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Rearing laying hens: the effect of aviary design and genetic strain on pullet exercise and perching behavior

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Introduction: To prepare laying hens for life in cage-free aviaries, they must be reared in aviaries that accustom them to the challenges of navigating a complex three-dimensional structure. Rearing aviaries vary in design and contain a brooding compartment in which chicks are confined during the first six weeks of age. These compartments vary greatly in their size and complexity. The present study aimed to examine the impact of environmental complexity during early life and birds' genetic strain on their space use and forms/types of exercise.

Methods: Four consecutive flocks of brown and white chicks were raised in three styles of rearing aviary with low, intermediate, or high complexity. Behavioral observations were performed at three ages during the brooding phase (weeks 1, 3, and 5) and the open phase when the brooding compartments were opened (weeks 7, 11, and 17). Behaviors observed were categorized as aerial locomotion, perching, dynamic load-bearing behavior (DLB), and wing-involved load-bearing behavior (WLB).

Results: During the brooding phase, chicks in aviaries of high complexity exercised most frequently (e.g., DLB events/minute: 1.75 in *High* vs 1.11 in *Mid* and 0.10 in *Low, p<0.0001*), and the effect remained for whites, but not the browns, during the open phase. White pullets exercised more than brown pullets both in brooding (e.g., 1.47 vs 1.28 DLB events/minute, *p<0.0001*) and open phases in *High* and *Mid* (e.g., 1.17 vs 0.93 DLB events/minute, housing x strain interaction *p=0.009*). Throughout rearing, whites had higher odds of perching than browns (brooding: 0.52 vs 0.45, *p=0.04*, open: 0.27 vs 0.17, *p=0.0007*).

Discussion: We concluded that rearing aviary design can affect behavior during the brooding phase; however, once the brooding compartments were opened, housing differences almost exclusively affected white pullets. The data suggest that genetic strain of birds must be considered in the design of pullet housing with the goal of maximizing space use and musculoskeletal development of laying hen pullets.

KEYWORDS

laying hen, rearing aviary, brooding compartment, locomotion, load bearing

1 Introduction

As an alternative to conventional cages, aviary housing is a concession towards improving laying hen welfare as it allows hens to perform a broader range of behaviors. Aviary systems promote behaviors that hens are highly motivated to perform and consist of elevated structures that accommodate perching (including night-time roosting), litter areas for dustbathing and foraging, and enclosed areas for nesting (Weeks and Nicol, 2006; Widowski et al., 2017). However, successful use of these 3-dimensional (3-D) spaces requires locomotion behavior including navigation (León et al., 2021), balancing (LeBlanc et al., 2016), and the proficient use of perches and ramps (Scott et al., 1997; LeBlanc et al., 2018). As land fowl (Galliformes), chickens are not inherently proficient in vertical locomotion, and the use of 3-D space must be learned (Gunnarsson et al., 2000). Additionally, hens housed in aviaries are known to have a high prevalence of keel bone fractures (KBF), some presumably from unsuccessful navigation attempts (Wilkins et al., 2011; Harlander-Matauschek et al., 2015; Toscano et al., 2018; Rufener and Makagon, 2020; Toscano et al., 2020). There is ample evidence for the negative impact of KBF on hen welfare, such as pain (Nasr et al., 2013), impaired mobility (Nasr et al., 2012; Rentsch et al., 2019), and potential signs of long-term negative affective states (Armstrong et al., 2020). The widespread occurrence of osteoporosis in laying hens likely contributes to their susceptibility to KBF.

Bipedal locomotion in chicks develops during their first two weeks of life, with running seemingly easier than walking due to a top-heavy body proportion that complicates balancing on a single leg (Muir et al., 1996). Exercise restriction through spatial limitations (cage: 8x8 cm, 20 cm^2/bird) in the first week of life shortened stride lengths and decreased the maturation rate of head-bobbing behavior (Muir and Chu, 2002). The use of perches and platforms by chicks vary by strain (see Habinski et al., 2017a) but starts as early as two weeks of age (Kozak et al., 2016b). Early access to 3-D structures, such as elevated perches, platforms, and ramps/ladders, has been reported to alter space use (Heikkilä et al., 2006; Colson et al., 2008; Brantsæter et al., 2016), improve spatial locomotion (Gunnarsson et al., 2000; Norman et al., 2021), and decrease the risk of injury later in life (Norman et al., 2021).

Several lines of research support aviaries in promoting musculoskeletal health in chickens (Fleming et al., 1997; Heerkens et al., 2015). Bone strength increased with access to vertical space (short-term: Enneking et al., 2012; long-term: Hester et al., 2013), but not in response to running (in roosters Judex and Zernicke, 2000) or after providing them with additional floor space (Whitehead and Wilson, 1992 as cited in; Whitehead, 2004). Hens housed in enriched cages during the lay period had fewer KBF when reared in aviaries compared to conventional cages (Casey-Trott et al., 2017), demonstrating the long-term effects of early life environment. Indeed, it is proposed that bone responds to mechanical stimuli as the difference between a newly imposed versus habitual mechanical loading (Frost, 1987).

Laying hen development is also influenced by genetic factors. White- and brown-feathered strains have different behavioral (Dudde et al., 2018; Nelson et al., 2020; Peixoto et al., 2020; Peixoto et al., 2021) and physiological (Habig et al., 2012; Dudde et al., 2018; Nelson et al., 2020; Peixoto et al., 2021) phenotypes. Brown-feathered egg-layers are known to be less active (hens: Hewlett and Nordquist, 2019; pullets: Pufall et al., 2021), perform fewer aerial transitions as adults (Ali et al., 2016; Garant et al., 2022) and pullets (Kozak et al., 2016a; Chew et al., 2021b; Pufall et al., 2021), and roost in lower places than white-feathered birds (Ali et al., 2016; Ali et al., 2019). Due to their larger body sizes, brown strains occupy more horizontal space than whites (pullets: Giersberg et al., 2017; hens: Riddle et al., 2018), while white hens use more space for wing flapping (Riddle et al., 2018).

During early rearing, laying hens are housed in environments with no structures for egg collection (e.g., nest-boxes or tilted floors) and with furnishings (e.g., waterlines or perches) that can be adjusted as birds grow (Nicol et al., 2017). The designs of commercial rearing aviaries vary significantly in their spatial arrangement and complexity, particularly during brooding when chicks may be confined in compartments (Widowski and Torrey, 2018). This brooding phase lasts from day-old to 3-13 weeks, depending on manufacturer recommendations and producer preference (Pufall et al., 2021 supplementary material), after which pullets are released to the entire rearing aviary (open phase). Pullets stay in the rearing aviary until they are grown (15-18 weeks old) and moved into adult housing set up for egg collection (Janczak and Riber, 2015). Commercial rearing aviaries can be generally grouped into three basic designs, with total space and complexity (number and arrangement of perches and platforms, see Figure 1) being low, intermediate, or high during the initial brooding phase (Widowski and Torrey, 2018; Pufall et al., 2021). While the design still differs during the open phase, the spatial complexity is more comparable with similar structural resources in all three aviary designs. In an exploratory study on commercial farms, Pufall and colleagues (2021) found that pullets housed in high complexity rearing aviaries during the brooding phase showed more locomotory behavior and aerial transitions than pullets in aviaries of low and intermediate complexity designs.

In this study we investigate the effect of rearing aviary design on the amount and type of exercise chicks/pullets perform throughout the rearing phase. Lohmann Selected Leghorn-lite and Lohmann Brown-lite laying hen pullets were reared in low, intermediate, and high complexity models of commercial rearing aviaries. We hypothesized that 1) chicks in brooding compartments of higher complexity would perform more exercise including dynamic load-bearing exercise (locomotion) and wing-involved load bearing activity, and make more use of perches during the brooding phase, 2) pullets in rearing aviaries with high complexity, particularly during brooding, would exercise and perch more during the open phase than those in low or intermediate complexity, and 3) the amount of exercise and perching behavior would be higher in white than brown feathered chicks/pullets in all housing systems.
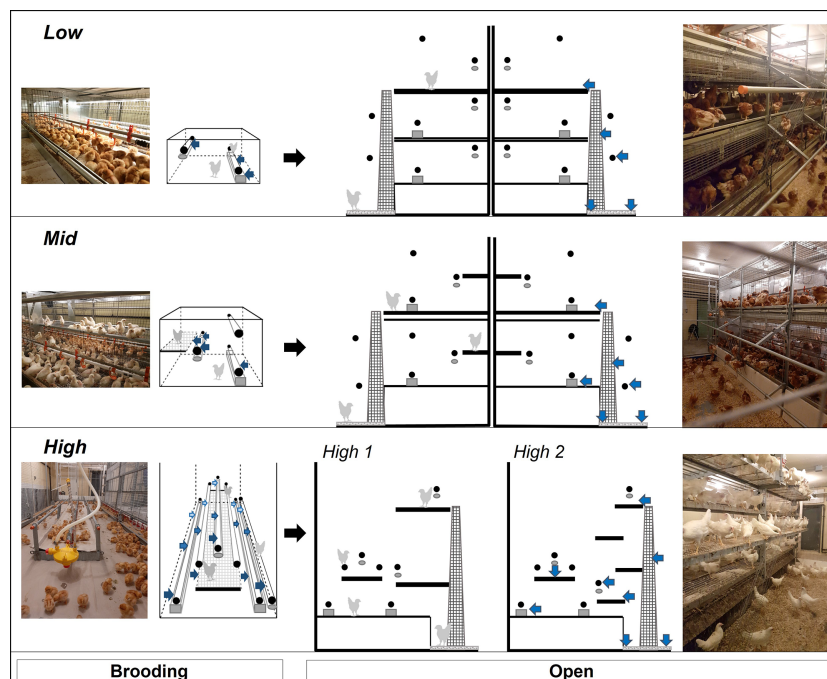
FIGURE 1
Photos and schematics of housing treatments varying in environmental complexity. Aviary style 1 (*Low*: Natura Primus), style 2 (*Mid*: Natura Filia), and style 3 (*High*: NivoVaria, '*High.1*'; Pullet Portal '*High.2*'). Design of brooding compartments (left) and design of aviaries during the open phase (right), black arrows indicate the transition from brooding to open housing. Elevated spatial structures are in black: perches (circles) and platforms (bars). Drinkers (ovals) and feeders (squares) are in grey. Arrows (blue) point to labels at pre-determined locations used for behavioural observations; focal birds were chosen based on proximity to labels.

2 Materials and methods

2.1 Ethical statement

All use of animals and procedures in this study were considered and approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #4127).

2.2 Animals and management

Between the summer of 2019 and 2021, four consecutive flocks of Lohmann Selected Leghorn lite (white) and Lohmann Brown Lite (brown) were hatched at a commercial hatchery (Archer's Poultry Farm Ltd., Ontario, Canada), beak treated with an infrared laser, and vaccinated against Infectious Bronchitis, Marek's, Infectious Bursal disease, Newcastle disease, and Coccidiosis before transport to the research station. Both strains are commonly used in the Canadian egg industry (van Staaveren et al., 2018). For each flock, approximately 1,378 chicks of both strains were hatched and transported simultaneously and immediately placed into their housing treatments. Chicks were fed *ad libitum* from chain feeders with a standard layer chick starter crumble diet for six weeks [wk], then switched to a standard laying hen grower crumble diet. An intermittent lighting schedule was used for the first four days after hatch (4 h light: 2 h dark) followed by 16 h light: 8 h dark until five weeks of age and then 10 h light: 14 h dark for the remainder of the trial. LED lights provided a light intensity of 40 lux on day one and decreased steadily to 10 lux by five wk of age. Temperature was set to 34° C at placement and gradually decreased to 20° C by six wk of age. Chicks were further vaccinated against Newcastle disease and Infectious Bronchitis at three, 11, and 17 weeks of age and against Fowl Pox, Infectious Laryngotracheitis, and Avian Encephalomyelitis at six wk of age. Illustrations of bi-weekly body weights and mortality rates by rearing housing system and strain can be found in the appendix of the PhD thesis by Rentsch (2023).

2.3 Housing

The white-and brown-feathered strains within each flock were randomly divided into three different rearing aviaries (n=8/aviary style). Each aviary represented one of three general styles of commercial rearing aviaries (Figure 1; Table 1). The major difference between aviaries was the design of the brooding compartments (low, intermediate, or high complexity, left column in Figure 1), where chicks were confined for the first few weeks of life, henceforth called the brooding phase. Following the brooding phase, pullets were given access to a litter area, multiple tiers, additional perches, and ramps during what will, from now on, be called the open phase (Figure 1 right column), where the amount and arrangement of the various resources (i.e., complexity) also differ but to a lesser extent than during brooding. In this study, the

TABLE 1 Bird numbers, replication for behavioral observations (statistical unit), and resource allowances per strain for the three rearing aviaries.

Aviary style complexity	Low[1]			Mid		High[2]	
	Weeks 1-3	Weeks 4-6	Weeks 7-17	Weeks 1-6	Weeks 7-17	Weeks 1-6	Weeks 7-17
Replicates/strain[3]	12	12	4	12	4	4	4
Birds/replicate	115	58	345	144	432	600	600
Floor space/bird [cm^2]	100.32	200.64	300.97	145.35	300.97	NV[4]: 327.1 PP: 351.42	645.16 614.58
Perch space/bird [cm]	2.08	4.15	8.18	3.12	8.61	NV: 5.54 PP: 6.05	6.35 7.16
Platform space/bird [cm^2]	–	–	–	39.05	–	NV[4]: 46 PP: 54.67	–
Litter space/bird [cm^2]	–	–	188.39	–	168.39	–	190.32
Birds/nipple	24.63	12.32	8.21	20.14	10.07	NV: 9.38 PP: 8.11	6.25 5.26
Feed trough/bird [cm] [5]	2.1	4.19	4.19	2.11	4.22	NV: 3.6 PP: 4	3.6 4

[1]At week 4, half of the birds in Low were moved to identical cages on the lower tier to reduce density. Only the original replicate cages were used for behavioral observations.
[2]Represented by two similar models from different brands; the NivoVaria (NV) and the Pullet Portal (PP). Both strains were replicated in both models twice. When no model is specified, values apply to both equally.
[3]Statistical units= the number of independent replications per strain
[4]This system uniquely had plastic grid flooring and platforms instead of a wire floor.
[5]Limiting factor for bird capacity per system.
A dash (-) indicates that a specific resource was not provided at that age.
Each aviary had two sections, one per strain.

brooding phase lasted six weeks for all pullets in all rearing systems. Space allowances and resource allocations for all three aviary styles are detailed in Table 1.

Aviary Style 1 (low early complexity 'Low') was represented by the Natura Primus system (Big Dutchman North America, Holland, Michigan), a three-tiered system located in a single room but divided in the center creating two separate aviary sections, each facing a wall (Figure 1). The two strains were housed separately, one per section, and were visually but not auditorily divided. The brooding compartment consisted of three cages on the middle tier with 115 chicks per cage (L: 122 cm x W: 94 cm x H: 50 cm). The cages were furnished with an automated feeder, a drinker line with nipples, and two perches (Figure 1). One perch was mounted on the drinker line and was raised with the drinker as chicks grew (max H: 40 cm). The other was mounted in a fixed position (H: 14 cm). Halfway through the brooding phase (at 3 wk of age), half of the chicks were moved to cages in the lower tier to decrease the stocking density. At 6 wk of age, the cages were opened so that pullets had access to all three tiers, litter floor (W: 176.53 cm x L: 368.3 cm), external perches (H: 77 cm and 150 cm), and ramps connecting the three tiers (Figure 1). At that time, pullets from specific cages became indistinguishable and were treated as one population per strain.

Aviary Style 2 (intermediate early complexity 'Mid') was represented by the Natura Filia system (Big Dutchman North America, Holland, Michigan), a two-tier system located in a single room, but divided in the center creating two separate aviary sections, each facing a wall (Figure 1). The two strains were housed separately, one per section, and were visually but not auditorily divided. Each system had three brooding compartments, with 141 chicks per cage (L: 147 cm x W: 111.5 cm x H: 90 cm). The three cages offered an automated feeder, a drinker line of nipple

drinkers, an elevated platform (L: 147 cm x W: 30.5 cm at H: 40 cm), and three perches. One of the perches was mounted to the drinker line and raised with the drinker as the chicks grew (max H: 40 cm), the other two were stationary (H: 19 cm and 68 cm). In the open phase, pullets could access a litter area (W: 161.5 cm x L: 442 cm) and two system tiers. Both tiers had three perches internal to the aviary structure (H: 19 cm, 40 cm, and 68 cm), two perches external over the litter area (H: 40 cm and 103 cm), and an external ramp that ran from the litter area to the second tier.

Aviary Style 3 (high early complexity 'High') aviaries were represented by two commercial aviaries, each located in a separate room (Figure 1). The Pullet Portal by Farmer Automatic (PP, Farmer Automatic, Georgia USA) was previously built at the research farm, but has since been discontinued. Therefore, a different but spatially similar model was chosen as the second replicate for High; the NivoVaria by Jansen (NV, VDL Jansen, formerly known as Jansen Poultry Equipment, Barneveld Netherland). Across the four consecutive flocks, the two strains were raised in both systems twice. Both High systems offered an open concept brooding compartment that held 600 chicks and spanned the length of the room (PP: L: 724 cm x W: 245 cm, NV: L: 726.5 cm x W: 236 cm, Figure 1). The brooding compartment was furnished with six perches and a swinging platform (PP: L: 567.5 cm x W: 58 cm, NV: L: 472.5 cm x W: 58.5 cm) which was raised as the chicks grew. Two perches were mounted over the automatic feeder (H: PP: 21 cm, NV: 26 cm), two were fixed over the edge of the platform (H: 26 cm), and two over drinker lines that were raised as the chicks grew (max H: 40 cm). In the open phase, chicks were given access to one more drinker line and perch (max H: 35 cm), a litter area (L: 726.5 cm x W: 157.5 cm), and either two or four tiers depending on the manufacturer (PP: L: 693.5cm x W: 127 cm H: 26

cm, 86 cm, 131 cm, and 176 cm, NV: L: 701 cm x W: 277 cm H: 60 cm, 170 cm, Figure 1).

2.4 Behavioral observations

Locomotory behavior and perching were quantified in all four flocks using focal animal sampling during live observations conducted at six time points: (wk 1 (days 3-4), wk 3 (days 14-18), wk 5 (days 28-32), wk 7 (days 42-46), wk 11 (days 70-74), and wk 17 (days 112-116)). Pre-determined locations were marked by taping labels to the housing structures and the number of labels was proportional to the number of birds in each system (during brooding: 9 in *Low*, 12 in *Mid*, 15 in *High*; in the open phase: 10 in *Low*, 12 in *Mid*, 16 in *High* per strain). Locations were chosen as evenly as possible between the different systems, with at least one at each of the following locations: the feeder, drinker, on a perch, on the platform (if available) or tier, and by the litter area (Figure 1). For each focal sample, the bird closest to the pre-determined location was selected and observed continuously for five minutes. Behavior was recorded by two observers using a behavior coding program (Pocket Observer 3.2 by 2012 Noldus Information Technology bv) on digital tablets (Samsung Galaxy Tab4 SM-T230NU) that allowed the recording of locations and all occurrences of behavior (Table 2) in real-time. During the brooding phase, the number of observations was doubled (per strain: *Low*: 18, *Mid*: 24, *High*: 30). To this end, each observer recorded the behaviour of a bird at every location, resulting in two observations per location. During the open phase, the observers were stationed at either end of the aviary where half the labels were visible, resulting in one observation per location. In wk 3, 5, 11

(flock 3 only), and 17, each aviary system was visited twice with two days between visits. No observations were recorded for flock 2 during wk 11 due to a COVID-19 research pause. Out of the 2880 focal bird observations planned during the brooding phase, 7.6% were lost to unforeseen circumstances such as observer illness or technical malfunctions. No observations were lost in the open phase. Overall, 2661 focal bird observations were logged during the brooding phase and 1216 during the open phase. All observations were conducted in the morning (09h-12h) or the afternoon (14h-17h). Morning and afternoon observations were balanced across ages and housing systems over all four flocks. During live observations, blinding was not possible, as both housing type and bird strain were visually distinguishable and only one observer was blinded to the hypotheses as the other was involved in designing the experiment. To assess inter-observer reliability, 15 individuals spread over all four flocks and different age groups were marked with livestock paint and observed simultaneously by both observers for the designated five minutes.

Aerial locomotion (AL) was analyzed as a frequency. Perching was analyzed as frequency and duration. Perching duration (in seconds; maximum 300sec) was analyzed with a reduced data set, only including observations when perching occurred: 1,367 out of 2,661 observations for the brooding phase, and 385 out of 1,216 observations for the open phase. Behavioral data were pooled into dynamic load-bearing behavior (DLB) and wing-involved load-bearing behavior (WLB) and analyzed as count data. At this point, there is still uncertainty about which forms of exercise load specific bones and to what degree (Tobalske, 2007; Vitienes et al., 2023). Our decision to pool types of exercise into DLB and WLB was based on the assumption that using specific muscles would load the bone said muscle is anchored to (Willie et al., 2020). The two

TABLE 2 Ethogram.

Behavior	Definition	Recorded as
Walking	Pullet has one foot on the ground or perch while the other is lifted, moving forward (Chen and Bao, 2012). *Start coding at 3rd step, stop when there was no step for 2 sec*	State behavior
Running	Pullet has one foot on the ground while the other is lifted and moving forward rapidly. Head and neck are stretched and pointing forward.	State behavior
Wing assisted running	Pullet is running while flapping her wings	State behavior
Sparring	Pullet actively postures and/or collides ventrally with another pullet, usually while flapping her wings (Kruijt, 1964). Two possible postures: 1) head lowered, neck stretched forward, and wings extended. 2) head held high, chest puffed out, and wings tucked in.	State behavior
Aerial locomotion	Pullet is transferring through the air by bending her legs and straightening them fast, propelling herself up- or downwards. She might flap her wings for ascent or use her wings to slow the descent	Point behavior
Step up or down	Pullet has one of her feet on one level while lifting or lowering the other foot to a differently elevated area, placing it there before following with the first leg, ending up on a different level.	Point behavior
Group run	Pullet is part of a group of five pullets or more, running in the same direction.	Point behavior
Wing flapping	Pullet moves her wings up and down in rapid succession (Pickel et al., 2010). *One bout is between 1 and 5 flaps, more than 5 flaps is the next bout.*	Point behavior
Perching	Pullet is located on the perch. Behaviors on the perch include walking, sitting, standing, wing-flapping.	Location

State behaviors were mutually exclusive and recorded as durations and frequencies. Point behaviors were recorded as frequencies only, and locations were recorded as durations and frequencies.

groups were not mutually exclusive; DLB was analyzed to assess the relationship between spatial complexity and locomotion, assuming there would be a link to skeletal development in general. WLB, on the other hand, exclusively included behaviors that exercise the wings (a distinct subgroup of exercise (Gatesy and Dial, 1996)), which might have relevance for keel bone development specifically. DLB included walking, running, aerial transitioning, stepping up/down, and wing-assisted locomotion. WLB included wing flapping, aerial locomotion, wing-assisted locomotion, and sparring.

2.5 Statistical analysis

Analyses were done in R and R Studio (R Core Team, 2021) version 4.1.0 using packages 'lme4', 'DHARMa', 'tidyverse', 'car',

'emmeans', and 'oddsratio'. The brooding and open phases were analyzed separately. As birds housed together cannot be treated as independent units, the statistical unit was brooding compartment for the brooding phase analyses (N= 24 for *Low* and *Mid*, N= 8 for *High*) and aviary system for the open phase analyses (N= 8 per aviary style). Data were analyzed by fitting (generalized-) linear mixed effect models (LMM and GLMM binomial, GLMM Poisson) depending on data distribution (Tables 3 and 4). LMM: DLB was normally distributed in the brooding and the open phase. GLMM binomial: Aerial locomotion and perching were analyzed as probabilities (binary) due to zero-inflation of the data and so were WLB during brooding and perching duration (the probability of exceeding 60 sec) during the open phase. GLMM Poisson: Perching duration during brooding was analyzed after rounding up or down to full minutes and WLB during open was

TABLE 3 Model estimates $\pm$ SEM, and *p-values* of generalized-/linear mixed models fitted to behavior variables in the brooding phase.

Brooding Phase	Aerial locomotion		Perching		Perching duration[1]	DLB[2]	WLB[3]	
	estimate [probability]	raw data [rpm] [4]	estimate [probability]	raw data [rpm]	estimate [minute]	estimate [rpm]	estimate [probability]	raw data [rpm]
1st, 3rd Quartile		0.00, 0.20		0.00, 0.20				0.00, 0.40
Median		0.00		0.20				0.20
Distribution	binary		binary		Poisson	normal	binary	
Aviary	*P=0.03*	*mean, max*	NA[5]	*mean, max*	*P=0.69*	**P<.0001**	*P=0.67*	*mean, max*
Low	0.39 ± 0.05	0.25, 2.0	0.65 ± 0.07	0.28, 1.0	2.41 ± 0.33	1.10 ± 0.12[a]	0.54 ± 0.04	0.28, 2.8
Mid	0.40 ± 0.05	0.30, 4.0	0.61 ± 0.07	0.29, 1.2	2.03 ± 0.25	1.11 ± 0.11[a]	0.59 ± 0.03	0.33, 4.0
High	0.39 ± 0.04	0.31, 3.2	0.29 ± 0.05	0.25, 1.0	2.38 ± 0.27	1.75 ± 0.10[b]	0.56 ± 0.03	0.34, 4.2
Strain	*P=0.0003*		**P=0.04**		*P<.0001*	**P<.0001**	*P=0.07*	
Brown	0.35 ± .04	0.25, 2.8	0.45 ± 0.05[a]	0.26, 1.0	2.58 ± 0.19	1.28 ± 0.08[a]	0.55 ± 0.03	0.31, 4.2
White	0.44 ± 0.04	0.34, 4.0	0.52 ± 0.04[b]	0.28, 1.2	1.98 ± 0.15	1.47 ± 0.08[b]	0.58 ± 0.03	0.34, 3.6
Age	*P=0.001*		*P<.0001*		*P<.0001*	**P=0.04**	*P<.0001*	
wk 1	0.22 ± 0.04	0.17, 2.8	0.06 ± 0.02	0.28, 0.8	1.33 ± 0.21	1.47 ± 0.11[a]	0.37 ± 0.04	0.15, 1.8
wk 3	0.49 ± 0.06	0.36, 4.0	0.57 ± 0.04	0.28, 1.2	2.15 ± 0.17	2.46 ± 0.10[a]	0.66 ± 0.03	0.42, 4.2
wk 5	0.39 ± 0.05	0.28, 3.2	0.68 ± 0.04	0.26, 0.8	2.49 ± 0.2	1.22 ± 0.10[a]	0.56 ± 0.04	0.31, 2.6
Interactions[6]								
aviary: strain	*P=0.78*		*P=0.32*		*P<.0001*	*P=0.66*	**P=0.04**	
aviary:age	*P=0.005*		**P<.0001**		*P=0.005*	*P=0.32*	**P=0.003**	
strain:age	*P=0.19*		*P=0.23*		*P=0.50*	*P=0.56*	*P=0.31*	
aviary:strain: age	**P=0.004**		*P=0.85*		**P=0.009**	*P=0.94*	*P=0.07*	

[1] Reduced data set excluding observations where no perching occurred. Estimate for duration is mean minutes within the 5-minute observation period.
[2] DLB, dynamic load-bearing behavior.
[3] WLB, wing-involved load-bearing behavior.
[4] rpm, rate per minute.
[5] NA, not available. Comparison is not meaningful, as the number of observations with perch as the starting location is not equal between aviary styles.
[6] Significant interactions are illustrated in Figures 2, 3, and 4.
Aerial locomotion, perching rate, and WLB were zero inflated and therefore analyzed binary (occurrence yes/no). Raw values (mean, max) are provided for binary outcomes variables. Bolded *p-values* indicate significant interactions or significant main effects not driven by an interaction according to the Wald chi-square statistics. For significant main effects, a-b values with different letters are considered statistically different.

TABLE 4 Model estimates $\pm$ SEM, and *p-values* of generalized-/linear mixed models fitted to behavior variables in the open phase.

Open Phase	Aerial locomotion		Perching		Perching duration[1]		DLB	WLB
	estimate [probability]	raw data [rpm] [2]	estimate [probability]	raw data [rpm]	estimate [prob. > 1 minute]	raw data [sec]	estimate [rpm]	estimate [count/5min]
1st, 3rd Quartile		0.00, 0.20		0.00, 0.20		26.0, 300		
Median		0.00		0.00		106.0		
Distribution	binary		binary		binary		normal	Poisson
Aviary	*P=0.11*	*mean, max*	NA[3]	*mean, max*	*P=0.09*	*mean, max*	*P=0.1*	*P<.0001*
Low	0.18 ± 0.04	0.26, 0.8	0.18 ± 0.08	0.21, 0.4	0.97 ± 13.16	202.8, 300	0.87 ± 0.10	4.22 ± 0.14
Mid	0.29 ± 0.05	0.33, 0.8	0.46 ± 0.12	0.29, 0.8	0.39 ± 0.11	122.4, 300	1.13 ± 0.10	5.35 ± 0.16
High	0.25 ± 0.04	0.33, 1.4	0.12 ± 0.05	0.24, 0.6	0.39 ± 0.00	148.7, 300	1.1 ± 0.95	5.38 ± 0.14
Strain	**P<.0001**		**P=.0007**		*P=0.36*		**P<.0001**	*P<.0001*
Brown	0.18 ± 0.02[a]	0.28, 1.0	0.17 ± 0.04[a]	0.24, 0.6	0.84 ± 40.32[4]	162.1, 300	0.93 ± 0.06	4.53 ± 0.10
White	0.31 ± 0.03[b]	0.34, 1.4	0.27 ± 0.06[b]	0.27, 0.8	0.43 ± 0.08	137.9, 300	1.17 ± 0.06	5.58 ± 0.12
Age	**P=0.03**		*P=0.66*		*P=1*		**P<.0001**	*P<.0001*
wk 7	0.30 ± 0.04[a]	0.35, 1.4	0.19 ± 0.05	0.30, 0.8	0.48 ± 0.10	144.8, 300	1.37 ± 0.07[a]	6.92 ± 0.18
wk 11	0.23 ± 0.03[ab]	0.31, 1.2	0.23 ± 0.05	0.24, 0.6	[3]0.84 ± 45.2	144, 300	1.00 ± 0.06[b]	4.91 ± 0.13
wk 17	0.21 ± 0.03[b]	0.30, 1.2	0.22 ± 0.05	0.25, 0.8	0.47 ± 0.09	156, 300	0.87 ± 0.06[b]	4.16 ± 0.12
Interactions[5]								
aviary:strain	*P=0.14*		*P=0.09*		*P=0.85*		**P=0.009**	*P<.0001*
aviary:age	*P=0.89*		*P=0.73*		*P=0.74*		*P=0.97*	*P=0.07*
strain:age	*P=0.44*		*P=0.68*		*P=0.34*		*P=0.11*	*P<.0001*
aviary:strain:age	*P=0.33*		*P=0.07*		*P=0.55*		*P=0.11*	**P<.0001**

[1] Reduced data set excluding observations where no perching occurred. Estimate is the mean probability of a perching bout exceeding one minute.
[2] rpm= rate per minute.
[3] Comparison was not meaningful (p-value not available [NA]), as the number of observations with perch as the starting location was not equal between aviary styles.
[4] A large variation made this estimate unreliable and overexaggerated the standard error.
[5] Significant interactions are illustrated in Figures 4 and 5.
Raw values (mean, max) are provided for binary outcomes variables. Aerial locomotion, perching rate and duration were zero inflated and therefore analyzed binary (occurrence yes/no or perching duration longer exceeding 1min yes/no). Bolded *p-values* indicate significant interactions or significant main effects not driven by an interaction according to the Wald chi-square statistics. For significant main effects, a-b values with different letters are considered statistically different.

analyzed as counts per minute. Fixed effects were aviary style, strain, and age, as well as all interactions. To make sure that birds living in the same housing unit would not be considered as independent from each other, the following random effects structure were incorporated into the analysis: i) start location (label number) nested in aviary style and flock to account for repeated measures in each compartment or aviary, ii) brooding compartment (for analyses of brooding phase) nested in aviary style and flock to account for the replication within each flock, iii) age nested in flock to account for the repeated measures taken at different timepoints within the same flock. All models were evaluated through visual assessment of model assumptions (homoscedasticity: Tukey-Anscombe plot and normalcy of residuals: Q-Q plot). Explanatory variables were tested with a Wald Chi-Squared test to establish the main effects explained in the model (Tables 3 and 4). The significance level was set at p<0.05. Post-hoc analyses were done for relevant pairwise comparisons using Tukey tests with Bonferroni

corrections of p-values for multiple testing and calculating odds ratios (OR) for binomial models. OR were considered significant if the 95%-confidence interval (CI) did not include '1'.

Aviary style was not included in the model for perching frequency, as the proportion of total pre-determined starting locations on perches varied between aviary styles (Figure 1), although it was considered in interactions when assessing effects of strain based on housing type. Perching duration followed a bimodal distribution with one narrow mode ranging from one to 60 seconds, peaking at five seconds (152 observations) and the second narrow mode at 300 seconds (233 observations). Hence, data were analyzed as the probability of perching duration exceeding 60 seconds (binary: perching duration = '0' ≤ 60 sec > '1'). Due to the rarity of group-runs in aviaries of *Low* or intermediate (*Mid*) complexity, a meaningful statistical analysis was not possible, but descriptive statistics for the *High* complexity aviary are provided.

Inter-observer reliability for focal bird sampling was assessed by calculating kappa statistics with Observer (The Observer® XT by Noldus, Version: 14.2) for behavior durations and frequencies.

3 Results

Inter-observer reliability for behavioral observations was almost perfect for behavior durations (mean kappa=0.93) and moderate for frequencies (mean kappa=0.78) (interpretation based on McHugh, 2012).

3.1 Brooding phase (weeks 1-6)

Model outputs and main effect estimates are described in Table 3.

3.1.1 Aerial locomotion

The probability of aerial locomotion was best explained by a three-way interaction of aviary style, strain, and age (p = 0.004, interaction Figure 2). White birds were more likely to perform aerial locomotion than browns in *High* during wk 1 (p<0.0001) and in *Low* during wk 5 (p = 0.008). Generally, the probability of aerial locomotion increased with age from wk 1 to 3, then decreased for browns from wk 3 to wk 5 in *Low* and *Mid* and stayed the same for whites (see Figure 2). During wk 1, whites were more likely to perform aerial locomotion in *High* than in *Mid* (p= 0.03).

3.1.2 Perching

Chicks' probability of being on the perch was affected by an interaction of aviary style and age (p<0.0001, Figure 3A), and by a strain effect (p= 0.04). White chicks had higher odds of perching

than brown chicks, although the 95%-CI of the OR indicates that the odds are only marginally different (OR=1.18, CI= 1, 1.4). Perching probability increased with in *Mid* and *High* (p<0.01) but in *Low*, odds of perching increased between wk 1 and wk 3 (p<0.0001) and then decreased between wk 3 and wk 5 (p=0.001). During wk 1 and wk 3, *High* chicks were less likely to perch than chicks in *Low* (wk1: p= 0.0001, wk3: p<0.0001) or *Mid* (wk1: p= 0.0004, wk3: p= 0.004). Perching duration was explained by a three-way interaction of aviary style, strain, and age (p= 0.009). Generally, perching duration increased with age and was longer in browns than whites (see Figure 3B).

3.1.3 Load bearing behavior

DLB frequency was affected by aviary style (p<0.0001), strain (p<0.0001), and age (p= 0.04). Chicks from *High* complexity aviaries performed more DLB than *Low* (p=0.006) and *Mid* chicks (p= 0.009). White chicks performed more DLB than browns. There were no statistical differences in DLB between the different ages in the post-hoc analysis (p>0.1). The probability of WLB was affected by an interaction of aviary style and age (p= 0.003) and an interaction of aviary style and strain (p= 0.04). WLB became more frequent by wk 3 relative to wk 1 but decreased in *Low* birds in wk 5 (Figure 4A). In *High*, white chicks had higher odds of WLB than browns (p= 0.02), while no strain differences were observed in *Low* (p=0.1) or *Mid* (p= 0.4, Figure 4B).

3.1.4 Group runs

During the brooding phase, 258 group run participations were recorded. No brown chicks in *Low* were observed in a group run, and this behavior was only observed in 0.3% of the total observations in the white chicks. In *Mid*, group runs were recorded in 2.5% of the observations of browns and 2.3% of white chicks. In *High*, 39.9% and 38.7% of total observations included group runs in the browns and white chicks respectively.
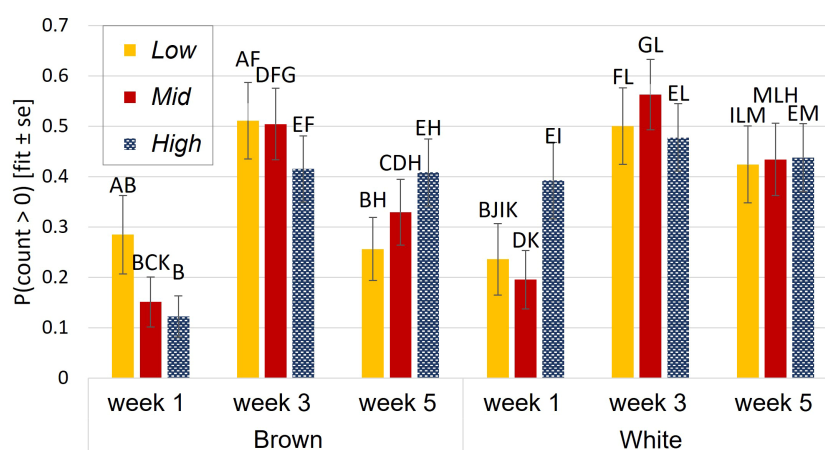

FIGURE 2
Aerial locomotion during brooding. Probability of aerial locomotion was affected by an interaction of aviary style complexity, strain, and age. Y-axis: the estimated probability of aerial locomotion and standard errors of the estimate. Different letters indicate statistical differences with p<0.05.
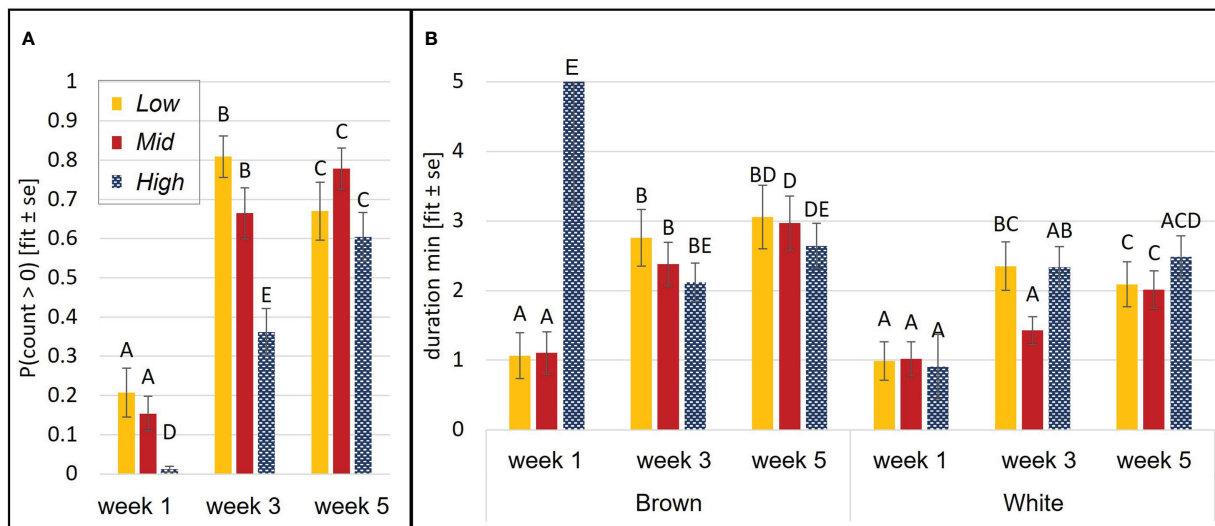
FIGURE 3
Perching behavior during brooding. Probability of perching was affected by an aviary style complexity by age interaction (A). Duration was affected by an interaction of aviary style complexity, strain, and age (B). Perching duration of brown *High* chicks in week 1 is likely overestimated due to a low n and large variance in this group. Y-axes: model estimates and standard errors. Different letters indicate a statistical difference with p<0.05.

3.2 Open phase (weeks 7-17)

Model outputs and main effect estimates are described in Table 4.

3.2.1 Aerial locomotion

The data show that strain (p<0.0001) and age (p= 0.03) impact the probability of observing aerial locomotion. White pullets had 1.95-fold higher odds of performing aerial locomotion than brown pullets (CI = 1.51, 2.53). Pullets had lower odds of performing aerial locomotion during wk 17 compared to wk 7 (OR = 0.68, CI = 0.49,0.95).

3.2.2 Perching

There was a strain effect on perching probability (p = 0.0007), with white pullets having 1.41-fold higher odds of perching than brown pullets (CI = 1.1,1.82). Perching duration was not affected by any of the main effects or their interactions in this phase.

3.2.3 Load bearing behavior

DLB was affected by an interaction between aviary style and strain (p= 0.009, Figure 5) and by age (p<0.0001). White pullets in *Mid* and *High* performed DLB at higher rates per minute (rpm) than browns (*Mid*: p= 0.0003, *High*: p= 0.0001, *Low*: p= 0.55 Figure 5) and more than *Low* white pullets (*Mid*: p= 0.02, *High*: p= 0.03). There was no aviary style effect on DLB rpm in browns (p>0.9). Pullets also performed more DLB during wk 7 than in wk 11 (p= 0.006) and wk 17 (p=0.0005). WLB frequency was explained by a three-way interaction of aviary style, strain, and age (p<0.0001). White pullets performed less WLB in *Low* than in *Mid* or *High* (p<0.0001 for all pairwise comparisons) (Figure 4C). Brown pullets housed in *Low* and *Mid* performed more WLB than

High during wk 7 (*Low*: p= 0.01; *Mid*: p= 0.008). During wk 11, this trend was reversed, whereby *High* pullets exhibited higher WLB than *Low* (p= 0.03, Figure 4C), and in wk 17, with more WLB in *High* than *Mid* (p= 0.04). The frequencies of WLB behavior generally decreased across all ages for brown pullets in all systems. For white pullets, frequencies of WLB generally decreased from wk 7 to wk 11 in all systems with similar values during wk 11 and wk 17 (Figure 4C).

3.2.4 Group runs

During the open phase, group runs were indiscriminately low with 23 occurrences in total (browns: *Low*=0.6%, *Mid*=0.5%, *High*=2.7%, whites: *Low*=0%, *Mid*=1.6%, *High*=4.3%).

4 Discussion

The present study aimed to assess whether chicks housed in spacious and complex brooding compartments exercised and perched more compared to chicks housed in less spacious and complex compartments during the brooding phase. The data demonstrate that increasing the brooding compartment complexity increased some forms of exercise, namely dynamic load-bearing behavior (DLB) and wing-involved load-bearing behavior (WLB); however, increasing complexity decreased the use of perches. Pullets raised in high-complexity brooding compartments were hypothesized to perform more forms of exercise and use perches more frequently during the open phase, and the data partially support this hypothesis for white birds (most WLB in *High*) but not for brown birds. Finally, whites generally exercised and perched more frequently than brown-feathered chicks and pullets in all three styles of rearing aviaries.
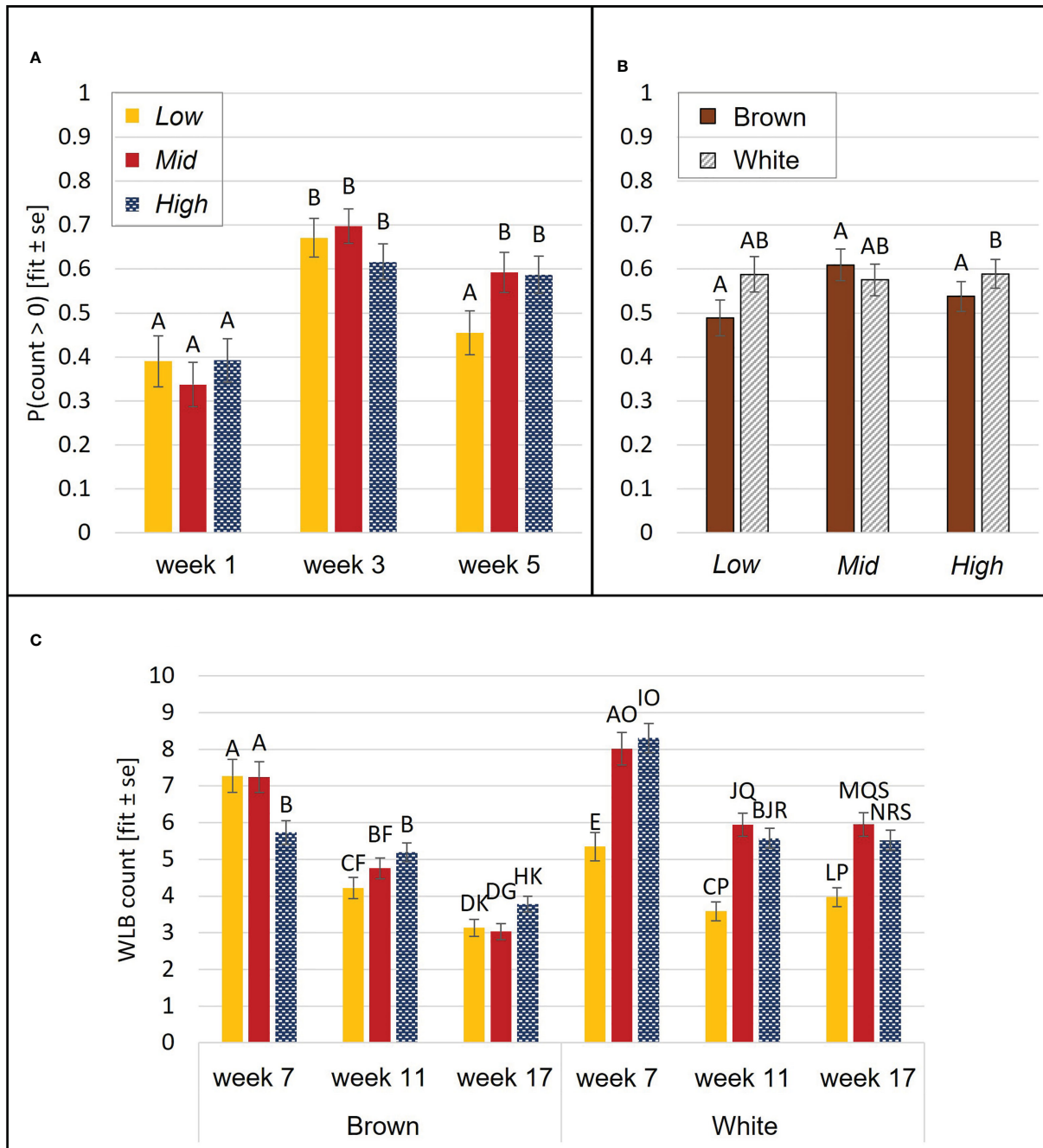
FIGURE 4
Wing-involved load bearing (WLB). During brooding, the probability of WLB was affected by an aviary style complexity by age interaction **(A)** and an aviary style complexity by strain interaction **(B)**. During the open phase, count (per 5 min observation) of WLB was affected by an interaction of aviary style complexity, strain, and age **(C)**. Y-axes: model estimates and standard errors. Different letters indicate a statistical difference with p<0.05.

Group runs were assessed with descriptive statistics only, but effects were clearly apparent as group runs were almost exclusively observed in the brooding compartment with *High* complexity, similar to data reported from commercial systems (Pufall et al., 2021). Since running requires less balancing skills compared to walking (Muir et al., 1996), chicks are better suited to run due to disproportionally large heads and short, thin legs. The benefits of group running in chicks are unclear, but it does appear to be the most vigorous form of locomotion at that age, and as it is usually

accompanied by wing flapping, it has the potential to strain long bones and the keel simultaneously.

Aerial locomotion increased after the first week and was generally highest during week three. There was a decrease in aerial locomotion for browns in *Low* complexity by the end of brooding, which could possibly be attributed to the birds growing and space becoming limiting. In the open phase, white-feathered birds performed more aerial locomotion than brown-feathered birds, but it decreased over time in all housing systems. The
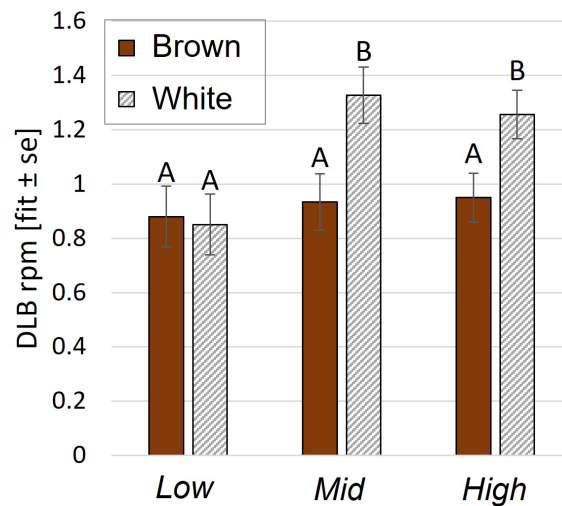
FIGURE 5
Dynamic load bearing behavior during the open phase. Rate per minute (rpm) of DLB behavior was affected by an aviary style complexity by strain interaction. Y-axis: Model estimates and standard errors. Different letters indicate a statistical difference with p<0.05.

literature corroborates these age (Kozak et al., 2016b; Pufall et al., 2021) and strain effects (Kozak et al., 2016a; Chew et al., 2021b; Pufall et al., 2021), and on commercial rearing farms, aerial locomotion was observed least in *Low* complexity systems (Pufall et al., 2021).

Perching was observed more often in *Low* and *Mid* complexity brooding compartments than in *High* in weeks one and three of brooding which was unexpected. A possible explanation could be that the *High* complexity proved to be too difficult to navigate for the young chicks though further investigation would be required to confirm this hypothesis. In addition, fewer but longer perching bouts were observed in browns than whites during brooding and whites perched more often in the open phase. Greater use of perches in white pullets than browns was also found in furnished cages (Habinski et al., 2017b), indicating consistent strain differences in perching independent of housing system.

Load bearing and potential skeletomuscular consequences

Similar to aerial locomotion, DLB and WLB increased during brooding (albeit not significantly for DLB) and decreased towards the end of rearing. As pullets age, space for locomotory and loading behavior becomes restricted at commercial densities. At the end of the brooding phase, *Mid* and *High* complexity brooding compartments allowed for more WLB than *Low* and chicks in *High* complexity compartments exhibited more DLB than chicks in *Low* or *Mid* for the whole of brooding. White birds performed more WLB than browns in *High* throughout brooding and more WLB and DLB in *High* and *Mid* during most of the open phase. Consequently, during the open phase, it was predominantly the white pullets that took advantage of the opportunities for WLB in *High* and *Mid* aviaries. This corroborates data from commercial farms, where the highest prevalence of locomotory behavior was between 9-10 wk of age, with more locomotion observed in *High* systems and in white versus brown pullets (Pufall et al., 2021).

Muscle and bone strength is expected to increase in complex aviaries which encourage more exercise. Based on the current data,

a more pronounced effect is expected in white pullets based on greater exercise levels, unless they reached a plateau in the response to bone loading where they no longer benefited from more bone loading behavior (Frost, 1987). On commercial farms, pullets from *High* had stronger leg bones than those raised in *Low* with no differences in leg or breast muscle mass or keel bone (KB) size (Pufall et al., 2021). Ross (2021) studied skeletal profiles of the birds used in the present study and found that white and brown pullets had stronger humeri (i.e., wing bones) in *High* complex brooding compartments relative to *Low* complex ones. Interestingly, no effect of aviary design was observed on the KB area (Pufall et al., 2021; Ross, 2021). It is possible that aviary-reared pullets may experience sufficient keel loading independently of aviary design. Unexpectedly, Ross (2021) reports that, at the end of the brooding phase, white pullets had larger length and radius of long bones (femur, tibia, humerus, radius) in *Mid* compared to *Low* and *High*. They hypothesized that the restricted horizontal space in *Mid* brooding compartments compared to *High* might force chicks to perform more jumping (e.g., aerial locomotion) to make use of the platform, however, this was not confirmed by the observational data on aerial locomotion presented here.

Based on differences in activity levels observed in the present study, white and brown pullets are expected to exhibit distinct skeletal characteristics, specifically, stronger keel and long bones in whites than in browns. The literature reports that in comparison to brown pullets, white pullets having stronger leg bones and larger keel areas in proportion to their body weight in all three rearing aviary designs (Pufall et al., 2021) and furnished cages (Fawcett et al., 2020), and they have proportionally thicker and longer tibiae in floor pens (Chew et al., 2021a). Post-mortem analysis of pullets from the present study at the end of brooding and open phases revealed skeletal differences between brown and white pullets, with whites having stronger femurs at the end of rearing and proportionally larger keel areas (Ross, 2021). However, browns had a higher bone mineral density (Ross, 2021). The WLB observed

in these studies was positively associated with the skeletal development of the humerus, and strain differences emulated differences found in keel size.

Early sensitive window? Previous work suggests an early-life sensitive window for developing spatial tasks, such as perching behavior (Appleby and Duncan, 1989) and navigation in 3-D space (Gunnarsson et al., 2000). Our data support this theory for white feathered laying hen development. We propose that the opportunity for engaging in spatial tasks (via complex housing during brooding) and birds' genetic background influence space use and behaviors during this sensitive developmental window. To confirm this hypothesis, candidate traits must be assessed at the end of rearing or during lay to test whether the design of the brooding compartment influences behavioral development.

4.1 Limitations and future directions

A more refined analysis of some recorded behaviors may have elucidated impacts of aviary design, bird strain and age. For example, distinguishing between upwards and downwards transitions and aerial locomotion with or without wing use (e.g., jumps) may demonstrate housing impact on a subset of behaviors. In the present study, WLB may be overestimated as it included aerial transitions without wing-use by default. Furthermore, separating upwards from downwards transitions, would indicate whether birds use wing-assisted locomotion predominantly for one or the other.

The unequal population sizes and system dimensions between the different aviary designs could not be accounted for in the statistical models and therefore was another constraint of the study by limiting comparison between treatment groups with differing space allowances and total floor space. It is notable, however, that such differences are also found in commercial settings that use the three aviary designs, so even if these factors cannot be disentangled, we would still argue that our findings are applicable to commercial settings. A strength of this paper is that it followed a field trial, thereby allowing comparison of a controlled experiment to a practical commercial setting, lending external validity to the conclusions drawn. Birds form this study were also used for further skeletomuscular analysis (Ross, 2021).

5 Conclusion

Rearing aviary design was most influential during the brooding phase, where brooding compartments differed substantially in their size and complexity. Once the brooding compartments were opened, housing design almost exclusively affected the behavior of white pullets. Overall, white chicks and pullets made more use of the available space. The data indicate that the optimal rearing aviary design depends on the genetic strain, as brown pullets appear not to utilize the spatial opportunities of high-complexity aviaries to their full extent. Further investigation of effect of the brooding compartment and open phase design on the development of pullet's spatial skills and their response to specific bone strains is needed to inform pullet rearing practices aimed at improving laying hen welfare in aviary housing.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://doi.org/10.5683/SP3/NAHIK3, borealis.

Ethics statement

The animal study was reviewed and approved by University of Guelph Animal Care Committee.

Author contributions

Study conception and design: TW, AR. Data acquisition and analysis: AR. Interpretation of data: All authors. Drafting of Manuscript: AR. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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