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A first approach to investigate characteristics of piling behaviour in individual laying hens

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ABSTRACT

Piling behaviour is defined as the dense clustering of laying hens and is frequently observed in loose-housed layer flocks. Despite these widespread reports, there is a lack of experimental evidence detailing how piles are formed, maintained, and dissolved, and sometimes lead to suffocation. The current effort sought to assess individual roles within the formation and dissolution of piles in an experimental setting previously shown to result in frequent piles. Our study used a commercially available radio frequency identification system in combination with video recordings to monitor the spontaneous formation of individual piles and determine which birds were involved over three specific days of a 14-week period. We predicted that involvement in the pile, as well as latency and duration, would be repeatable measures. A total of 210 piles were detected by video across eight pens and three observation days, of which 89 piles were used following our own exclusion criteria. A total of 86 hens (out of 568 in the study) were never recorded on the antennas during piles occurring in the three video focus days suggesting they did not participate in piles on these days. Random regression models were used to analyse individual variation in plasticity of piling behaviour. Repeatability of piling characteristics was also calculated. Piling frequency, duration, and latency to join were affected by age, though only the former two were associated with time of day. Hens were repeatable in how often they piled (pile frequency: $R = 0.134$ [0.074, 0.157]) and their duration in the pile ($R = 0.106$ [0.065, 0.146]), although repeatability estimates for both characteristics were low. Birds were not consistent in their latency to join an existing pile ($R = 0.033$ [0.002, 0.064]). We believe the current manuscript is the first to demonstrate repeatability in piling behaviour, a finding that has important implications for reliably establishing causal factors, as well as long term solutions including targeted breeding.

1. Introduction

Piling has become a large problem within cage free housing systems where occurrences can lead to high mortality (>30 hens per day), likely as a result of suffocation with obvious welfare and economic implications. Piling behaviour is defined as the dense clustering of laying hens and is frequently observed in loose-housed layer flocks (Barrett et al., 2014; Gray et al., 2020; Winter et al., 2021, 2022a; Chowdhury et al., 2024). Despite these widespread reports, there is a lack of experimental evidence detailing how piles are formed, maintained, and dissolved, and sometimes lead to suffocation. Gray et al. (2020) suggested that the initial accumulation of unevenly distributed hens could result from different conditions including attraction or repulsion from a stimulus, hen-specific routines, or even random events. Additional hens can then become attracted to this core pile causing it to grow. In support of this proposed process, detailed observations within commercial settings have indicated that there is often an identifiable cause associated with the pile's initiation such as a spot on the wall or person walking through the barn (Winter et al., 2021, 2022a). However, controlled experimental studies failed to induce piling with a frequency different than an adjacent control area (Winter et al., 2022b) confirming the difficulty in predicting or replicating the phenomena.

Under the assumption that piles begin with an initial stimulus leading to successive waves of individual hens joining the pile, it is also

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unclear if specific individuals are more likely to be involved in piles and, if so, whether they adopt particular roles such as initiator or follower. For comparison, feather pecking is believed to be a repeatable and heritable trait initiated by a relatively small subpopulation of hens (Piepho et al., 2017; Iffland et al., 2020) which then spreads through the general population. As with investigations of feather pecking, a key methodological element to determine if involvement and key roles are repeatable is the ability to observe individual hens longitudinally. Unfortunately, this is often difficult as traditional methods to distinguish and follow individual hens (e.g. marking or backpacks) often influence the animal's behaviour (Dennis et al., 2008). Alternatively, advances in sensor and computer vision technology offer the ability to track individual hens with minimal disturbance (Siegford et al., 2016; Yang et al., 2023). Our group (Rufener et al., 2018; Gómez et al., 2022; Montalcini et al., 2023c; a; Perinot et al., 2025) and others (Stadig et al., 2018; Ruhnke et al., 2019) have used such technology to demonstrate clear and consistent patterns in animal movement within their housing. Given that occurrence of piling is likely a combination of environmental (including key locations, as well as stimuli associated with those locations) and individual animal factors, tracking technology offers a unique solution to isolate these factors.

The current effort sought to assess individual roles within the formation and dissolution of piles in an experimental setting previously shown to result in frequent piles (Winter et al., 2022b). Our study used a commercially available radio frequency identification (RFID) system in combination with video recordings to monitor the spontaneous formation of individual piles and determine which birds were involved over three specific days of a 14-week period. Unlike our previous study, we used no specific stimulus to encourage the formation of piles. We also determined whether birds were present from the piles' beginning (i.e., latency to join) and the duration to remain in the pile. Based on the similar movement patterns mentioned earlier, we predicted that involvement in the pile, as well as latency and duration, would differ between individuals and be repeatable measures. We also were keen to determine how variation in these measures would vary at different ages within peak lay and time of day. As the frequency and duration of piling was recorded both at the pen (i.e., group) and individual levels, we hereafter refer to the group level metrics as group frequency and group duration.

2. Materials and methods

2.1. Ethical note and availability of data and code

Approval to conduct the study was obtained from the Veterinary Office of the Canton Bern in Switzerland (approval number BE104/2021). The experiment complied with the Swiss regulations regarding the treatment of experimental animals. All data and utilized code is available at: 10.17605/OSF.IO/DX6ZF within the Open Science Framework (OSF) platform.

2.2. Animals and housing

The study was conducted at the Centre for Proper Housing of Poultry and Rabbits (ZTHZ) in Zollikofen, Switzerland. White laying hen chicks (N = 600) of four distinct parental crosses identified by coloured wing tags attached in the hatchery were delivered at one day of age and reared in one pen of a semi-commercial rearing barn on site. Although parental crosses were used, estimates of heritability were not a goal of the study and not possible with too few sires represented. At 18 weeks of age, birds were transferred to an on-site experimental barn which was equipped with eight identical pens each holding 67–73 birds (N = 568 birds in total). Uneven group sizes were a result of mortality during rearing but otherwise unintended. Unused birds were sold commercially. Birds were separated according to the parental cross type (i.e., not mixed between crosses) resulting in two pens per parental crosses. Pens (9.0 ×2.0 m) were made of metal mesh wire with visual isolation between pens using opaque plastic sheets up to 1.5 m from the floor. Each pen was equipped with a grid area (4.0 ×1.5 m) above which three perches (each 4.0 m in length) were installed at three different heights. Group nests (Vencomatic, Krieger AG, Switzerland, size: 113.5 ×51.5 cm) were placed next to the grid area in a way that eggs could be collected from outside of the pen (Fig. 1). A drinker line was installed underneath the first perch and feed was provided using two automated feeding troughs running perpendicular to the grid area (Fig. 1). All pen furnishings (e.g., perches) were positioned in the middle of the pen in order to leave both pen sides open as corners were previously identified as areas prone for piles to occur (Winter et al., 2021). The areas where piling was expected to occur (and RFID and video data was collected) are hereafter referred to as piling areas. Litter was provided as wood shavings on the whole floor. Birds were fed a standard layer diet (Granovit AG) *ad libitum.* A lighting schedule based on the standard management guide was used with 10 h of light provided at 18 weeks of age and increasing to 15 h of light at 26 weeks of age. Lights were provided in each corner as well as in the middle of the pen with an onset period of five minutes in the morning and 20 min of dimming during the dusk period in the evening. The experiment continued until 35 weeks of age after which hens were either sold or used for a separate experiment. During population of the experimental pens, each bird received a pen-specific coloured leg ring (Fieger AG) in case it was found outside the pen and an adjustable leg band (Roxan, Selkirk, Scotland) containing a RFID transponder (Hitag S 2048 bits, 125 kHz, diameter: 4.0 mm, length: 34.0 mm) for individual tracking (described in section 3.3.2).

2.3. Data collection

2.3.1. Video observations

A combination of RFID technology and video observation was used to describe piling behaviour of individual hens. In order to detect piling behaviour as a first step, video cameras (Ipolis SNO-L6083RP, Samsung, Suwong, South Korea) were installed over both piling areas of each pen (i.e., two cameras per pen, positioned with an overhead view pointing towards the litter) covering an area of approximately 2 m^2. Video recordings were made continuously during the entire light period from 18 to 33 weeks of age using a customized video recording software (Multieye Hybrid Greenwatch, Artec Technology, Diepholz, Germany). After preliminary observations determined that piling was occurring regularly in all pens and inclusion of video observations would require limited observation days, we chose three single days (the first day of 20, 27 and 33 weeks of age) for which a single observer manually scanned the entire day to identify piles occurring in both pen corners. The chosen ages were based on previous work from our group that examined piling at 20 and 30 weeks of age (Winter et al., 2021a, 2022a). The definition of piling behaviour was adapted from Winter et al. (2021) and included the following criteria: 1) a minimum of three immobile hens (maximal movement durations > 5 s); 2) standing with overlapping body outlines ($> 25\%$ of the body outline with either other hens in the pile or pen wall); 3) facing in the same direction along the sagittal line; 4) not showing any other behaviours. Piles were recorded if all conditions were met and lasted for a minimum of 60 s. The end of a pile was defined as one of the criteria being absent for at least 30 s. The time cutoffs were chosen to eliminate brief collection of hens and instead focus on those with a motivated need to pile, e.g., a group of hens could find themselves clumped but then disentangle themselves, a phenomenon we believed would last less than 60 s and thus not be piling. For each detected pile, the pen side, date, time and pile information (i.e., start and end times, duration (calculated as end time – start time), and size (number of birds included in the pile by counting visible combs and/ or tails)) were recorded. All videos were analysed by the same person who was trained prior to video analysis. To assess reliability of video analysis, a set of 21 videos (30 min per video) recorded during the experiment was analysed

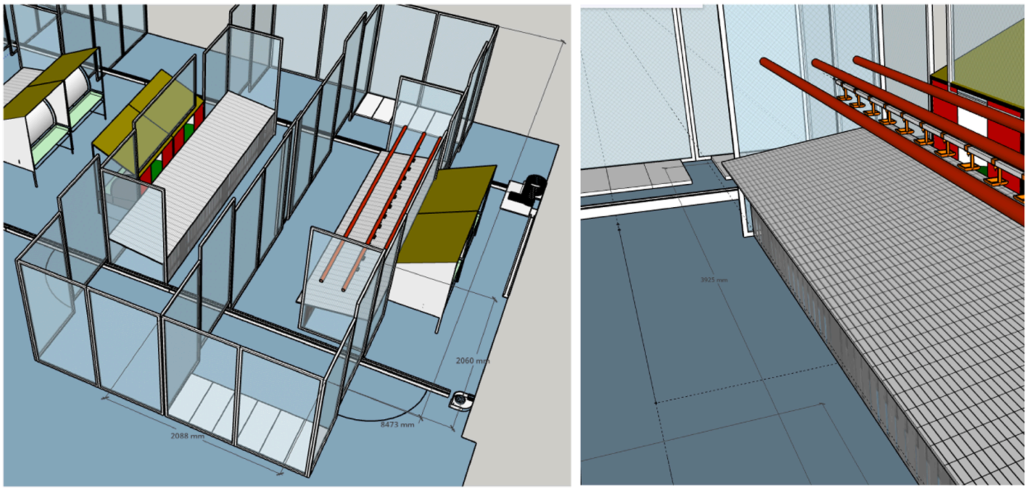

Fig. 1. Schematic of two pens used to hold animals. The nestbox and faecal pit were located at one side in the centre with the RFID panels shown as white rectangles on either extreme side of the pen. Each side of the pen was then further divided into two sections or quadrants containing three antennae i.e., six antennae per piling area and 12 antennae per pen.

by two independent examiners. Inter-observer reliability was assessed by calculating the intra-class correlation coefficient for the pile variables: start time, end time, and duration (IBM SPSS statistical package Version 29, SPSS Inc, Chicago, IL) based on a mean-rating ($k = 2$), absolute-agreement, and a two-way mixed-effects model. Reliability outcomes were very high for each variable (Table 1).

The final dataset resulting from video analysis included information on the number of piles occurring on the three days (i.e., one day each at 20, 27 and 33 weeks of age) per pen and specific pen-level information (pile number, duration, and number of birds per pile) to provide an overall picture of piling behaviour.

2.3.2. *Tracking of individual hens*

Tracking of individual hens was conducted using RFID technology (M1 antennae system, Gantner Pigeon Systems GmbH, Austria). At either pen side, six RFID antennae were placed on the floor to cover the extreme edges of the pen side (i.e., 12 antennae per pen, see Fig. 1). Antennas were covered with a wooden board (i.e., providing protection from bird faeces and so that staff could walk over the area) that was also covered with litter. The tracking system has been used in several studies and the exact functioning of the system is described elsewhere (Gebhardt-Henrich et al., 2014; Guerrero-Bosagna et al., 2020), including a validation of general movement in a commercial aviary (Gebhardt-Henrich et al., 2023). The antennae covered the piling area that was also visible to the cameras. The RFID antennas scanned for presence of leg band transponders in their immediate vicinity (~10 cm) in a continuous sweep every 1–2 s. Within each antenna, there were 12 uniquely identifiable registration coils that were evenly spaced (i.e., six on each side in a 2 ×6 grid formation) along the antennae's length. If a bird was standing on top of a coil at the time of scan, the transponder was energized, allowing the transponder's unique identification number to be read by the antenna alongside the associated time stamp and the ID of the antenna in a.csv log file on a connected computer (custom designed software "Chicken tracker", Gantner Pigeon Systems GmbH). In normal function, the individual coil-level data is not reported, i.e., only presence of transponders on the antennae unit is recorded. After discussion with the manufacturer, they adapted the firmware to provide the additional coil-level information so that each registration included the antennae identifier as well as the registering coil. The recording interval of each antenna was set to 10 s, though repeated detections of the same transponder/antenna pair were suppressed for 10 s instead of being reported every sweep to keep the data throughput manageable. As a consequence, not every change in coil was reported. If a transponder's position was not reported again within 15 s, i.e. within five seconds after the same-antenna suppression, the bird was marked in further data processing as non-detected from that point until the next positive detection. Additionally, while every detection is certain (the bird was near that coil within approximately 2 s of that time), lack of a record does not indicate with certainty that the bird is not on a trackable position due to limitations of the system. For instance, we believe the coils can become overwhelmed with too many transponders in close proximity leading to individual transponders being cancelled out. The RFID system was continuously running for the entire experiment, i.e., 20–33 weeks of age.

Table 1
Intraclass correlation coefficients and 95% confidence intervals for pile start and end time, and duration across two independent observers.

Variable	Intra-class correlation	95% confidence interval Lower bound	Upper bound	F	P-value
Pile start time	1.0	1.0	1.0	2.92×10^6	< 0.001
Pile end time	1.0	1.0	1.0	1.01×10^5	< 0.001
Pile duration	0.95	0.87	0.98	21.19	< 0.001

2.3.3. *Combining video with RFID data*

Video recordings were integrated with RFID data to identify individual hens in each of the detected piles. Each pile detected on video was divided into five timepoints using the video's timestamp T1 (start of the pile, i.e., the time after which all criteria were met for at least 60 s); T5 (end of the pile, i.e. when at least one criterion was not met), as well as 25%, 50%, 75% through the pile duration (i.e., T2, 3, and 4, respectively). For each timepoint within a piling event, the location of the piling animals in relation to antennae and coil was noted. The location was noted by dividing the piling area of each pen side into two sections or quadrants. Each quadrant contained three antennae i.e., six antennae per piling area and 12 antennae per pen. A coordinate grid made up of 36 coordinates (6 ×6; corresponding to the estimated position of the antennae coils) was layered over each quadrant on the video screen and used to identify the centre of each pile at each timepoint. The centre was also used to calculate each hen's Euclidean distance from the centre based on the detected coil. Based on the detailed video analysis, the final dataset also included for each pile at each timepoint: pile centre (i.e., grid coordinates), associated time stamp, antennae at which piles were detected, maximum radius of piles (cm) in relation to grid coordinates, and number of birds in the pile. Based on this information, the log files produced by the RFID system were extracted to identify bird IDs recorded on the antennae for each bird within pile timepoint. To validate whether the number of piling birds per timepoint and location matched between video and RFID data, values were compared for each timepoint and the difference calculated (i.e.: # of birds from video – # of birds from RFID). During analysis, it became apparent that the time stamps from the video and RFID system were not synchronized as the former was added by the camera and not the associated computer. To correct for this offset, on each day in which analyzed piles occurred and for each camera, videos were analyzed to detect a distinct movement across antennas and synchronized with a similar pattern in the RFID data. The effort produced an offset value in seconds that aligned RFID and video timestamps.

2.3.4. Data processing

From the combined video and RFID dataset, a code was written to extract all birds considered to be on antennae when and where piles occurred, as well as within the recorded maximum distance to the centre for each pile and time point. Since no pile moved between antennae, the identified centre was valid for all timepoints and in calculation of maximum pile size. In practice, this meant that the last detection of the transponder prior to the time point had to be on coils close to the pile centre and less than 15 s before the time point, or else the bird would be marked as non-detected. After that, the code ordered all transponders by distance to the centre of the pile on the 6 × 6 grid based on the last reported coil. If there were more distinct transponders than expected from video data, the assumption is transponders furthest away are not in the pile. When examining the number of birds assigned to a pile event at each time point in comparison to the number gathered from synchronized video, the pile was not included for analysis at the individual level if there was a difference greater than two hens. Based on this validation, we ultimately used 89 out of 210 detected piles.

Latency to pile for individual hens was defined as when the hen was first detected during the pile period within the defined distance (i.e., maximum pile radius as determined above) to the centre minus the start time of the pile. Duration in a pile for individual hens was equal to the sum of all durations where the hen is considered detected in the pile (considering non-detection-after-15-seconds intervals). Frequency was defined as presence during at least one time point of a pile.

Additional information was collected including exact timestamp of when hens were first and last registered on antennae where piles occurred, how many birds were already piling at the time when a specific hen joined a pile, and other data. However, given the uncertainty of the dataset and the large number of piles that had to be rejected due to differences with counts of pile size from video data, we decided to be more conservative and not use this data.

2.4. Production

Production parameters collected from hens between 18 and 33 weeks of age included daily numbers of eggs laid and dead birds per pen from which hen-day egg production was calculated (Supplementary Table 1). Production data are presented as descriptive data only to confirm animals were in a good state of health.

2.5. Statistical analyses

All analyses were conducted in R (version 4.2.1, R Core Team, 2022) with RStudio as the user interface (Posit Team, 2022).

2.5.1. Pen level analyses

A total of 210 piles were detected by video across the eight pens and three observation days. Pile characteristics obtained at pen level included: pile frequency, duration, and size. As the main focus of the study was the piling of individuals, pen level data was assessed only descriptively with the average, minimum, and maximum values for each piling characteristic calculated according to pen and age. Reasons for exclusion of piling events included a difference of more than two birds between the RFID detected and video counted values. Pile characteristics of excluded vs. included pile events were average duration (309 ± 153 s vs. 223 ± 157 s) and average maximum size ((T2) –13 ± 6 birds vs. 9 ± 3 birds)).

2.5.2. Individual level analyses

The individual level analyses were based on the validated dataset of 89 pile events. For piling frequency, data were extracted including all birds (i.e., birds that were not piling as well as those that were) and all piles to determine how often a hen was involved in a pile. For duration and latency to join a pile, the data included only birds that were engaged in piling at a minimum of one piling event and at any given timepoint per pile, i.e., birds that never engaged in piling were excluded from the dataset. In order to investigate a time-of-day effect, the lighted period was divided into three phases, which differed depending on age as the light hours increased between 20 and 27 weeks of age. At 20 weeks of age, the lighted period was split into three, four-hour phases (i.e., 0500 – 0859, 0900 – 1259, 1300 – 1700). At 27 and 33 weeks of age, the lighted period was split into three, five-hour phases (i.e., 0215 – 0714, 0715 – 1214, 1215 – 1700). A total of 86 hens (out of 568 in the study) were never recorded on the antennas during occurring piles of the three video focus days suggesting they did not participate in piles on these days.

Individual variation in piling behaviour: Random regression models were used to analyse individual variation in plasticity of piling behaviour (see detailed protocol by Hertel et al., (2020)). For each response variable, we considered three models. One model, being the simplest, included: 1) a random intercept (ri) for hen ID only. A second model contained a random intercept for hen ID and a random slope (rs) over age. The third model contained a random intercept for hen ID (as in the second model) and time of day as a random slope. R codes are provided for all models. Models two and three (i.e., models including random intercept (ri) and random slope (rs)) were each compared to model one to determine which had the better fit. Fit was based on the Akaike Information Criterion (AIC) and conditional R-squared (R^2c) values, measures that represent the proportion of the variance for a dependent variable that is explained by an independent variable in a regression model. As the variable piling frequency had a Poisson distribution, the package MuMIn was used to calculate conditional R-squared values using the r-squaredGLMM function.

Repeatability of piling characteristics: Repeatability of each piling characteristic was calculated for the individual hen using the package rpt. Due to model convergence issues, the ri models were used for further analyses (also see below). We fit a Gaussian distribution for both latencies to join a pile and duration. A Poisson distribution was used for piling frequency (using rptPoisson as explained in Stoffel et al., 2017). As external factors may influence how consistent individual birds behave over time (Montalcini et al., 2023a; b), we also accounted for age. The repeatability estimate with 95% confidence intervals (CI) were based on 1000 bootstraps.

Effects of explanatory variables on piling behaviour: Similar to the repeatability estimation of piling characteristics, the ri models were used to analyse the effects of age and time of day on each piling characteristic. Data were analysed using generalized (GLMM) and linear mixed-effects (LMM) models applying the package lme4 (Bates et al., 2015). Model assumptions were checked visually using q-q plots for LMM and Dharma for GLMM to check for a normal error distribution and homoscedasticity of the residuals. Piling frequency was analysed with GLMM using Poisson as the underlying data structure; latency to join a pile was square-root transformed in order to meet model assumptions. Models (one model for each piling characteristic as response variable, i.

e., frequency, latency to join, and duration) were fit with age (categorical with three levels: 20, 27 and 33 weeks of age) and time of day (categorical with three phases: first, second, and third portions of the lighted periods) as explanatory variables. Hen ID nested in pen was included as a random term in each model. For the variables latency to join pile and duration, a pile-specific identifier was included as a crossed random term. Model comparisons were performed using a parametric bootstrap approach with the function "PBmodcomp" from the "pbkrtest" package (Halekoh and Højsgaard, 2014). P-values were obtained by comparing the full model, including all main effects, to models each reduced by one main effect only. Model estimates and their 95% confidence intervals (CI) were calculated for the full models and displayed using the package "effects" (Fox, 2003).

3. Results

3.1. Group-level characteristics

Group piling frequency, duration, and size values are reported in Table 2 across the three ages based on observations from the video-based data. Overall pen frequency rose to 14.1 piles at 27 weeks of age, a value double that of the other ages. The maximum number of piles observed was 18 piles in pen 5 at 27 weeks of age, although this pen also had relatively infrequent piles at the other weeks of age. Overall pile size increased reaching its largest overall value at 33 weeks of age. The largest average pen value was 18.7 in pen eight, which had the one of the lowest overall values of seven animals at 20 weeks of age. Overall average pile duration was greatest at 20 weeks of age with a value of 294 s although the 33 weeks of age value was similar at 290 s.

3.2. Piling frequency

Individual hens differed in how often they piled by age as shown when comparing the rs and ri models as the former had a better fit (AIC rs model: 5368 vs. AIC ri model: 5450). In addition, the better fit of the rs model was supported by the R-squared value showing that the rs model explained around 23.4% of the variability observed in the data compared to the ri model, which only explained 11.2%. Individual hens also varied in how often they piled by time of day as supported by the AIC and R^2 values (rs model: AIC value: 5373, R^2:16.0% vs. ri model: AIC value: 5450, R^2: 11.2%).

The analysis of the explanatory variables showed that piling frequency was affected by age ($p < 0.001$) and time of day ($p < 0.001$) (Fig. 2). Birds piled more at 27 weeks of age compared to the other two ages (model estimates and 95% CI for 20 weeks of age: 0.38 [0.31, 0.46]; 27 weeks of age: 0.60 [0.51, 0.71]; 33 weeks of age: 0.39 [0.33, 0.47]). Hens piled more often during the second and third phase of the day compared to the first (model estimates and 95% CI for first: 0.32 [0.26, 0.39]; second: 0.58 [0.49, 0.69]; third the light phases: 0.44 [0.37, 0.52]).

3.3. Latency to join a pile

Hens varied their latency to join a pile differently by age as the rs model had a better fit compared to the ri model (AIC rs model: 23148 vs. AIC ri model: 23229). The better fit of the rs model was supported by the conditional R-squared value showing that the rs model explained 39% of the variability compared to the ri model which explained 17%. The negative correlation between the random intercept and random slope of R= -0.99 (between 20 and 27 weeks of age) indicates that those hens that had a greater latency to pile (i.e., took longer to join a pile) at 20 weeks of age decreased their latency at 27 weeks of age compared to hens that already had a reduced latency at 20 weeks of age. In addition, the positive correlation of R= 0.85 between the random intercept and random slope for 27 and 33 weeks of age indicates that those hens that joined piles earlier at 27 weeks of age increased their latency more so at 33 weeks of age compared to hens that already had a longer latency at 27 weeks of age (Fig. 3). Individual hens did not vary in their latency to join a pile by time of day as supported by the AIC and R^2c values (rs model: AIC value: 23241, R^2c: 0.19 vs. ri model: AIC value: 23229, R^2c: 0.17).

The analysis of the explanatory variables showed that latency to join a pile was affected by age ($p = 0.009$) but not time of day ($p = 0.70$). Latency to join a pile decreased from 20 weeks of age to 27 weeks of age and 33 weeks of age (model estimates of the mean and 95% CI for 20 weeks of age: 27.8 [15.1, 44.4] sec; 27 weeks of age: 13.0 [6.6, 21.6] sec; 33 weeks of age: 13.1 [4.9, 22.3] sec, Fig. 3). Fig. 3 shows the effect of age and variation between individuals for latency to join a pile for Pen 1. As the pen effect was small, the plots showing latency to join an existing pile for the other pens are provided in the supplementary material. In comparison to age, latency to join a pile was not affected by time of day (model estimates and 95% CI for first: 13.3 [3.6, 29.0] sec; second: 16.3 [8.8, 26.1] sec; third light phases: 17.0 [8.8, 27.9] sec).

3.4. Pile duration

Hens varied their duration in a pile differently by age as the rs model had a better fit compared to the ri model (AIC rs model: 23145 vs. AIC ri model: 23279). In addition, the better fit of the rs model was supported by the conditional R-squared value showing that the rs model explained 51% of the variability observed compared to the ri model which explained 34%. The negative correlation between the random intercept and random slope of R= -0.99 between 20 and 27 weeks of age and R= -0.78 between 20 and 33 weeks of age indicates that those hens that had a long piling duration in 20 weeks of age decreased their piling duration more in both 27 and 33 weeks of age compared to hens that had a short piling duration in 20 weeks of age. The positive correlation of R= 0.87

Table 2
Pile frequency and characteristics including pile size at peak (i.e., number of hens involved per pile) and pile duration per pen and age of all video-observed piles. The mean values and standard deviations (SD) are presented per piling characteristic. The global values (last row) are presented as averages across all pens per week of age.

Week of age	20			27			33		
Pen	Frequency	Peak size ø ± SD	Duration (min)ø ± SD	Frequency	Peak size Ø ± SD	Duration (min)ø ± SD	Frequency	Peak size ø ± SD	Duration ø ± SD
1	10	10.5 ± 3.1	4:20 ± 00:53	16	11.75 ± 5.5	4:32 ± 02:25	8	14.25 ± 3.4	6:17 ± 03:02
2	4	9.75 ± 5.7	6:26 ± 04:11	14	13.93 ± 6.6	5:33 ± 02:35	9	15.2 ± 6.2	4:41 ± 02:24
3	0	NA	NA	16	11.68 ± 7.0	4:22 ± 02:23	9	16.78 ± 8.2	5:46 ± 02:09
4	7	10.7 ± 3.6	3:20 ± 02:14	10	6.8 ± 2.1	2:39 ± 01:33	5	11 ± 5.6	4:54 ± 03:04
5	2	10 ± 7.1	1:40 ± 00:56	18	9.2 ± 3.9	2:42 ± 01:17	3	16 ± 3.0	8:12 ± 03:22
6	7	10.6 ± 2.4	7:38 ± 01:52	12	8.9 ± 2.7	4:08 ± 02:45	7	15 ± 8.7	6:07 ± 02:56
7	7	11.4 ± 4.4	6:50 ± 01:55	13	9.8 ± 5.5	3:40 ± 02:35	9	13 ± 5.7	4:17 ± 02:32
8	4	7 ± 3.4	4:10 ± 04:13	14	9.3 ± 2.8	3:24 ± 02:20	6	18.7 ± 8.3	4:38 ± 03:16
Total	5.13	10	4:74	14.13	10.17	3:47	7	24.15	5:42

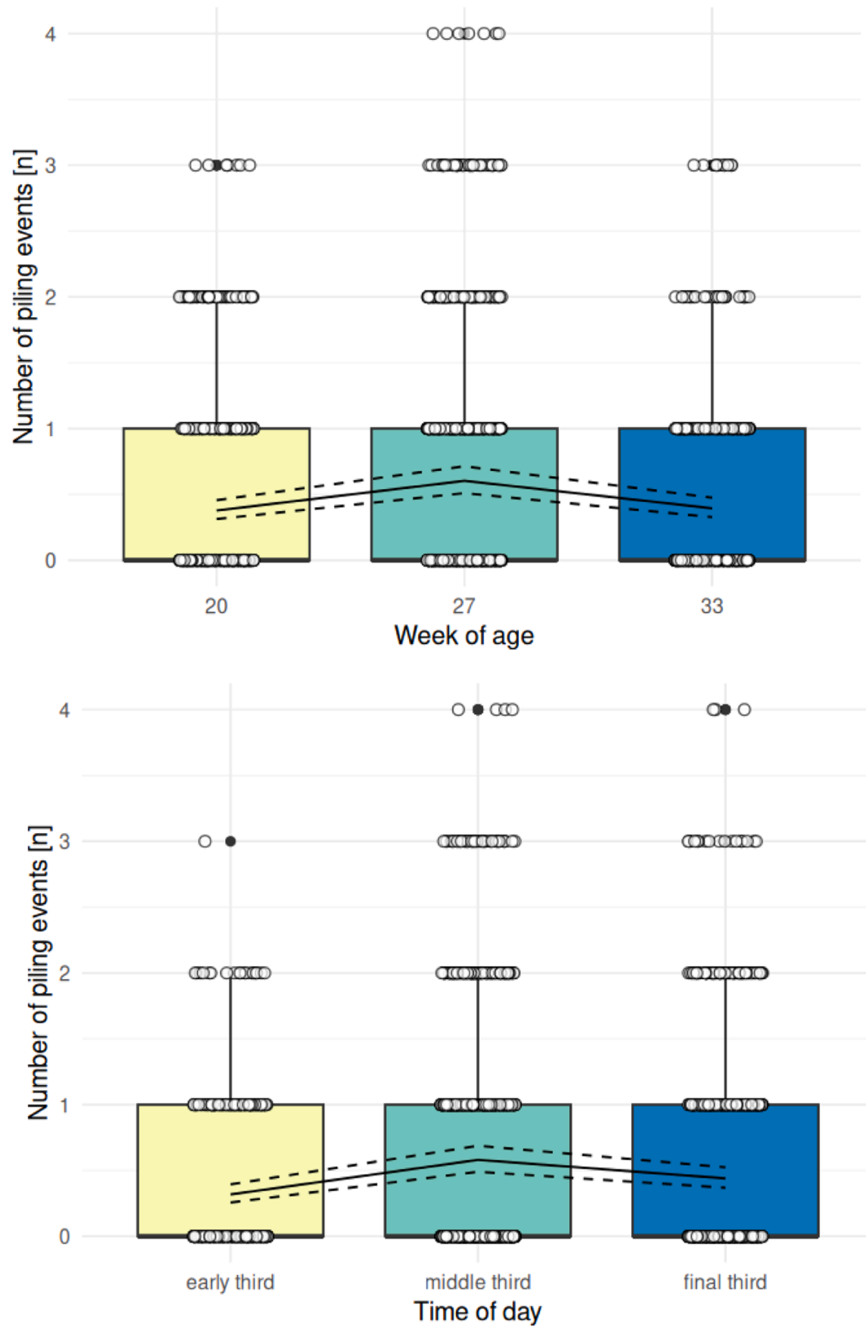

Fig. 2. The piling frequency of individual hens across week of age (top) and time of day (bottom). Boxplots represent distribution of raw data, white circles represent raw data of individual birds and solid and dashed lines represent model estimates and their 95% confidence intervals.

between the random intercept and random slope for 27 weeks of age and 33 indicates that those hens that piled for shorter durations at 27 weeks of age increased their duration in 33 weeks of age as compared to hens with a longer duration at 27 weeks of age (Fig. 4). Individual hens did not vary in their piling duration by time of day as supported by the AIC and R^2c values (rs model: AIC value: 23287, R^2c: 0.34 vs. ri model: AIC value: 23279, R^2c: 0.34).

The analysis of the explanatory variables showed that piling duration was affected by age ($p = 0.007$) but not time of day ($p = 0.11$). Piling duration decreased from 20 weeks of age to 27 weeks of age and increased again at 33 weeks of age (model estimates and 95% CI for 20 weeks of age: 94.9 s [66.1, 123.6]; 27 weeks of age: 76.0 s [62.7, 89.3]; 33 weeks of age: 111.4 s [83.4, 139.4], Fig. 4). In comparison to age, piling duration was not affected by time of day (model estimates and 95% CI for first: 83.1 s [54.6, 111.5]; second: 97.1 s [82.0, 112.2]; third portion of the light phase: 79.7 s [62.4, 96.9]).

3.5. Repeatability

Hens were repeatable in how often they piled (pile frequency: $R = 0.134$ [0.074, 0.157]) and their duration in the pile ($R = 0.106$ [0.065, 0.146]), although repeatability estimates for both characteristics were low. Birds were not consistent in their latency to join an existing pile ($R = 0.033$ [0.002, 0.064]).

4. Discussion

Investigations to date have shown that occurrence of piling at the

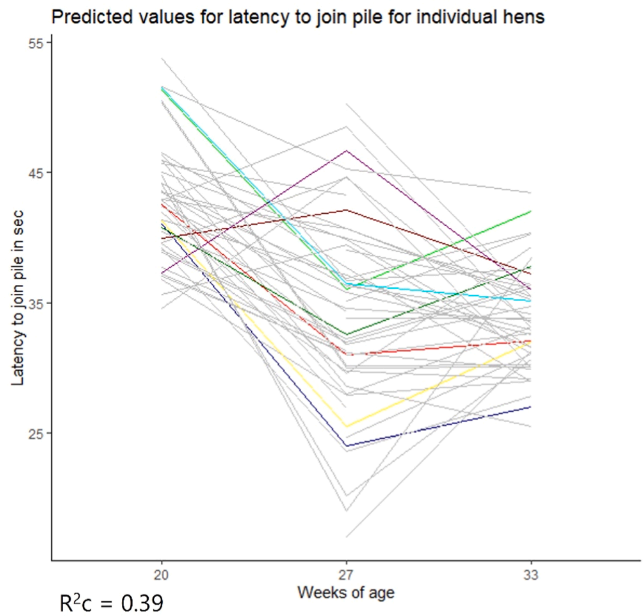

Fig. 3. The latency to join an existing pile (in sec) for week of age 20, 27 and 33 in a single representative pen. Predicted values are shown for pen 1 with each line representing an individual hen. Coloured lines represent examples of single hens for better visualization. The overall age effect ($p = 0.009$) is shown with varying responses by individual hens.

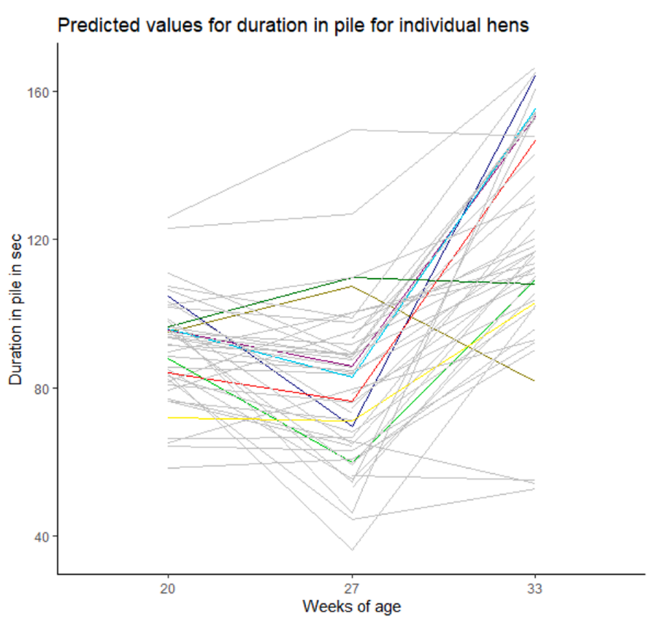

Fig. 4. The duration of staying in a pile (in sec) for week of age 20, 27 and 33 in a single representative pen. Predicted values are shown for pen 1 with each line representing an individual hen. Coloured lines represent examples of single hens for better visualization (same individuals coloured as in Fig. 3). The overall age effect ($p = 0.007$) is shown with varying responses by individual hens.

group level (using commercial flocks (Winter et al., 2021, 2022a) and experimental (Winter et al., 2022b) settings) are fairly consistent in terms of some characteristics, e.g., time of peak occurrence. The current study was undertaken to determine whether piling behaviour was repeatable within individual hens using relatively controlled experimental conditions with a combination of video observations and automated tracking. By allowing for monitoring of individual piling propensity, we can better appreciate the variation across individuals and over time, including subgroups of animals within the overall flock. We believe the current manuscript is the first to demonstrate repeatability, a finding that has important implications for reliably establishing causal factors, as well as long term solutions including targeted breeding. At the study's conception, our hope was that piling detection could be performed entirely with RFID technology by identifying a continued presence of animals with a high density on the antennae system. However, this was not possible due to the antennae becoming overwhelmed with the concentration of tags requiring us to develop the combination of video and RFID tracking protocol described.

Our results suggest that the frequency of an individual being present in a pile as well as their duration to remain in that pile were repeatable. Of interest was the correlations across ages where piling duration and latency to join a pile at 20 weeks of age was negatively correlated with values at 27 weeks of age, and that the age-specific pattern was unique to individuals. The reversal of this pattern with respect to age may offer important information as to the reasons for why hens pile. For instance, environmental factors such as differences in local (i.e., specific to a barn area) temperature have been proposed following on-farm observations (Campbell et al., 2016; Winter et al., 2021), although subsequent testing within a controlled setting proved no difference compared to a control area (Winter et al., 2022b). In this vein, others have proposed a role for social facilitation (Gray et al., 2020), which is supported by Winter et al. (2021) who observed that hens showing interest early on in some pen aspect (e.g., pecking at a screw in the wall) led others to also approach the area. In these scenarios, we could expect that an initial hen would be attracted to a stimulus or area which would then attract other hens. If we assume that shorter latencies indicate birds more likely to be attracted to the initial stimulus or area, it is feasible that the birds initially attracted at 20 weeks of age were no longer likely to be in that first wave of birds at 27 weeks of age as the novelty of the stimulus diminishes. However, those birds with shorter latencies at 20 weeks of age may still have been attracted nonetheless to the group of birds gathering (i.e., not the initial stimulus) at later ages in the area in a process initiated by other birds in the pen. Beyond the correlations which support this position, the repeatability of frequency and duration, but not latency, also support this explanation for variations across ages.

Alternatively, the repeatability of piling frequency and duration could suggest that the initiation of piling is more a consequence of random events or processes (Gray et al., 2020). Unlike the study by Winter et al. (2022b) which the pen design of the current study was based on, there was no effort to diversify pen areas. All pens were intended to be replicates with the same ventilation and lack of natural sunlight. If some animals were more prone to initiate piling, we would have expected repeatability in the latency to join a pile, i.e., higher values. The fact that this was not observed could indicate that piles are initiated by random hens. In this scenario, independent of who begins a pile (i.e., the latency to join), the consequent pile repeatedly attracts the same group of individuals who remain for their specific durations. Given this new information, future studies should consider repeatability towards key stimuli, such as novel objects or persons in the barn. Ideally such efforts could identify if there were consistency in individual hens' appearance at the stimuli and whether there are general characteristics which drive pile formation. To aid determining the role of social facilitation, it would also help to determine if hens that move together in non-piling situations are more likely to be found in the same pile. A growing body of research suggests that there are patterns of repeatable movements within hens and that groups of hens may even move together in subgroups (Gómez et al., 2022) and with variable, non-random associations (Perinot et al., 2025). Inclusion of this coordinated movement outside of piling events could suggest the piling stimulus is less important than remaining within a subgroup.

Despite the repeatabilities being statistically significant, their relatively low values compared to other studied behaviours in laying hens bring into question their biological significance. For instance, general and aggressive feather pecking were 0.44 and 0.22, respectively (Bennewitz et al., 2014). Focusing on movement specific variables, reported values have ranged from 0.24 to 0.81 for space use behaviors in relation to key events such as vaccination (Montalcini et al., 2023a) or 0.44 for a composite variable of movement in an aviary (Montalcini et al., 2023c). Durations in specific aviary areas (e.g., litter, wintergarden) had repeatability ranging between 19 and 53 and heritability from 5% to 27% (Makanjuola et al., 2024). Based on these comparative values, further work is needed to determine the accuracy of our reported piling characteristic repeatability values. We believe additional time points of observation would allow for greater confidence in the values, however the time required for this additional data was logistically not possible. Our focus was around peak lay based on previous efforts (Winter et al., 2021a, 2022a), though Campbell et al. (2015)indicated piling was highest at 75 weeks of age confirming the need for expanded observations. Methodological advances, such as use of machine learning techniques and computer vision (Valletta et al., 2017; Liu et al., 2024), will also aid efforts. We have sought to address some of these concerns towards our specific tracking challenges below.

We freely admit that our tracking methodology suffered from serious methodological issues. Besides that the majority of piles were not included in the individual analysis, the lack of individual hen identifiers that were visible during the recording (as with backpacks or some other system) made verification that hens were in the pile not possible. We had considered using some type of backpack system but were concerned that this would interfere with the hens' behaviour and affect their motivation to pile. Despite this lack of validation, the comparable durations and maximum pile sizes for excluded and included piles suggest our methods were reasonable. An additional problem had less to do with tracking technology but more as to how to define the behaviour of individual birds. While it would be clear that a pile was taking place from video footage, it was more difficult to classify whether individual birds were piling, especially for birds on the fringes. These birds could be motionless but facing the opposite direction or meeting the definition of piling for a brief (e.g., five seconds) period. Beyond our definition of piling at the group level, we also had to devise definitions for the individual level which is inherently more subjective and would be expected to have a large effect on the number of birds in the pile. For these reasons, while we believe our methods are important in suggesting that piling characteristics are repeatable, we also recommend continued efforts to improve how piling in individual birds is identified.

The current study was conducted in a controlled environment using relatively small groups of hens in contrast to the diversity and group sizes of actual commercial conditions. Beyond the aforementioned technical problems in determining individuals that were indeed piling, there are also concerns that findings lack external validity to commercial conditions. Although future efforts would benefit from being conducted in commercial conditions, patterns of piling in relation to time of day did match that of previous observations on farm, i.e., reduced appearance in the morning and more frequent piles at 27 weeks of age (Winter et al., 2021). We believe these patterns suggest that the repeatability of behaviour observed in the current study is relevant for commercial conditions. Moreso, given the need for phenotyping required within commercial breeding operations to be done in controlled settings that allow for focused observations with minimal disturbances to the hens, we believe our described techniques could aid the selection against piling behaviour.

5. Conclusions

Our study sought to identify the repeatability of piling behaviour within individual hens using a combination of automated tracking and video observations. Although our tracking system had clear methodological issues requiring a large number of piles to not be considered, the retained piles suggest that piling is repeatable in terms of frequency and duration, although not latency to join. Future efforts with improved technology will help to confirm the validity of these findings. Our results offer interesting directions to consider how piles may be initiated and propagated, including how individual hens may be attracted leading to secondary hens being attracted, group movement patterns, and other scenarios. Although repeatability was relatively low, we believe our techniques offer raw information for breeding companies to evaluate the incorporation of these methods into their respective breeding programs.

Contributions

A Stratmann was involved in conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, and revising it critically for important intellectual content

A Kashev was involved in analysis and interpretation of data, drafting the article, and revising it critically for important intellectual content

M Rice was involved in acquisition of data, analysis and interpretation of data, and revising the article critically for important intellectual content

M Toscano was involved in conception and design of the study, acquisition of data and funding, interpretation of data, drafting the article, and revising it critically for important intellectual content

CRediT authorship contribution statement

Ariane Stratmann: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Toscano Michael:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Fogarty Maxine:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Kashev Alexander:** Writing – review & editing, Visualization, Resources, Methodology, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2026.106967.

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