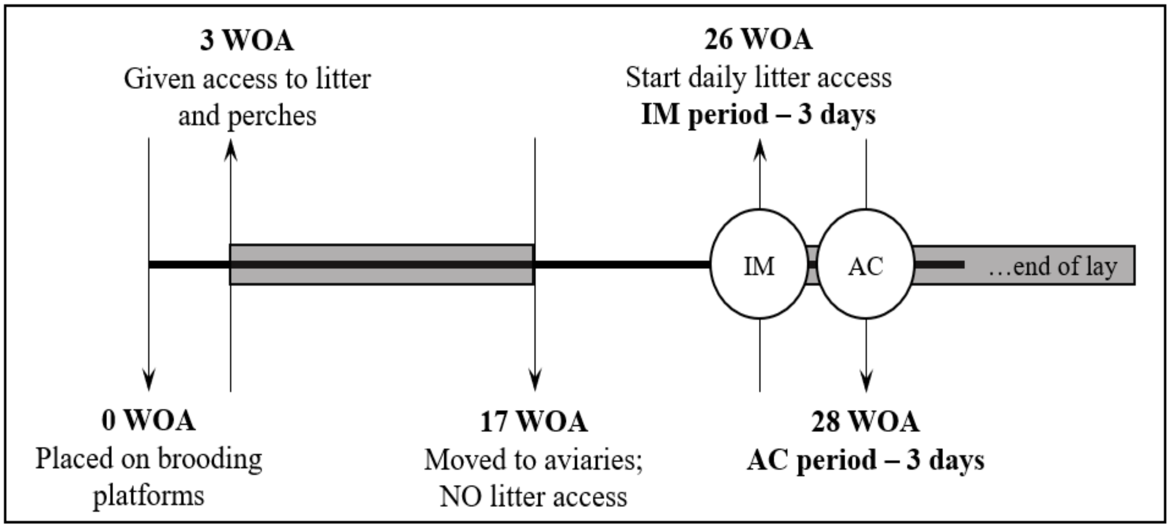

Figure 3.2. Timeline of the flock used in the study. Grey highlights indicate when hens had access to litter. Chicks were placed in rearing pens at 0 WOA where they had constant access to litter from 3-17 WOA. At placement in aviaries, hens were confined within tiers and did not have access to litter area until peak lay was reached at 26 WOA (9 weeks without litter access), at which point they had daily access. Video footage of hens in the open litter area was collected for three consecutive days immediately (IM) after hens accessed litter, and two weeks later once they were acclimated (AC) to the schedule of daily litter access. Please note that only ages and management changes pertinent to this study are shown; the flock continued through a full lay cycle after data collection.

Starting at 26 WOA, doors on the lowest tier in each unit opened at approximately 11:35 to allow hens to access litter areas and closed at 01:00, when hens were roosting in tiers. Lights in each room turned on at 05:00, meaning hens spent the first 6.5 hours of each light period restricted within the tiers. Farm staff generally collected eggs while hens were enclosed in the aviary, though personnel periodically walked through the rooms during the later hours in the day. Lights in the system shut off at 20:00 after a 30-minute period of dimming. For both IM and AC, footage was reviewed only during those hours that hens had access to litter and the lights in the systems were on (11:35-20:00, approximately 8.5 hours). To assist with analysis, these hours of footage were separated into six equal time segments, labelled A through F (each time segment encompassed 85 minutes). See **Figure 3.3** for a daily schedule.

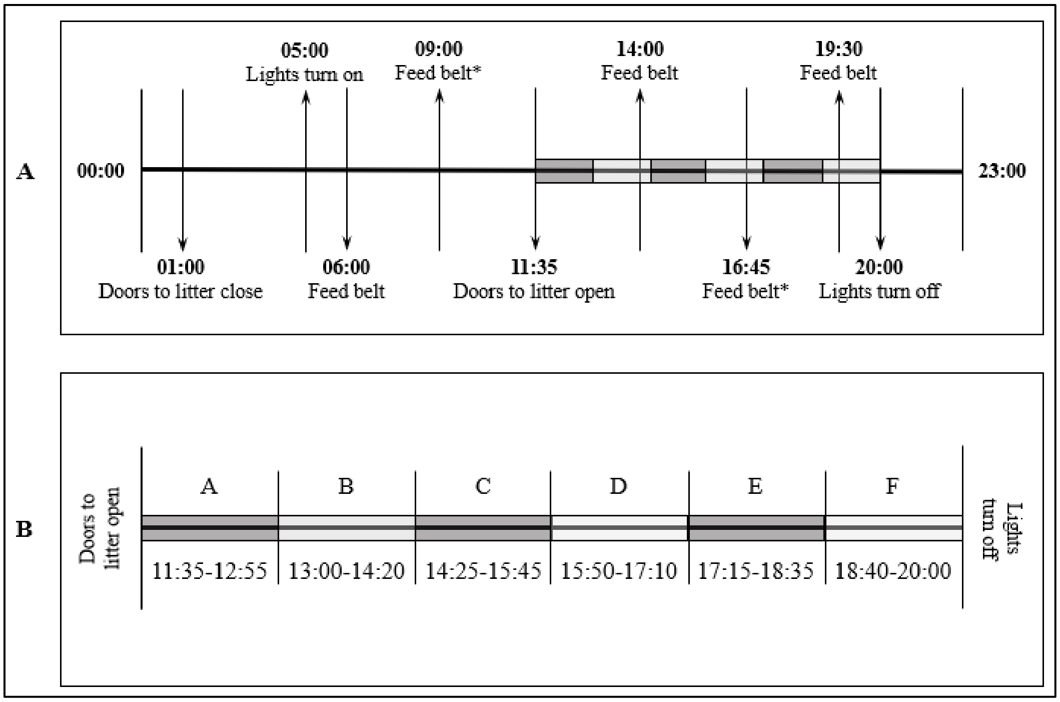

Figure 3.3. A. Daily schedule used throughout the study in all 16 aviary units. Lights turned on at 05:00 and shut off at 20:00 after dimming for 30 minutes. Hens had litter access from 11:35 until 01:00 daily, as denoted by the grey highlight; thus, hens could access litter for 8.5 hours while the lights in the system were on. Feed belts delivered feed three times a day at 06:00, 14:00, and 19:30. *Feed belts ran for approximately 10 seconds at 09:00 and 16:45 to entice hens into the system to feed between deliveries of fresh feed. **B.** Timeline expanding upon those hours each day that hens had access to litter and lights in the system were on. For ease of analysis, the time of day in each day of video footage was separated into 6 85-minutes time segments, A-F.

Video Decoding

A total of 3 trained human observers counted the number of hens occupying the open litter area as well as the number of hens actively dust bathing every 5 minutes. During each 5-minute scan, observers paused the video to count all hens on the open litter, including those dust bathing. Hens were counted as occupying the open litter area if they were on the floor and their bodies were at least one-third of the way past the outer perch (see **Figure 3.4**). Hens that were roosting on the outer perch at the time of the scan were not counted as being on the open litter area. Since it is difficult to accurately determine if a hen is dust bathing from a still image, observers watched approximately 10 seconds of video prior to each 5-minute scan to determine if a hen was actively dust bathing. If a hen was actively dust bathing during those 10 seconds but stopped dust bathing or moved out of view at the precise time of the scan, she was not counted as dust bathing (nor was she counted as being on the open litter area if she moved out of view). Using the "ICC" package in R, we found a high degree of reliability among the observers for the counts of hens on litter (0.79) and for counts of hens' dust bathing (0.89).

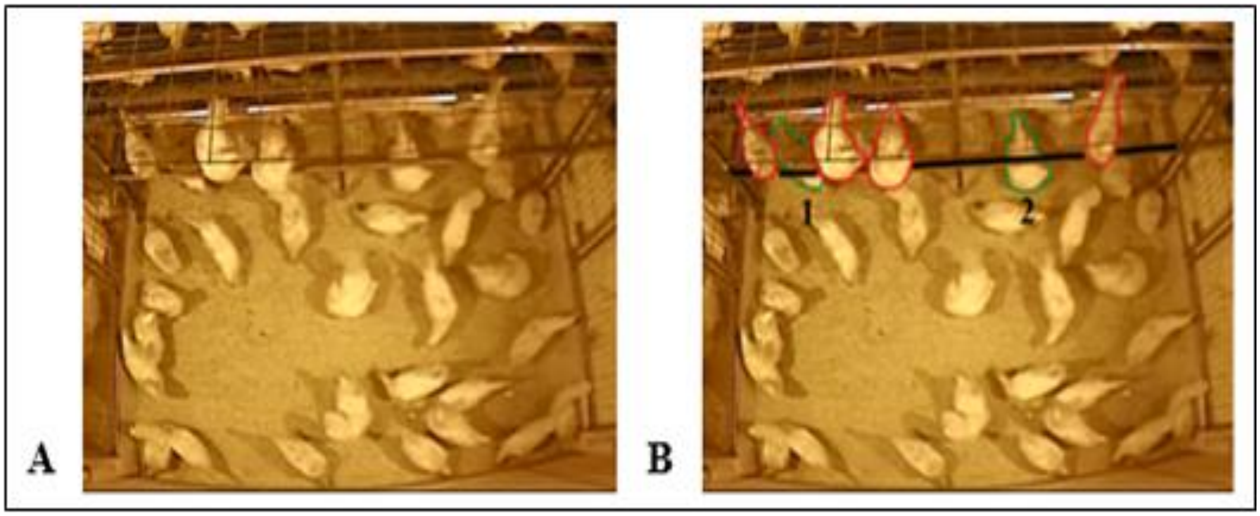

Figure 3.4. **A.** Example screen capture from video footage of white-feathered hens on open litter area. **B.** The black line emphasizes the location of the outer perch, which hens use to enter and exit the tiers in their aviary unit. Hens outlined in red are sitting on the outer perch at the time of the screen capture and are not counted as occupying the open litter area. Hens outlined in green are physically on litter, but only hen 2 would be counted as occupying the open litter area because her body is more than one-third of the way past the outer perch. All other hens that are not outlined would be counted as occupying the open litter area.

Statistical Analysis

Linear mixed models were used to analyze counts of hens on the litter and counts of hens' dust bathing for three consecutive days at two time periods (IM and AC). We ran a total of 4 models (2 time periods and 2 counts of hens): IM litter occupancy, IM dust bathing, AC litter occupancy, AC dust bathing. No statistical comparison was conducted between IM and AC.

Main effects for analysis included the strain of laying hen (BB, HB, DW, or W36), time segment (i.e., time of day broken into 6 equal segments, A-F), and day (either IM1-IM3 or AC1-AC3, depending on the data set). Furthermore, all possible 2-way interaction between strain, segment, and day were fitted as well. Random effects included room and various interactions thereof with strain, segment, and day in order to ensure proper specifications of experimental units or replication. Mean separation was examined further using Sidak post hoc analysis.

Results

IM: Litter Occupancy

During the IM period (the first 3 days of litter access for hens in aviaries), litter occupancy was influenced by a significant three-way interaction of strain by time by day (P=0.0002), as well as significant two-way interactions of strain by time (P<0.01), time by day (P<0.01), and strain by day (P=0.045). There were also significant main effects of strain (P=0.001), time (P=0.007), and day (P<0.01).

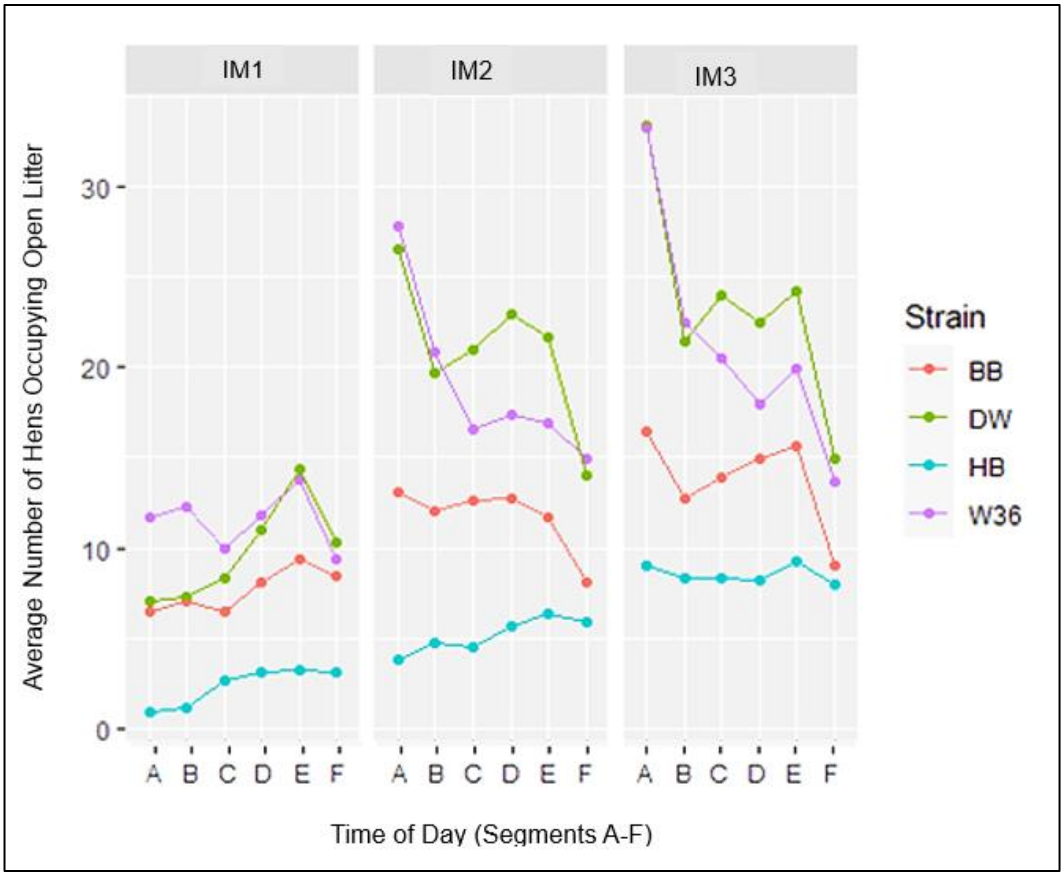

Figure 3.5. Average number of hens in open litter area for each strain during IM period (the initial 3 days after hens gain litter access in aviaries). BB and HB are brown-feathered strains; DW and W36 are white-feathered strains. Segments, or time of day, are located on the x-axis (each day constitutes 8.5 light hours of litter access; each segment accounts for 85 minutes (A 11:35-12:55, B 13:00-14:20, C 14:25-15:45, D 15:50-17:10, E 17:15-18:35, F 18:40-20:00)). Feed belts delivered feed within aviary tiers during time segment B.

Litter Occupancy Among Strains

Strains of the same feather color (white or brown) showed similar patterns of litter occupancy to each other across IM (**Figure 3.5**). Hens of both white-feathered strains, DW and W36, had similar mean counts of hens on litter on IM1 (P>0.42 for all time segments), IM2 (P>0.33 for all time segments), and IM3 (P>0.51 for all time segments). Brown-feathered strains, BB and HB, accessed litter in similar numbers to each other on IM1 (P>0.27 for all segments), though more BB hens occupied litter in segment A on IM2 than HB hens (approximately 13 hens versus 4 hens, respectively; P=0.04). However, other than segment A, litter occupancy was similar in both brown strains for the rest of IM2 (P>0.07 for segments B-F) and remained similar through all of IM3 (P>0.11 for all segments). Counts of brown-feathered hens, especially HB, were generally lower than counts of both white strains (**Figure 3.5**). BB hens tended to occupy litter in numbers above HB hens and below both white-feathered strains. Significantly more DW and W36 hens were counted on litter than HB hens in segments A through E on IM2 (P<0.01 for all comparisons), and IM3 (P<0.01 for all comparisons). There was no significant difference in the number of hens of all strains counted on the open litter area in segment F on any IM day (P>0.07 for all comparisons, except IM2 with more W36 hens than HB hens (approximately 15 hens versus 6 hens, respectively; P=0.04).

Day to Day Variation Within Strain

Overall, litter occupancy increased for all strains across IM, though counts of white-feathered hens increased more quickly than counts of brown-feathered hens (**Figure 3.5**). When looking at overall counts of hens on litter each day (all time segments), litter occupancy increased significantly from IM1 to IM2 in both DW hens (P<0.0001) and W36 hens (P=0.001). Both white-feathered strains maintained this level of litter occupancy from IM2 to IM3 (DW: P=0.41; W36: P=0.47). Brown strains did not show a significant change in litter occupancy from IM1 to IM2 (BB: P=0.09; HB: P=0.31), nor was there a significant difference in either strain from IM2 to IM3 (BB: P=0.52; HB: P=0.19). However, the overall number of brown-feathered hens counted on litter in IM3 (for all time segments) was significantly higher than the overall count in IM1 (P=0.008 for both BB and HB). Across time segments, there was greater variation in litter occupancy in white-feathered strains than in brown-feathered strains. The most pronounced change in litter occupancy was seen from IM1 to IM2 in time segment A when counts of white-feathered hens increased significantly (segment A IM1: 7.0 DW hens and 11.6

W36 hens versus segment A IM2: 26.5 DW hens and 27.8 W36 hens). Comparatively, counts of brown-feathered hens in time segment A did not increase as drastically (segment A IM1: 0.9 HB hens, 6.4 BB hens; segment A IM2: 3.8 HB hens, 13.0 BB).

IM: Dust Bathing

For dust bathing patterns in IM, there was a significant three-way interaction of strain by time segment by day (P=0.004), as well as significant two-way interactions of strain by time segment (P<0.01) and time segment by day (P=0.0002). There was also a significant effect of strain (P=0.0004) and of time segment (P=0.0004).

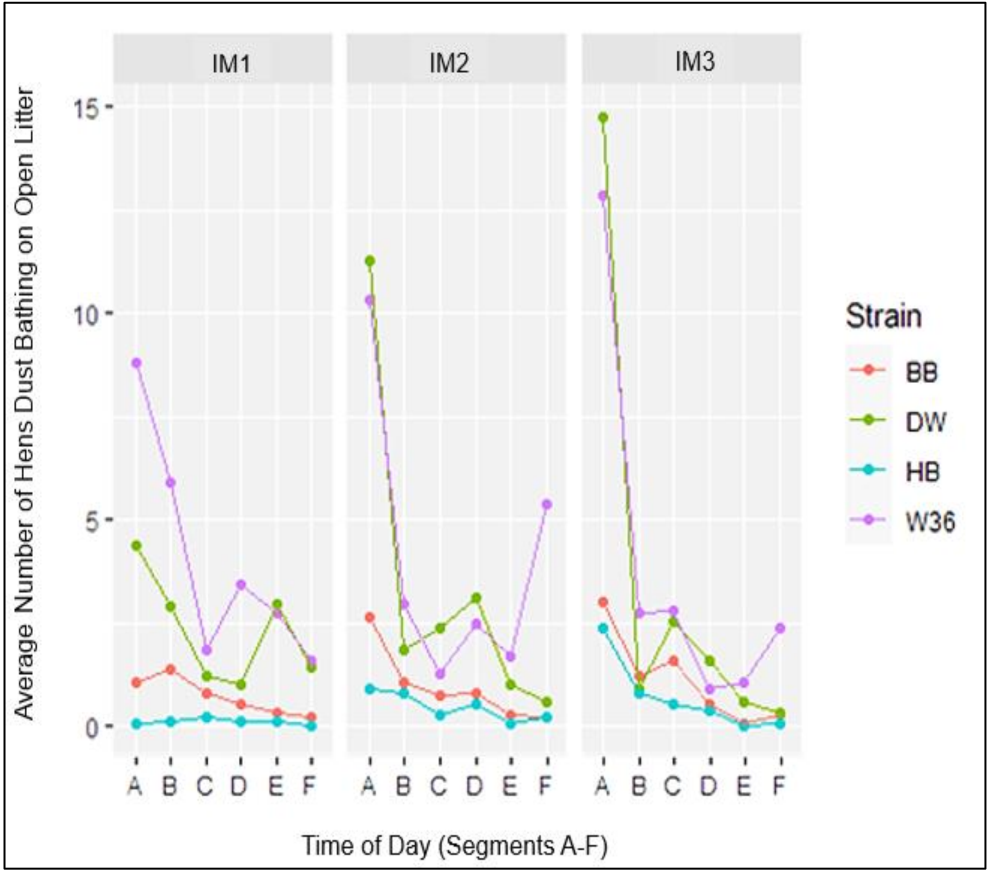

Figure 3.6. Average number of hens dust bathing in open litter area for each strain during IM period (the initial 3 days after hens gain litter access in aviaries). BB and HB are brown-feathered strains; DW and W36 are white-feathered strains. Segments, or time of day, are located on the x-axis (each day constitutes 8.5 light hours of litter access; each segment accounts for 85 minutes (A 11:35-12:55, B 13:00-14:20, C 14:25-15:45, D 15:50-17:10, E 17:15-18:35, F 18:40-20:00)). Feed belts delivered feed within aviary tiers during time segment B.

Dust Bathing Patterns Among Strains

Patterns of dust bathing differed depending on strain and time segment, with significant discrepancies between white and brown strains in segment A on all days in IM (**Figure 3.6**). In segment A on IM1, more W36 hens were observed dust bathing than any other strain (8.8 W36 hens versus 4.4 DW hens, 1.0 BB hens, and <1.0 HB hens; P<0.02 for all comparisons). By IM2, the number of W36 and DW hens dust bathing in segment A was comparable (11.3 hens versus 10.3 hens, respectively; P=0.91), and there were more white hens dust bathing at this time than brown hens (2.6 BB hens and <1 HB hens; P<0.0001 for all comparisons). This pattern continued in segment A of IM3, with W36 and DW hens dust bathing in similarly high numbers (14.8 W36 hens versus 12.9 DW hens; P=0.58) compared to low numbers of both brown strains (3.0 BB hens and 2.4 HB hens; P<0.0001 for all comparisons). The number of HB and BB hens dust bathing in segment A was comparable across all 3 days (IM1 P=0.9; IM2 P=0.64; IM3 P=0.97). With minor variation, there were no differences in the number of hens of each strain dust bathing for the remainder of each day (P>0.40 for all comparisons, except for more W36 hens than both brown strains in segment B on IM1 (5.9 W36 hens versus 1.4 BB hens and <1 HB hen; P<0.01 for all comparisons) and in segment F on IM2 (5.9 W36 hens versus 1.4 BB hens and <1 HB hen; 1.6 W36 hens versus <1 BB hen and <1 HB hen; P<0.01 for all comparisons)).

Day to Day Variation Within Strain

When looking total counts of hens' dust bathing averaged across all time segments each day, there was no significant difference in the overall number of hens within each strain dust bathing during IM (**Figure 3.6**, P>0.31 for all comparisons).

AC: Litter Occupancy

In AC, litter occupancy was influenced by significant two-way effects of strain by time (P<0.01) and time by day (P=0.004), as well as a significant effect of time (P<0.01).

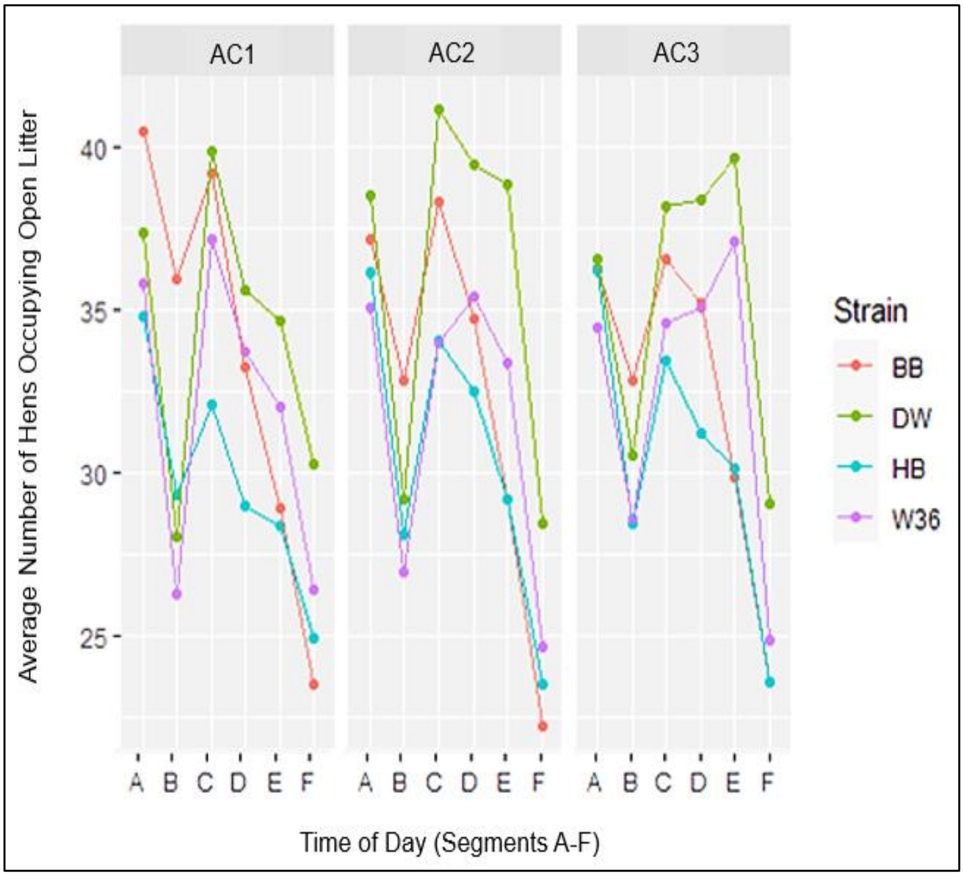

Figure 3.7. Average number of hens in open litter area for each strain during AC. BB and HB are brown-feathered strains; DW and W36 are white-feathered strains. Segments, or time of day, are located on the x-axis (each day constitutes 8.5 light hours of litter access; each segment accounts for 85 minutes (A 11:35-12:55, B 13:00-14:20, C 14:25-15:45, D 15:50-17:10, E 17:15-18:35, F 18:40-20:00)). Feed belts delivered feed within aviary tiers during time segment B.

Litter Occupancy Among Strains

Hens of all 4 strains showed similar patterns of litter occupancy across AC, and the number of hens counted on the open litter area was fairly consistent day-to-day (**Figure 3.7**). However, the number of hens occupying litter varied depending on the time of day. In time segment A, hens of all strains accessed litter in similarly high numbers (AC1: 34.8 HB hens, 35.8 W36 hens, 37.3 DW hens, and 40.4 BB hens; AC2: 36.1 HB hens, 35.0 W36 hens, 38.5 DW hens, and 37.1 BB hens; AC3: 36.2 HB hens, 34.4 W36 hens, 36.5 DW hens, and 36.3 BB hens (P>0.24 for all comparison)). There was a noticeable decrease in birds on litter for all strains in segment B (P>0.10 for all comparisons, note: the feed belt ran during segment B), before an increase again in segment C (P>0.10 for all comparisons). Litter occupancy was comparable across all strains through segment D (P>0.08 for all comparisons). There was slight variation in segment E, with more DW hens counted on litter than both BB and HB hens on days AC2 (P=0.02) and AC3 (P<0.02). There were also more BB hens occupying the open litter than W36 hens in segment B on AC1 (36 compared to 26, respectively; P=0.02). All strains had similarly low counts in segment F (P>0.13 for all comparisons).

Day to Day Variation Within Strain

In general, hens of all strains maintained a consistent daily pattern of litter occupancy in all 3 days of AC (**Figure 3.7**). There were a few instances of variation, such as more BB hens accessing litter in segment A on AC1 compared to AC3 (P=0.03), and DW hens had higher litter occupancy in segment D on AC2 compared to AC1 (P=0.03). In segment E, however, fewer DW hens accessed litter on AC1 than AC2 (P=0.03) or A3 (P=0.001). There were also fewer W36 hens counted in segment E on AC1 than AC3 (P=0.05).

AC: Dust Bathing

Counts of hens dust bathing in AC were influenced by a significant two-way interaction of strain by time segment (P<0.01) as well as significant effects of strain (P=0.0003) and time segment (P<0.01).

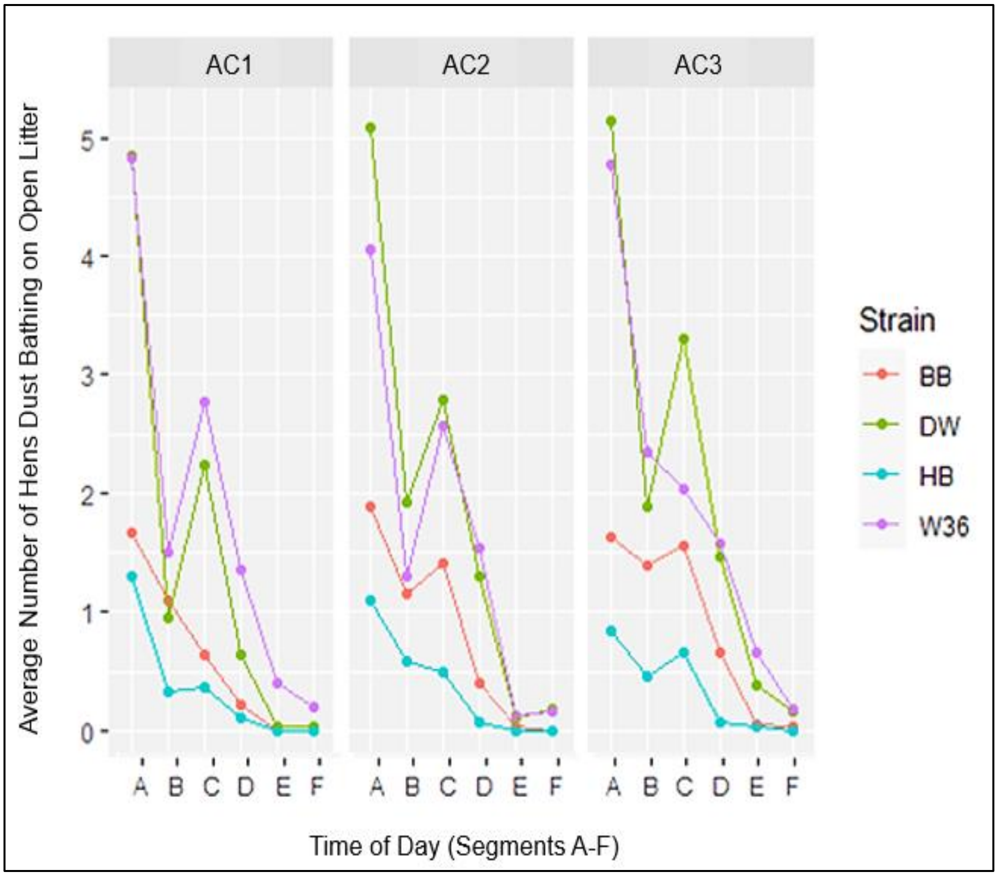

Figure 3.8. Average number of hens dust bathing on open litter area for each strain during AC period. BB and HB are brown-feathered strains; DW and W36 are white-feathered strains. Segments, or time of day, are located on the x-axis (each day constitutes 8.5 light hours of litter access; each segment accounts for 85 minutes (A 11:35-12:55, B 13:00-14:20, C 14:25-15:45, D 15:50-17:10, E 17:15-18:35, F 18:40-20:00)). Feed belts delivered feed within aviary tiers during time segment B.

Dust Bathing Patterns Among Strains

More white-feathered hens than brown-feathered hens were counted dust bathing in AC (**Figure 3.8**). Similar to IM, differences in patterns of dust bathing were most significant in segment A with more white-feathered hens dust bathing in segment A than brown-feathered hens on all 3 days in AC (DW and W36 daily average 5 hens; BB and HB daily average 1-2 hens; P<0.0001 for all comparisons). Both white strains also dust bathed more than both brown strains in segment C each day (P<0.001 for all comparisons), though this difference was more pronounced in HB hens than BB hens (DW and W36 daily average 2-3 hens; BB daily average 1 hen; HB daily average <1 hen). All strains had comparably low counts of dust bathing in segment E and F each day in AC (P>0.72 for all comparisons).

Day to Day Variation Within Strain

Hens of all strains maintained fairly consistent patterns of dust bathing in AC (**Figure 3.8**) though there were more DW dust bathing on AC2 and AC3 compared to AC1 (P<0.04 for both days).

Discussion

The purpose of this study was to examine patterns of litter occupancy and dust bathing in aviary-housed laying hens following periods of litter restriction. We used hens of 4 genetic strains (BB and HB: both brown-feathered hens; DW and W36: both white-feathered hens). Hens were observed for 3 consecutive days at 2 different time periods: IM, immediately following hens' initial access to litter in aviaries (at 26 WOA), and AC, 2 weeks after hens began experiencing daily litter access (at 28 WOA). We hypothesized that white-feathered hens would occupy litter and dust bathe in greater numbers compared to brown-feathered hens, and that white-feathered hens would acclimate to litter access in aviaries more quickly than the brown-feathered hens. Given that all hens in the present study were reared in the same facility with identical furnishings (i.e., litter and perches), it is likely that differences among strains regarding use of the open litter area are related to genetic predisposition.

Litter Occupancy

The overall number of hens counted on the open litter area increased in all 4 strains throughout IM. These results agree with previous findings that hens access litter gradually following a period of complete restriction (Alm et al., 2015), and suggest that hens in the current study were starting to acclimate to the daily schedule of aviary doors opening and accessing litter on the floor level. However, as expected from prior research (Ali et al., 2016), patterns of litter occupancy differed significantly depending on strain. Counts of white-feathered hens on the open litter area were generally higher than those of brown-feathered hens. Hens of the 2 white strains also showed greater variation in litter occupancy each day compared to hens of the 2 brown-feathered strains. The fact that significantly more white-feathered hens accessed litter on the second day compared to the first day may indicate these hens were more quickly adapting to the schedule of daily litter access and were anticipating doors opening, or that these strains were generally more motivated to access the litter resource compared to the brown-feathered strains. White-feathered hens have been reported as more socially motivated than brown-feathered hens (Dudde et al., 2018), and it is also possible that the sight of other hens occupying the litter was more enticing to white strains than to brown strains.

By AC, patterns of litter occupancy appeared more stable within and across strains and hens of all strains had a similar pattern of moving on and off the open litter area depending on the time of day. Minor discrepancies in these patterns are likely due to random variability each day, especially given that all strains had similarly low numbers by time segment F. The lack of significant daily variation in litter occupancy suggests hen of all strains had acclimated to the daily schedule of aviary doors opening for litter access and were able to develop a consistent pattern of litter occupancy within the 2-week period between IM and AC. However, visual analysis of litter occupancy in IM (**Figure 3.1**) compared to AC (**Figure 3.3**) suggests different rates of acclimation between white and brown strains. Hens of brown strains were slower to initially access litter and had lower but more consistent litter occupancy rates within and across days in IM. In addition, brown strains were not as influenced by time segment as white strains, especially HB hens who showed the least significant variation in litter occupancy over the day.

However, hens of the 2 brown strains were eventually able to establish a pattern of litter occupancy during the 2 weeks between IM and AC. These results were anticipated based on prior research in our lab (Ali et al., 2016), as well as growing evidence of genetic variability in spatial distribution and behavior patterns (Kozak et al., 2016; Giersberg et al., 2019). In addition, brown-feathered hens have been documented to need at least one week to acclimate to multi-tiered systems (Cheon et al., 2020), and it has been suggested that white-feathered hens are better suited for multi-tiered aviaries than brown-feathered hens due to their greater utilization space and resources (Purdum et al., 2020).

It is noteworthy that the time of day influenced litter occupancy in all strains. Several studies have established that hens maneuver to specific resources for commodity-dependent behaviors (Carmichael et al., 1999; Mishra et al., 2015; Campbell et al., 2016). Results from the current study show an obvious and nearly universal decrease in litter occupancy for all strains in time segment B, which is likely indicative of hens being drawn back into tiers in response to the cue of the feed belt delivering fresh feed. Hens in commercial production systems are often housed in large groups and may experience crowding during periods of peak movement where multiple conspecifics attempt to access the same area at the same time (such as a hens' attempting to eat freshly delivered feed from a limited amount of feeder space).

Patterns of Dust Bathing

Patterns of dust bathing differed between brown-feathered strains and white-feathered strains in both IM and AC. White-feathered strains (DW and W36) generally dust bathed in higher numbers than brown-feathered hens (BB and HB). These results are consistent with other findings (Grebey et al., 2020) and may indicate white-feathered hens were more inclined to dust bathe at the same time as other flock mates or that their motivation to dust bathe was higher overall than in brown-feathered hens. However, in both IM and AC, hens of all strains had the highest counts of dust bathing in time segment A, the first 85 minutes they could access litter each day (between ~11:35-12:55). The high proportion of dust bathing events counted in segment A may suggest that hens desired to dust bathe during those hours when they were enclosed in tiers and may also be indicative of rebound effect. Past research using similar aviaries and management schedules as those in the current study also reported high counts of hens' dust bathing soon after aviary doors opened at 11:00 and continuing for the first few days following initial litter access (Campbell et al., 2016).

It is commonly considered that dust bathing activity peaks in the afternoon (Vestergaard, 1982), and it has been suggested that hens may be more motivated to lay eggs during early to mid-morning hours than they are to dust bathe (Oliveira et al., 2019). However, the time of day with which we base many assumptions on hens' behavioral preferences are ultimately regulated by human-controlled husbandry practices. For example, humans decide when lights turn on and off and the availability of certain resource, and this control may influence hens' perception of time of day or behavior patterns. This variability is further emphasized by differing methods of observing and recording behavioral data. For instance, Campbell and colleagues (2016) reported peaks in dust bathing activity in either late morning ("morning" was defined as 11:00-13:00) or afternoon ("afternoon" was defined as 15:00-17:00), depending on the flock studied at the time. Authors (Campbell et al., 2016) noted that lights in the aviaries turned on at 06:00 and hens were unable to access litter until 11:00 each day (a total of 5 light hours within tiers to promote oviposition in designated nests). In another study using a multi-tiered aviary configuration in which lights turned on at 07:00 and doors to litter consistently remained open, litter occupancy was reported to peak at 08:00, with more hens on litter than in any other location, and some strains maintained this peak until 12:00 (Purdum et al., 2020). Both aforementioned studies provide valid and useful insight into how hens use litter in aviaries, and also highlight the need to consider observational methods and management practices in tandem with results. In addition, notable differences in the timing of oviposition between white- and brown-feathered strains have been reported (Tumova et al., 2017; Villanueva et al., 2017), and it is probable that the motivation to dust bathe also varies among strains at different times of the day.

Additionally, though there was no statistical comparison between IM and AC data sets, a visual analysis of dust bathing patterns (**Figure 3.2** and **Figure 3.4**) indicates that average counts of dust bathing decreased from IM to AC in all strains. For example, the highest number of dust bathing events was seen in DW hens in time segment A on IM3 (~ 15 hens), while in AC, DW hens again dust bathed the most in segment A on AC3 (~ 5 hens). From this visual analysis, it could be theorized that hens experienced a rebound effect from prolonged restriction from litter and that hens may have been frustrated during the 9-week period of time in which they were fully enclosed in tiers. However, the motivations behind dust bathing are complex and involve both internal and external cues (Hemsworth and Edwards, 2021), and more research should be done to assess the impacts of long-term litter restriction.

Limitations

We were unable to see the entirety of the litter area and hens were likely occupying the litter area underneath the tiers. Counts of hens could have been influenced by the possibility that hens moved in and out of view across multiple scans, including multiple scans within the same dust bathing bout. However, with the video available it would have been impossible to follow specific hens once they moved underneath the tiers (and out of view). In addition, video quality was not as sharp as it could have been and it was challenging to obtain an accurate count of hens on litter at certain points (especially when large proportions of hens were occupying litter and dust bathing at the same time). Additionally, the videos did not have audio capacity and it is likely that hens were influenced by sounds that we were unaware of within the systems. Previous research has identified that hens within the same flock do not behave in a uniform manner, and there are subpopulations of hens that maneuver among various resources throughout the day as well as those that may never fully explore the system (Sibanda et al., 2020). In the present study, we were unable to track individual hens and it is possible that the same hens were consistently accessing litter while some rarely left the tiers. Future studies should examine behavior patterns of individual hens within the greater flock.

Conclusion

Hens of different genetic strain behave differently when initially accessing litter in multi-tiered aviaries. Following a 9-week period of complete litter restriction (i.e., hens were relegated to aviary tiers, away from litter, until they reached peak lay), hens of two white-feathered strains (DW and W36) occupied litter in greater numbers and at a faster rate than hens of two brown-feathered strains (BB and HB). After 2 weeks, hens of all strains acclimated to the daily schedule of litter access, and litter occupancy rates were constant both within and among strains in AC. Overall, there were more white-feathered strains dust bathed at the same time compared to brown-feathered strains, though all strains showed a tendency to dust bathe soon after gaining access to litter each day. These results suggest that management tactics, like limiting when hens can access litter in cage-free systems, may prevent hens from performing behaviors based on their own preferred temporal pattern. Additionally, it important to note that standards for cage-free egg production differ internationally and many countries forbid the use of restrictive management practices. In Australia, for example, hens must be given continuous litter access no later than 3 weeks after their initial placement in adult housing systems (AU Poultry Welfare Standards and Guidelines, 2022). To ensure results can be extrapolated for global use, future studies in aviaries should examine hen behavior both with and without restrictive practices.

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CHAPTER 4.

UNFLAPPABLE: WING FLAPPING OF AVIARY-HOUSED LAYING HENS FOLLOWING SPATIAL RESTRICTION

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Abstract

Commercial housing systems are becoming larger and more complex to accommodate more positive hen behaviors, including wing flapping. Though ethologists do not fully understand the motivations behind wing flapping, research indicates hens need substantial three-dimensional space to flap their wings and housing system configurations likely influence this behavior. This was a pilot study examining the timing and frequency of wing flapping among 4 laying hen strains (2 white-feathered and 2 brown-feathered; 576 hens/strain) housed in commercial-style multi-tiered aviaries. Hens were separated by strain into 16 Natura60 aviary units within 4 rooms (4 units/room, 1 unit/strain/room). Each unit contained a wire enclosure above a litter-covered floor; enclosures were comprised of 3 tiers throughout which hens could access feed, water, perches, and nests. Doors on the bottom tier in each unit opened and closed to determine when hens could access litter; hens were confined within wire enclosures from 01:00-11:35 daily (~8.5 hours of litter access before lights turned off at 20:00). Ceiling-mounted cameras in each unit captured hen behavior on litter. Observers watched 1 day of video footage when hens were 28 weeks old and recorded every wing flapping event when the hen was not locomoting. For analysis, time of day (11:35-20:00) was broken into equal time segments, A-F. A linear mixed model compared counts of wing flapping among the 4 strains. Main effects were strain and time segment; random effects were room and unit location. We found significant effects of strain ($P=0.0126$) and time ($P<0.001$) on performance of wing flapping. Brown-feathered hens flapped their wings more than white-feathered hens. More wing flapping events were counted in segment A (11:35-12:55) compared to any other time segment, suggesting hens' daily confinement within wire enclosures may have influenced their motivation to wing flap once they had room to do so.

Introduction

Egg-laying chickens used in commercial animal agriculture have historically been housed in conventional cages, which do not provide enough room for birds to perform many behaviors (Hemsworth, 2021). Consumers in many countries have indicated concern for hen welfare in restrictive housing systems (Gautron et al., 2021; Sinclair et al., 2022), and global egg production is trending away from cages in favor of larger and more complex cage-free housing systems. The use of cage-free systems should ideally provide hens with more freedom of movement and enrichment to allow for the performance of behaviors hindered in cages (Hemsworth and Edwards, 2020). However, it is imperative to consider hens' performance of these behaviors in the context of intricate configuration of cage-free systems and other conditions on commercial farms, like potential effects of surrounding conspecifics, or management and husbandry practices.

Many features commonly seen in cage-free systems have been scientifically studied and implemented with the intent of improving hen welfare by allowing hens to perform ecologically important behaviors (like the provision of perches to allow for roosting), and recommendations for space and resource allocations are often specific numerical values (i.e., at least 6 inches of usable linear perch space per hen). Some other cage-free spacing demands are based on hens' ability to perform a behavior. In the United States, for instance, farm animal welfare legislation from California, Massachusetts, Michigan, and Rhode Island stipulates that hens in cage-free systems must be able to fully extend their limbs (as would occur during wing flapping) without touching the side of their enclosure (Animal Welfare Institute, 2023). Wing flapping is a dynamic behavior, and research on space use found that hens require a considerable amount of both vertical and horizontal space to flap their wings (Mench and Blatchford, 2014), and that different strains require different amounts of space (Riddle et al., 2018). However, applied ethologists do not yet fully understand the causation or function of wing flapping.

Chickens are heavy-bodied birds in the Galliformes order, and though Gallinaceous species have wings, true aerial flight is not a dominant form of locomotion. Instead, many Galliformes locomote primarily using their legs to walk or run and their wings to assist with certain behaviors (Tran et al., 2022). Red junglefowl, the extant wild ancestor of domesticated chickens, roost off the ground in trees or other foliage and use their wings to assist with vertical movement and balancing when looking for a satisfactory perch, or during escape behavior to move away from a perceived threat (Desta, 2019). Domestic laying hens have maintained many behavioral similarities to red junglefowl (Ferreira et al., 2022), including the utilization of their wings when navigating three-dimensional environments. Hens in cage-free systems employ the use of their wings to assist with jumping up or down from various tiers or perches, or when moving up a steep incline (often referred to as “wing-assisted incline running” or WAIR) (LeBlanc et al., 2018).

Chickens and junglefowl use their wings for non-locomotive purposes as well, including during social interactions like sparring among conspecifics and courtship (Kruijt, 1964), and during play behavior, which has been studied in young broilers (Baxter et al., 2019; Rayner et al., 2020). It is also suggested that chickens stretch or flap their wings for the purposes of maintaining or improving physical comfort (Nicol, 1989), and many ethological studies categorize wing flapping as a comfort behavior and a behavioral indicator of positive welfare (Sokolowicz et al., 2020; Rayner et al., 2020). There may be several reasons as to why hens flap their wings, and more research is needed to understand the motivations behind the behavior. Currently, producers using cage-free systems have no practical means to accommodate hens’ ability wing flap. As an initial investigation, we conducted a pilot study to examine when and how often hens flap their wings using pre-recorded footage of white-feathered and brown-feathered strains of laying hens housed in multi-tiered aviaries.

Materials and methods

Overview of Study Design

This study used 2,304 laying hens of 4 genetic strains: 2 brown-feathered, Hy-Line Brown [**HB**], Bovans Brown [**BB**], and 2 white-feathered, Hy-Line W36 [**W36**], and DeKalb White [**DW**] (n=576 of each strain). Several papers on have been published based on research on the same 4 genetic layer strains and video footage used in the current study (e.g., Ali et al., 2016; Grebey et al., 2020; please see Ali et al., 2020 for a review of findings). These studies generally focused on variation in resource use and spatial distribution among white-feathered and brown-feathered hens in commercial aviaries. The current study focused only on the behavior of wing flapping among the different strains, which had not been examined in past studies.

Housing System

Hens were reared and housed at the Michigan State University Poultry Teaching and Research Center in East Lansing, MI. Hens were separated by strain throughout their lay cycles. At 17 WOA, hens were moved from rearing pens to Natura60 aviary units (Big Dutchman, MI). There were 16 aviary units, distributed equally among 4 rooms (each room held 4 separate aviary units). Each of the 4 strains were stocked in one unit within each room, and the location of each strain was different in each room to avoid potential location bias. All aviary units had the same configuration, consisting of a wire enclosure comprised of three tiers, suspended above the floor. The floor level served as a litter area for each aviary unit and encompassed the open area directly in front of the tiers as well as the area directly underneath the tiered enclosures. Each tier had an internal ceiling height of 61 cm, and the enclosures sat approximately 50.80 cm above the litter area. Resources such as feeders, drinkers, and perches were interspersed among the tiers, and tier 3 contained nest boxes. See Ali et al. (2016) for additional details on the housing or management of the hens on this study.

Video cameras were fastened to the ceiling above the open litter area to record hen behavior at different ages, based on the initial study design (see Ali et al., 2016). Cameras were unable to capture the litter area that extended underneath the tiered enclosures. However, based on prior research on vertical space requirements for wing flapping of 49.5 ± 1.8 cm ((±2.54 cm to avoid touching sides of the enclosure); Mench and Blatchford, 2014), it is unlikely that hens would be able to fully flap their wings underneath the enclosure used in the current study due to the height of the internal ceiling (50.8 cm vertical space from floor to bottom of lowest tier).

System Management

Hens were unable to access litter when first placed in aviaries, being confined to the tiered enclosure until peak egg production was reached at 26 WOA. After this point, doors on the lowest tier opened daily at 11:35 to allow hens access to the litter area; a perch was located near doors to assist hens with jumping between the lowest tier and the floor. Lights in each room turned on at 05:00 and began dimming 30 minutes prior to shutting off at 20:00. This schedule allowed hens to access litter for approximately 8.5 hours of the light period. At 01:00, when the hens were in the tiers to roost for the night, doors to the litter closed. Feed belts within the tiers delivered feed at 06:00, 14:00, and 19:30, and ran for 10 seconds at 09:00 and 16:45 to encourage hens to eat. Researchers collecting data for other aspects of the project generally walked through each aviary unit twice, around 14:30 and 17:30.

Video Decoding and Counts of Wing Flapping

This study used video footage when hens were 28 WOA, after they had acclimated to daily litter access in aviaries. Trained human observers decoded hen behavior during the same 8.5-hour period that hens in each unit had litter access and the lights were on (11:35-20:00). Since wing flapping has a short duration and we did not want to miss instances of the behavior, we used continuous observation methods to decode a total of 136 hours of footage (8.5 hours x 16 aviary units). Future research should ideally examine sampling strategies to capture wing flapping behavior across multiple days (Daigle and Siegford, 2014).

Footage was divided into 5-minute segments to assist in decoding and analysis. Observers watched the entirety of each 5-minute segment (i.e., 11:35-11:45; 11:45-11:50) and, using a tally counter, recorded every instance a stationary wing flapping event on the open litter area. Our definition of a stationary wing flapping event (adapted from Riddle et al., 2018) was that a hen was standing upright in a stationary position (i.e., not actively moving; a hen could take a step or two for balance during the flapping event but generally remained in place) while extending both of her wings away from her body at least one time. In this case, a stationary wing flap is different from wing-assisted locomotion when the hen uses her wings to help her move through her environment (LeBlanc et al., 2018). See **Figures 4.1** and **4.2** for examples of white and brown hens wing flapping while stationary. Using the “ICC” package in R, reliability among the observers was found to be 0.8 for counting wing flapping events.

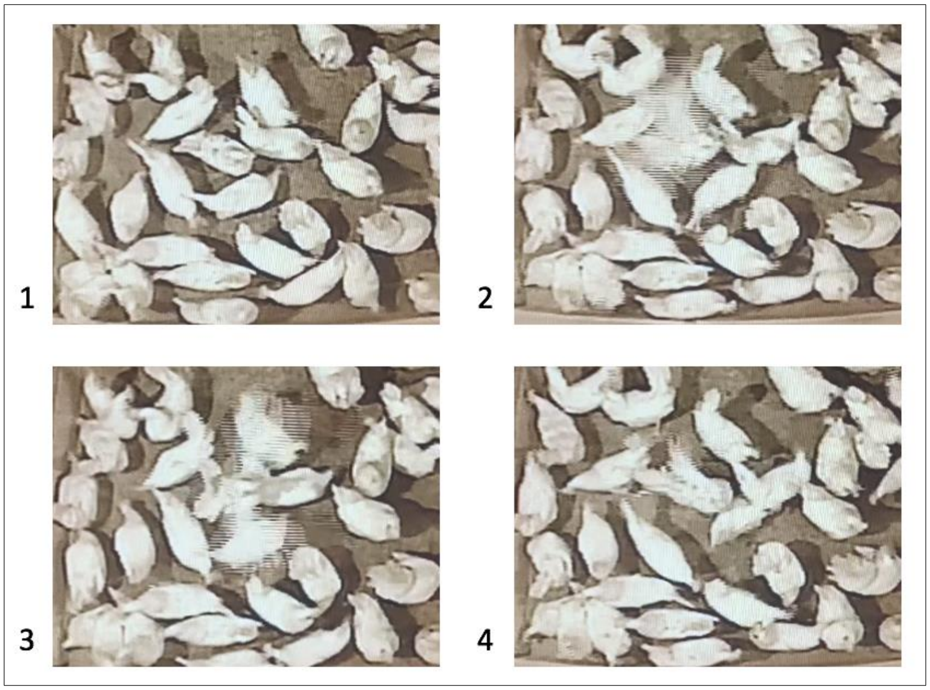

Figure 4.1. Sequence of a white-feathered hen standing stationary while wing flapping among conspecifics on the open litter area in a multi-tiered aviary; wings are raised up above and over the surrounding hens.

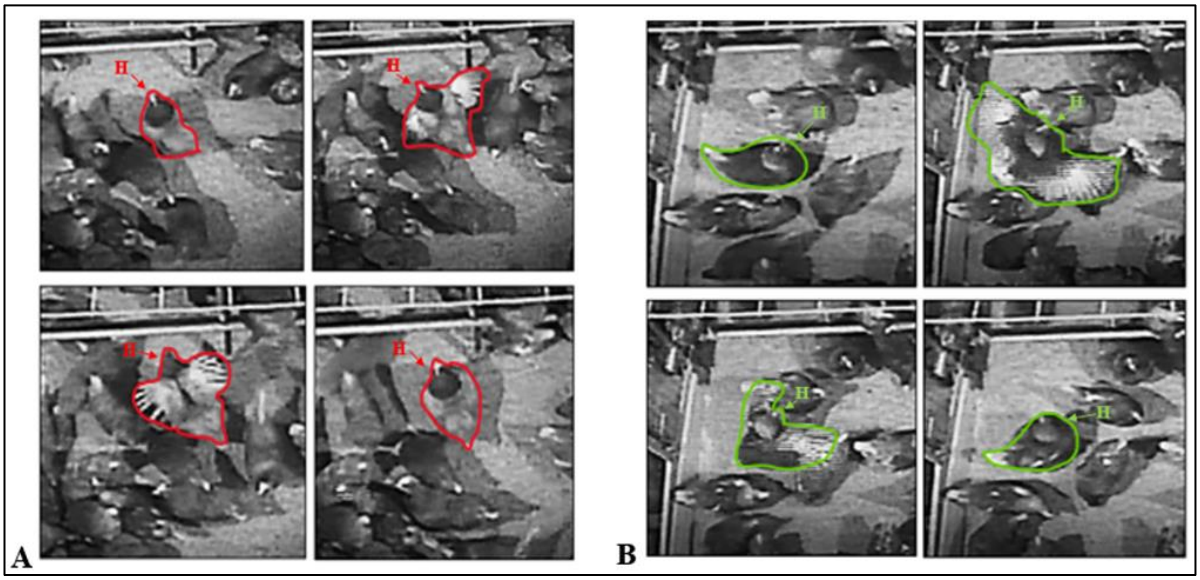

Figure 4.2. A. Image outlining a brown-feathered hen raising her wings; H indicates location of the hen's head. As these wings are not fully extended and never extend away from the hens' body, it would not count as a stationary wing flapping event. **B.** Example of a brown-feathered hen standing stationary while wing flapping; H indicates location of the hen's head. The wings are fully extended away from the body at least one time.

Statistical Analysis

We ran a linear mixed model to compare counts of wing flapping over the course of one day (8.5 hours) among the 4 strains. Main effects for analysis included strain (BB, HB, DW, W36) and time segment (i.e., the time of day broken into 6 equal segments, A-F.) Random effects were the room number and pen (or the location of the strain's unit within each room). Mean separation was examined further using Sidak post hoc analysis. Descriptive statistics are also presented; data collected by Grebey et al., 2020 were reutilized in the current study to provide approximate litter occupancy during the same 8.5 hours of video footage.

Results and discussion

This study was a preliminary examination of the timing and frequency of wing flapping in 2 brown-feathered and 2 white-feathered layer strains. We found a significant effect of time ($P<0.001$) as well as a significant effect of strain ($P=0.0126$) on performance of wing flapping (**Figure 4.3** and **Table 4.1**).

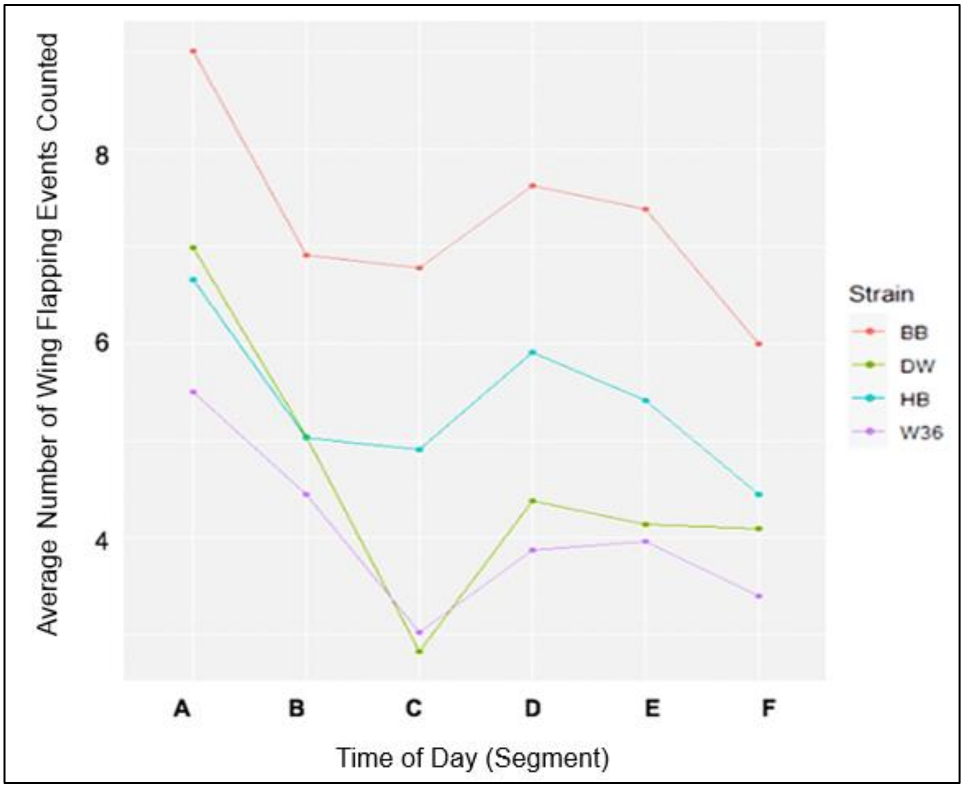

Figure 4.3. Graph depicting average number of stationary wing flapping events in each time segment for each of the 4 strains (white-feathered strains: DW and W36; brown-feathered strains: BB and HB). Each time segment (A-F) represents 85 minutes, for a total of 8.5 hours when hens had access to litter and system lights were on.

	A 11:35-12:55	**B** 13:00-14:20	**C** 14:25-15:45	**D** 15:50-17:10	**E** 17:15-18:35	**F** 18:40-20:00
DW	0-20 6	0-17 4	0-10 2	0-12 4	0-13 3	0-11 3
W36	1-18 5	0-16 4	0-9 3	0-15 3.5	0-11 3.5	0-10 3
BB	1-25 8	0-21 6	0-25 6	2-21 6.5	2-19 7	0-17 5
HB	0-25 5.5	0-18 4	0-21 4	0-16 5	0-20 5	0-14 4

Table 4.1. Range and median counts of stationary wing flapping events in each time segment (A-F) for each strain (white-feathered strains: DW and W36; brown-feathered strains: BB and HB). Each time segment (A-F) represents 85 minutes, for a total of 8.5 hours when hens had access to litter and system lights were on.

Timing of Wing Flaps

More hens of all strains were counted wing flapping in time segment A (11:35am-12:55pm) compared to any other time segment. This result may be explained by restrictive management practices, as hens' daily confinement within tiers may have influenced their motivation to wing flap once they had room to do so (Nicol, 1987). When doors to litter open, not only do hens obtain access to a desirable resource, but also stocking density within the system decreases and hens acquire more usable floor space as well as more vertical space than is available within the tiers (Campbell et al., 2016). Consideration should be given to the influence of temporary confinement on hens' performance of wing flapping. If hens are physically prevented from flapping their wings at desired times, it is possible that multiple hens may simultaneously flap at once (or within a short span of time) once they have room to do so. This could lead to additional questions as to whether concentrated bouts of wing flapping by many birds are due to rebound, social facilitation or temporal patterns that need to be investigated more directly. Additionally, even if a hen did flap her wings within the tiers, she may not be able to do so successfully without touching their enclosure, the furnishings within, as stipulated by cage-free legislation. The configuration of cage-free structures themselves may also influence hens' freedom of movement (Nannoni et al., 2022). In an unrestrictive enriched environment, it was found that ISA brown hens performed the majority of their wing flaps in the corridor between various resources that did not contain any furnishings (Mishra et al., 2005). Authors (Mishra et al., 2005) suggest this behavior requires a substantial amount of open space (and what a hen perceives as "enough room" may differ from what we assume).

Overall counts of wing flapping events appear low given the number of hens in each aviary; however, our methods of analysis may influence the interpretation of results. As seen in

Table 4.2, median counts of wing flaps within time segments are much higher than average counts (e.g., the median count of wing flapping events for HB hens in times segment A was 5.5, but the maximum number of flaps counted was 25 in the same segment). **Figure 4.3** shows patterns of wing flapping overlayed with average counts of litter occupancy (collected by Grebey et al., 2020). It is important to note that, although the frequency of wing flapping varies, all strains maintain at least some performance of the behavior across time segments, and this indicates that at least some hens within a flock are motivated to wing flap for one reason or another throughout a day. However, it is also possible that the same hens were counted flapping their wings. Due to the fact that hens in the current study were indistinguishable from one another, and that hens could move out of view of video cameras, there is no way of knowing that all stationary wing flaps counted came from different hens. Future studies should examine the frequency of wing flapping in individual hens within a flock, as well as flock behavior overall.

Strain Differences

Hens of both brown strains flapped their wings more than hens of both white strains. This finding was unexpected based on prior studies that suggest white-feathered hens show more synchrony during dynamic behaviors than brown-feathered hens (Ali et al, 2016; Grebey et al., 2020). Further, white-feathered hens have lower wing loading (ratio of weight to surface area of wings) compared to brown-feathered hens (LeBlanc et al., 2018), and white-feathered hens perform more wing-assisted behaviors than brown-feathered hens (Pufall et al., 2021). Interestingly, Riddle and colleagues (2018) reported that the area required for white-feathered hens to wing flap was greater than that of brown-feathered hen; and authors commented that white strains, though generally more narrow-bodied than brown strains, may have larger wingspans or extend their wings more fully than brown-feathered hens. In the current study, hens of both brown strains, especially BB, may have been more motivated to wing flap than hens of white strains. It is also possible that the larger-bodied brown strains show a greater rebound effect once litter access is available because they perceive tiers to be more constricting to their ability to behavior than smaller-bodied strains.

Future Research

Results from the current study indicate a higher degree of simultaneous wing flapping in brown-feathered hens compared to white-feathered hens; however, more research should be done before definite strain differences can be identified. We only examined one day of video footage, and it would be important in follow-up studies to observe hen behavior over multiple days to assess if wing flapping has a diurnal pattern. Research should also focus on any effects of group size or stocking density on hens' performance of wing flapping. Further, studies may want to investigate wing flapping in different areas of cage-free systems, such as within aviary tiers. Mench and Blatchford (2014) calculated that current spacing standards are insufficient if more than one hen at a time must be able to wing flap. In a separate study, we are utilizing depth cameras to assess the amount of vertical space a hen occupies while wing flapping. Determining more precise three-dimensional space requirements for dynamic behaviors, like wing flapping, will help egg producers and aviary manufacturers to better manage hens and configure systems to improve welfare and accommodate behavioral requirements in a practical manner.

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CHAPTER 5.

USING DEPTH CAMERAS TO ASSESS VERTICAL SPACE USE IN WING FLAPPING LAYING HENS

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Abstract

Cage-free legislation in several U.S. states mandate hens be able to fully extend limbs without touching their enclosure. This requirement is ambiguous and does not specify if hens must be able to extend limbs horizontally or vertically as well, and little research has looked at how much vertical space a hen occupies while extending her limbs (i.e., flapping her wings). Therefore, this study looked to establish a to measure the maximum height reached while wing flapping. Subjects used were 28 individually caged Hy-line W36 hens at 45 WOA. A ceiling-mounted Intel RealSense Depth Camera D435 was centered approximately 250cm above a black plywood board affixed to the floor. The depth camera was calibrated to that surface before a foldable test pen (121.9 by 121.9 by 121.9 cm) was placed around the plywood. During testing, one hen at a time was placed in the test pen and video recordings started. Recordings stopped once each hen flapped her wings. From depth footage for each hen, frames were cropped and processed to increase image quality and fill missing/dead pixels and a binary mask separated hens from background. The minimum distance between pixels was obtained for each frame, and maximum height reached by each hen was computed. The binary mask was overlayed on depth footage, superimposing a dot on the highest point reached in each flapping event, to visually verify the computed maximum height corresponded to the highest position for each hen. Initial results yielded an average maximum height during wing flapping of 51.02 ± 4.7 cm. Hens used in this study were from a single strain, old enough to have keel damage or poor feather condition, and were cage-reared and housed (likely with minimal muscular strength or experience wing flapping), limiting our ability to generalize our findings to a vertical height standard for cage-free hens. However, these methods may provide a useful approach for others to measure space requirements in laying hens of varying strains, ages, and rearing/housing methods.

Introduction

In the United States, there are no federal regulations on housing systems used in the egg industry, and most laying hens in commercial production are housed in conventional caged systems. However, initiatives to prohibit the intense confinement of farm animals have risen across the U.S., and several states have implemented legislation to ban conventional cages in egg production (Scrinis et al., 2017; Vogeler, 2020). The push to end the use of caged systems is largely driven by those advocating for improved welfare for hens on commercial farms (Vogeler, 2020; Molnar et al., 2020). As of March 2023, roughly 70% of the estimated 383 million laying hens in the U.S. are housed in caged systems (USDA Chickens and Eggs); the remaining ~30%, approximately 120 million hens, are housed in cage-free systems (USDA Cage-Free Shell Egg Report). The percent of hens in cage-free systems is expected to grow rapidly in coming years as producers work to meet goals set by state legislation and corporate commitments.

As the egg industry transitions to becoming cage-free, standardized welfare guidelines or regulations and terminology promulgated within state legislation could support the flow of interstate commerce in lieu of a national regulation and promote consistency in the design and manufacturing of cage-free systems. Many states with cage-free laws require producers to follow the per hen space requirements in the United Egg Producers (UEP) Guidelines for Cage-Free Housing (2017). The UEP is a collaborative group of U.S. egg farmers whose guidelines on management and resource provision are crafted by a scientific advisory committee and are updated periodically based on applicable findings from peer-reviewed behavioral research. Producers following guidelines such as those mentioned above are afforded science-based rationale for industry changes and specific guidance on how to effectively manage hens as intended by legislation. However, legislative demands from some states remain vague and require more clarification or consideration before producers can effectively fulfil requirements.

Laws passed in California, Massachusetts, Michigan, and Rhode Island stipulate that hens must be able to fully extend their limbs without touching the side of their enclosure. Experts raised concern with the terminology of this requirement when it was first presented in California's Proposition 2 (2008), largely due to the lack of more precise language and the space allocations were based on a description of hens' ability to perform a behavior (Sumner et al., 2008). A hen is likely fully extending her limbs while she is wing flapping, which involves horizontal and vertical spreading of both wings in front of and around her body. Ethologists do not fully understand the motivations behind wing flapping, and it is hard to accommodate this behavior without a more explicit understanding of why or how frequently hens desire to flap their wings. This law also does not specify whether housing systems must allow enough space for a single hen to extend her wings at one time, or if all hens in a flock must be able to do so simultaneously without interference from other hens (the latter would require far more space). Due to this ambiguity, egg producers in several states have no concrete basis for what to do from a practical perspective to comply with this legal mandate as it is written.

Two studies have identified that wing flapping is space-intensive in comparison to other behaviors (Mench and Blatchford, 2014; Riddle et al., 2018). Mench and Blatchford (2014) published findings regarding vertical space use in laying hens, showing that Hy-Line W36 hens had an average height of 49.5 ± 1.8 cm while flapping their wings; however, authors noted that if a hen must be able to wing flap without touching the enclosure, as specified in state law, she may would require additional space than just what her physical body occupies to ensure full extension of her wings without hindrance. Since state legislation intends for cage-free systems to accommodate dynamic behaviors like wing flapping, consideration should be given to the variety of environmental complexities within cage-free systems, as these features may inadvertently inhibit hens' full range of movements. For instance, multi-tiered aviaries are comprised of open tiers or partial enclosures layered in several levels positioned atop one another and hens are able to freely navigate between upper or lower levels with the help of ledges or ramps. These levels also contain resources including feed, water, and furnishings perches. It is possible that these internal furnishings or the surrounding sides and ceiling of aviary tiers prohibit the full extension of a hen's wings or impede her ability to flap without touching the enclosure in some manner.

The use of technology has allowed new insights into the study of animal behavior (Chen et al., 2019; Gomez et al., 2021). Modern imaging technology has the capacity to capture high speed motions in three dimensions and can be used to assess the amount of space occupied by a hen while wing flapping. Given that more producers will need to abide by these legislated standards after transitioning to cage-free systems, it is important to know how much space a hen occupies while wing flapping to determine whether they are in compliance with the law. Therefore, the objective of this study was to explore a method to measure the amount of vertical space needed for laying hens to wing flap using a depth camera. Though there may not be a gold standard when it comes to gleaning measurements of dynamic behaviors, findings from this study may provide some idea of three-dimensional space requirements for wing flapping as well as open doors to technology-based methods to assess space use in laying hens.

Materials and methods

Subjects and Home Housing

This study took the physical and kinetic measurements of 28 Hy-Line W36 hens that were used as part of the fertile egg flock at the Michigan State University Poultry Teaching and Research Center in East Lansing, MI. At the time of testing, hens were 45 weeks old.

Hens were individually housed in cages that measured 30.5 x 46.5 x 42 cm; the floor in each cage sloped to allow eggs to be easily collected, resulting in slightly more vertical space (~3 cm) near the front of the cages compared to the back. All subject hens were cage reared.

Physical measures of hens

In addition to measuring hens with a depth camera, two trained persons collected physical wing measurements from each hen. Hens were removed one by one from their home cages and held upright by one person, so that their right side was pressed against the person holding them and their left wing could be handled freely by the other person taking the measurement. Physical measurements were taken only from the left wing of each hen. First, a ruler was held gently over the front of each hen’s bent wing to assess the length from carpal joint to the tip of the longest primary feather. The wing was then fully extended horizontally from the hen and the ruler was held underneath the wing to record the length of the physical structures of muscle and bone. This was done by looking up from underneath the wing so that ceiling lights shone through primary feathers to indicate where the muscle and bone ended. Lastly, with the left wing still fully extended, a yardstick was placed against the hen’s body underneath the wing and the wing was gently flattened on top to get measurements to the tip of the longest primary feather. Each hen was then weighed using a digital scale before being returned to her home cage.

Depth Camera and Test Set Up

We used an Intel RealSense Depth Camera D435 to film hens wing flapping. This depth camera (90 x 25 x 25 mm) was mounted to the ceiling in the same barn as hens’ home cages. A black plywood board (121.9 x 121.9 cm) was affixed to the floor so that it was centered directly underneath the camera; this board contrasted better against the white-feathered hens. The face of the camera was approximately 250 cm from the surface of the plywood. Lights were placed around the testing area to prevent shadows in the camera's field of view. Prior to collecting any data, the depth camera was calibrated to the flat surface of the plywood board and we took measurements of objects of known size (such as a 5-gallon bucket) to ensure the camera was reporting correctly the depth. For testing purposes, a foldable, wire pen (121.9 x 121.9 x 121.9 cm) was secured around the sides of the plywood after we calibrated the camera to prevent hens from wandering or moving out of view from the depth camera. The pen was open on top so that the depth camera had an unobstructed birds-eye-view of each hen during testing, and the sides were tall enough so that hens would not fly out. A video camera was positioned on a tripod, facing the test pen to film each hen as she flapped her wings from a side-view.

Regrettably, there is no foolproof method to make a hen flap her wings. Given we were exploring an efficient method to assess space use, we encouraged wing flapping by placing hens individually into small, hard-sided pet carriers (45.5 x 29.2 x 30.5 cm) to restrict their space prior to moving them into the pen for testing. These carriers were smaller than hens' home cages, and we were hopeful that gently handling hens and temporarily, but strictly, confining them to the pet carriers would entice them to stretch and flap their wings more promptly once they had room to do so. During data collection, hens were removed from home cages and placed individually into carriers for a minimum of 10 minutes, while the testing procedure was being set up, and for a maximum of one hour, before being returned to home cages.

Test Procedure

A total of 2 people handled hens and conducted the testing procedure. Hens were habituated to the procedure at least twice prior to data collection. Testing of all hens took place on the same day for consistency in lighting and depth calibration.

Hens were removed from home cages in groups of four and were placed individually into one of the four small carriers. These carriers were situated around the test pen so that each group of four hens had visual access to one another. Hens remained in these carriers for at least 10 minutes to allow study personnel to note which hens were being tested and to gently cramp hens to encourage wing flapping. After space restriction, one person removed the first hen in the group from her carrier and placed her into the test pen, near the center of the black plywood. As soon as that person stepped away from the test pen to prevent casting shadows, the second person would begin recording on the depth camera and video camera simultaneously. Each subject hen remained in the test pen until she flapped her wings, or a maximum time limit of 10 minutes elapsed. If a hen did not flap her wings within 10 minutes, she was returned to her carrier and her corresponding video files were deleted. Once the other hens in that group had been tested and successfully flapped her wings, the non-flapping hen would then be placed back in the test pen for a second test attempt. When a hen successfully flapped her wings within the allotted time, the depth camera and video camera were stopped and both video files were saved and appropriately labelled to keep track of each hen’s corresponding video. The hen was then returned to her carrier. See **Figure 5.1** for a visualization of the testing area. Once all 4 hens in a group were tested and successfully flapped their wings, they were returned to their home cages and another group of 4 hens were removed for the test procedure. This was repeated until all 28 hens were recorded flapping their wings. No hens were kept away from their home cages (which contained feed and water) for more than 1 hour.

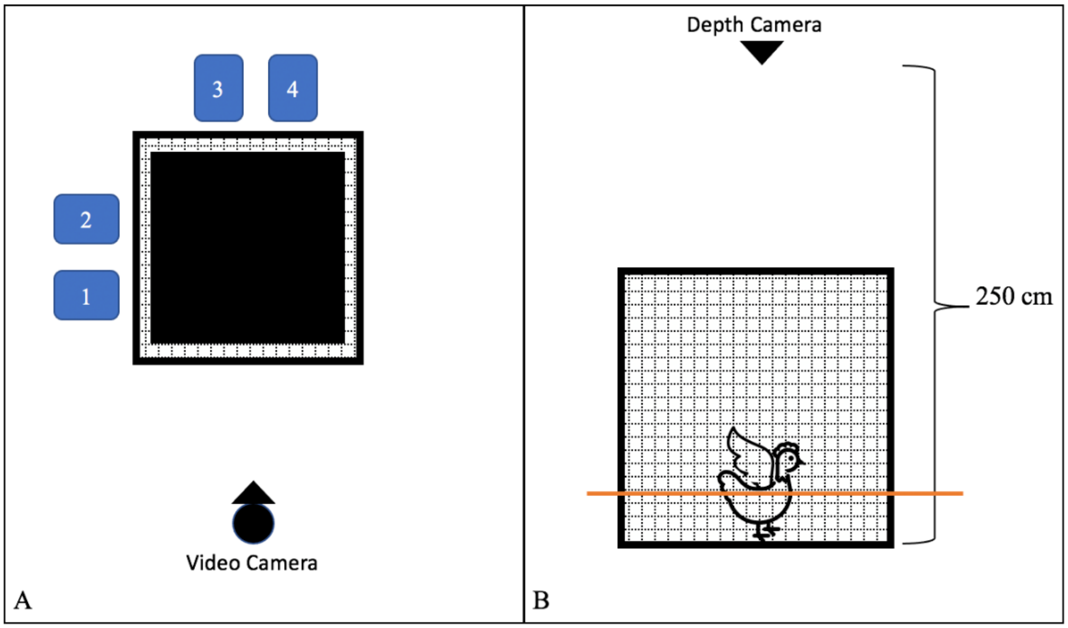

Figure 5.1. A. Top-down view of the test area, showing the video camera set up to record hens in the test pen. The blue rectangles labelled 1-4 represent the small carriers that hens were placed in prior to testing; these carriers are facing the black plywood surrounded by wire-test pen. **B.** Side view of test area, showing a hen in the test pen. The depth camera is directly above the test pen, approximately 250 cm above the surface of the plywood. The orange line represents the threshold applied for depth video analyses (described below).

Analyzing Depth Videos

Each of the 28 depth video files included RGB and depth streams. MATLAB (MathWorks) was utilized to control the video recording process where both the RBH and depth streams were aligned and frames from each video file were cropped to only feature the hen standing on the black plywood while flapping. We then performed a post-hoc analysis of depth streams and applied a distance-based threshold so that each depth video contained only those instances (pixels) when the depth values of the pixels fell in the threshold range. In this case, the depth camera was approximately 250 cm above the ground and the threshold was set to 245 cm. This threshold generated a binary mask to identified whether each pixel in the video was to be included for analysis (i.e., hens' bodies reached a high enough vertical point to be captured by the depth camera and was separated from the further away black plywood). The binary mask was then applied to the RGB frame for visual verification that no pixels had been missed. Finally, all overlapped depth frames, overlapped RGB frames, and the masked RGB frames were concatenated and saved into a new .mp4 video. In the masked RGB, a red dot was placed to note the pixel that represented the highest point for each frame. In this way, when a video of a hen wing flapping is played, the red dot moves around the hen to indicate which part of her body was at the highest vertical point while wing flapping (see **Figure 5.2**).

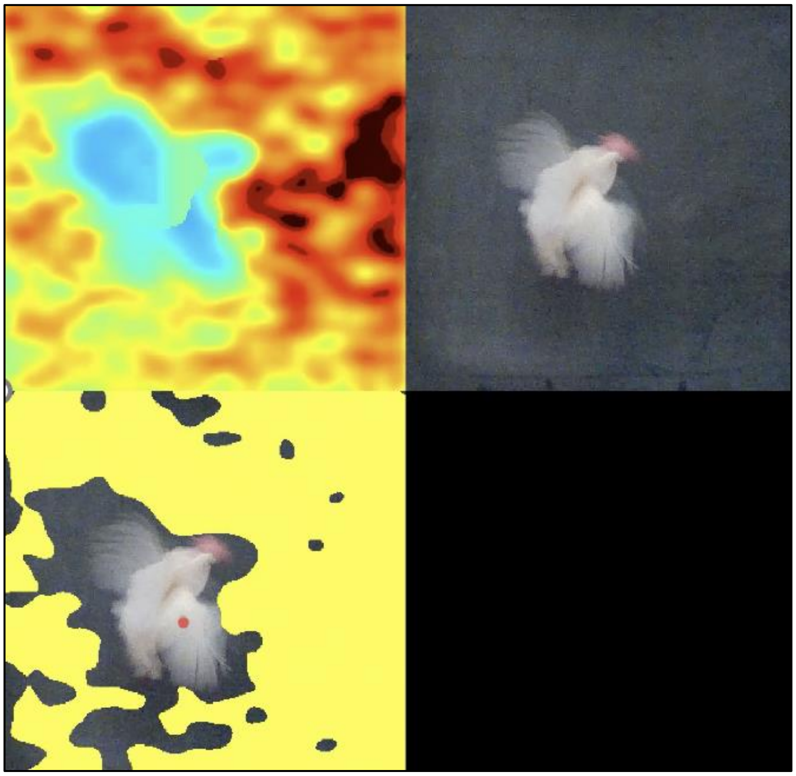

Figure 5.2. Example image taken from the final depth video file for a hen while wing flapping while standing on the floor. The video on the lower left side shows the binary mask in yellow, which indicates that those pixels were not included in depth analysis. The red dot is located on the part of the hen's body that is at the highest vertical point in each frame of the flapping event.

Results

During data collection, only 2 out of the 28 did not flap their wings within the 10-minute time limit and had to be retested. Once placed in the test pen, hens took anywhere from 35 seconds to over 7 minutes to successfully flap their wings. Average physical measurements from the 28 subject hens are presented in **Table 5.1**. Further analysis is being done on footage from the video camera, as well as assessments on any correlation among physical measurements and the maximum height of each hen while wing flapping.

Weight (kg)	Length of bent wing (carpus to primary tip) (cm)	Length of extended wing (to phalanges) (cm)	Length of extended wing (in)
1.62 ± 0.18	23.93 ± 0.6	16.84 ± 0.98	13.57 ± 1.03

Table 5.1. Average physical measures of the 28 Hy-Line W36 hens used in this study. All measurements were taken from the left wing of each hen (excluding weight).

While wing flapping, hens in the current study reached an average maximum height of 51.02 ± 4.7 cm (measurements ranged from 42 to 63 cm). Using footage from the depth camera to assess the number of pixels that contained hen's bodies in the test pen, we were able to assess the floor space occupied by each hen while flapping. At their highest vertical point during a wing flapping event, hens in our study occupied an average floor area of 2803.1 ± 483.3 cm^2 (measurements ranged from 2036 to 3739 cm^2).

Discussion

Hens tested in this study required an average of 51.02 ± 4.7 cm of vertical space while flapping their wings. These results are similar to those reported by Mench and Blatchford (2014), who also used Hy-Line W36 hens, and found their hens reached an average maximum height of 49.5 ± 1.8 cm while wing flapping. Based on these two reported findings of vertical space use while wing flapping, it can be assumed that multi-tiered systems that offer < 50 cm of vertical space between each tier probably do not allow hens of this strain to fully extend their limbs without touching the ceiling. However, hens do not behave in the exact same manner, nor are they physically identical to one another. It should be noted that hens used in both this current study and that by Mench and Blatchford (2014) were older (45 and ~78 WOA, respectively), and were housed in cages, and it is probable these hens had limited or impaired mobility due to prolonged confinement and age. Moreover, it is possible that hens from our study were never afforded enough three-dimensional space to lift their wings other than the brief time they spent in the test pen, and the manner in which they flapped may not be representative of hens with more muscle development and experience with wing flapping. In cage-free systems, hens have more opportunity to use and develop their muscles (Hartcher and Jones, 2017), including breast muscles that assist with wing flapping (Casey-Trott et al., 2017). Future studies utilizing depth cameras should focus on space requirements for dynamic behaviors in younger hens, especially those that were cage-free reared and housed.

When a hen flaps her wings, she does not only require vertical space but also adequate free space to her sides. In the current study, hens used an average of 2803.1 ± 483.3 cm^2 of floor space when their wings reached their highest vertical point during a flapping event. Other studies on space use have reported W36 hens to occupy an average floor area of 3344.5 ± 92.32 cm^2 (Riddle et al., 2018), and 1693.0 ± 136.0 cm^2 (Mench and Blatchford, 2014). The variation in these results may be explained by the rearing and housing of the hens used: Riddle and colleagues (2018) collected their measurements from hens who had been cage-free reared and housed. Mench and Blatchford (2014), in addition to using hens that likely had less muscle development than their cage-free counterparts, also collected wing flapping measurements as hens were jumping from a perch. Different wing-based behaviors require varying amounts of three-dimensional space, and it is likely that hens need less room to use their wings for jumping purposes as opposed to stationary flapping when they may more fully extend their wings.

The amount of space a hen occupies when performing dynamic behavior also varies depending on genetic strain (Riddle et al., 2018). There are also notable genetic differences in hens' skeletal and musculature structure that may influence how they perform specific behaviors, like wing flapping. White-feathered hens tend to have heavier breast muscles and larger keel bones, whereas brown-feathered hens have heavier leg muscles (Fawcett et al., 2020; Pufall et al., 2021). More research must be done to assess space requirements in multiple strains of laying hen before any useful guidelines can be provided, and it is possible that a range of height guidelines are necessary for hens of different strains.

Additional Considerations

It remains unclear if spacing requirements in cage-free legislation require that a hen be able to flap her wings at all times. Hens may not need or want to flap their wings at certain times because they are performing other behaviors that cannot occur concurrently, such as eating or laying eggs. Additionally, laying hens do not space themselves evenly throughout a given area. When hens congregate within a housing system, they effectively increase the stocking density of that area and reduce their freedom of movement (Appleby, 2004; Widowski et al., 2016). Crowding may limit a hens' ability to wing flap, or her perception that she is able to do so in that area at that time given the close proximity of her flock mates (Keeling, 1995). Explicit consideration should be given to assess the function and causation of wing flapping so that legislative demands can better reflect practical improvements to cage-free husbandry that genuinely benefit hens.

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CHAPTER 6.

GENERAL DISCUSSION

Analysis of DW, W36, BB, and HB Strain Differences

The overarching focus of this project was to examine how laying hens of different genetic strains perform dynamic and space-intensive behaviors in a multi-tiered aviary system. The specific behaviors chosen for examination were dust bathing and wing flapping, both of which have been deemed important by cage-free legislation. These studies were done not only to assess space use in multiple layer strains, but also to understand the effects of conspecifics on hens' ability to perform dynamic behaviors in an aviary system, as well as potential effects of litter restriction (ranging from 9 weeks of full restriction to a daily allowance of litter access following morning hours). We also explored a method to assess the amount of vertical space a hen occupies while flapping her wings.

Results from Chapter 2 show that hens of the two white strains had smaller inter-bird distances compared to hens of the two brown strains. This could indicate that hens of the 2 white strains tolerated closer conspecifics than brown strains while dust bathing, or that the brown hens were attempting to maintain a certain inter-bird distance while dust bathing and may therefore need more space allowance to successfully perform a bout. Previous research shows that brown-feathered hens occupy more room while dust bathing than white-feathered hens (Riddle et al., 2018), making it possible that brown-feathered hens ideally require a larger space allotment for the litter area not only for their physical body but also to maintain their preferred inter-bird distances. In addition, more white hens were on the open litter at any one time compared to brown hens, and more white hens dust bathed at the once compared to brown hens. These results were also seen in Chapter 3. In response to more hens occupying the litter area or dust bathing at the same time as a focal hen, white strains were more likely to decrease the amount of area they occupied while dust bathing, possibly in an attempt to complete a bout under less-than-ideal circumstances. Conversely, when presented with those same parameters, brown strains were more likely to decrease the duration of their dust bathing bout. The results of these two studies fit together well with previous studies looking at spatial distribution and nest use in these same strains in the same aviary systems. During peak lay, Ali and colleagues examined how the 4 strains of laying hens distributed themselves within the aviary tiers and litter areas (Ali et al, 2016). Put together, these findings suggest that hens of these white-feathered strains prioritize the ability to perform certain behaviors or access resources, even if they are in suboptimal conditions. Brown-feathered strains, on the other hand, are potentially more sensitive to the presence of other hens and may prioritize maintaining that inter-bird distance while roosting and dust bathing.

Results from Chapter 4 indicate that hens perform a high number of stationary wing flapping events in the first 85 minutes after accessing litter (and unfettered vertical space) each day. It is possible that hens are unable to successfully wing flap within the tiers of aviaries, and the fact that they flap when they are able to access more three-dimensional space on the open litter area suggests they experience some kind of rebound effect. It is also interesting to note that counts of wing flapping events were higher in the 2 brown strains compared to the 2 white strains, especially because white-feathered hens were previously found to use their wings more than brown-feathered hens (Pufall et al., 2021). Additional research should be done before definite strain differences can be identified, as well as any effects of group size or stocking density on hens' performance of wing flapping.

In Chapter 5, it was discovered that Hy-Line W36 hens required an average of 51.02 ± 4.7 cm of vertical space while flapping their wings. These results are similar to those found previously (Mench and Blatchford, 2014). Given that our hens were cage reared and housed, the manner in which they flapped may not be representative of hens with more muscle development and experience with wing flapping. However, this method of assessing vertical space requirements in dynamic behaviors like wing flapping may open the door for further assessments of hens from a variety of strains, ages, and backgrounds. Finally, explicit consideration should be given to assess the function and causation of wing flapping so that legislative demands can better reflect practical improvements to cage-free husbandry that genuinely benefit hens.

Space Guidelines

The United States will be producing predominantly cage-free eggs in the near future, following in the footsteps of countries around the globe. It is important to understand the implications that come from these systems. The results of these above studies not only involve aviaries, one of the more commonly used non-cage system, but also identify behavioral differences among laying hen strains. Our research is adding to the growing number of studies indicating that certain strains may be better suited to different management techniques or housing styles. By identifying behavioral differences among commonly used strains, we may be able to assist producers to more accurately select hens and management or cage-free system styles that best match one another. In addition, housing guidelines for cage-free systems stipulate a certain amount of space per hen. However, those guidelines do not account for the space needed to perform dynamic behaviors or the effects of conspecifics on hens' behavior. Our research showed that strains of laying hens occupy litter and perform dust bathing differently, and some strains may prefer to dust bathe at the same time to a greater extent than other strains.

Further, innate behavior patterns, coupled with typical management practices, may make it difficult to suitably provide hens enough freedom to dust bathe or perform other resource-specific needs at their preferred times, while also preventing unwanted behaviors, like laying eggs in litter. Space allowances may influence which behaviors hens are able to or desire to perform (Keeling, 1995). Future work is needed to determine the space requirements of hens for particular resources rather than blanketed guidelines for space as a whole. Differences in social distancing, behavioral synchrony, and the time-of-day around specific resources need to be identified. One broad value of space provided on a per hen basis may not be sufficient for hens of different strains and sizes, or it may not encompass variation in the amount of space occupied while hens perform dynamic movements. It is also important to remember that ethological studies help us interpret hen behavior in the context of these systems, not in the context of their true innate rhythms. We can only conclude so much from studies on hens in restrictive housing systems. Restrictive management tactics can range from limiting access to resources to limiting the amount of space a hen has to move within throughout her lifetime – no housing system can compare to the natural environment of the red jungle fowl (and accompanying pressures of living in the wild, which differ from those pressures in captivity).

Additional Thoughts

The common consensus that hens in multi-tiered aviaries and other complex cage-free systems have a higher prevalence of keel damage may not be entirely factual (Ruefner and Makagon, 2020). Further, keel bone health is likely influenced by our selection for high egg production (Eusemann et al., 2020). Creating appropriate housing standards for laying hens will help alleviate many aspects of their current welfare concerns, but consideration must also be given to the robustness of the hens themselves. Current selection methods are not sustainable long-term and further selection for production traits may be reaching biological limits in layers (Trixier-Boichard, 2020); selection for traits to improve robustness and otherwise benefit hen welfare should be considered as well (Fernyhough et al., 2020).

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