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Piling behaviour in Swiss layer flocks: Description and related factors

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ABSTRACT

Smothering is a major concern within the Swiss layer industry as it can lead to a high number of animal losses. The underlying cause is piling behaviour (PB), a phenomenon where hens densely cluster together in the litter area. The aim of this study was to describe PB and events preceding PB. Furthermore, we investigated the relation of the number of piles, pile duration, and number of hens involved in a pile with time of day, flock colour, flock age, environmental factors, and flock responses to behaviour tests. We video recorded the corners of litter areas (floor) inside the barn and winter garden of 13 commercial Swiss layer flocks (5 white, 5 brown, 3 mixed layer hybrids), which were known to previously experienced problems with smothering. We recorded environmental data (air speed, spot temperature) in the observed corners and assessed flock-level responses to two behaviour tests (novel object test, stationary person test). From the video recordings, events preceding piling and piling characteristics were assessed at 20 and 30 weeks of age (w) at three times per day (0–5 h, >5-10 h, >10–15 h after lights on). Statistical analyses included generalized and linear mixed-effects models and Spearman correlations. Results showed that piling events were mainly preceded by single hen activities (77.9%) and non- hysterical mass movements (7.6%). More piles and the largest numbers of animals involved in piling occurred in white and brown flocks at >5–10 h after lights came on. The number of piles was lower and the number of involved animals and pile duration higher in 20 w compared to 30 w. No correlation was found between environmental factors and flock behaviour test responses with piling characteristics. Potential underlying causes for PB are numerous, though we provide and discuss likely mechanisms, including response facilitation, individual stimulus response, and anti-predation behaviour, based on our findings. Furthermore, PB could relate to diurnal behaviours, for example, dustbathing and hens laying floor eggs in the litter area.

1. Introduction

Dead hens found along walls and in corners of layer barns are seen as a major and unpredictable problem in the loose-housed egg production (Bright and Johnson, 2011) and represents economic and welfare-related concerns (Barrett et al., 2014). The explanation is "smothering" or death likely due to suffocation. In Switzerland, where all commercial layer flocks are loose-housed, smothering regularly occurs with losses of 6–20 animals per incident (Stratmann and Winter, 2017). Responses of laying hen farmers in a non-representative interview on smothering let suggest that piling behaviour (PB) is the behaviour preceding smothering (Bright and Johnson, 2011). However, to the authors' knowledge, PB has been studied only in two commercial US layer flocks that were kept in the same barn (Campbell et al., 2016) and a relation between PB and smothering was not investigated. In that study, PB occurred randomly throughout the day, with a difference in pile duration and peak number of piling hens ("pile size") at different flock ages. The observed PB was described to be stimulated by non-fear related preceding events e.g. the "interest in other hens", "hens pecking at something" or "non-hysterical mass movements of hens" (Campbell et al., 2016). In addition to behavioural aspects, environmental factors such as sunlight (Gebhardt-Henrich and Stratmann, 2016) and spot temperature, light, or drafts are considered as contributing (Campbell et al., 2016) but have not yet been investigated in relation to PB. These factors might increase or decrease the attractiveness of barn areas, which in turn might result in animal distributions that facilitate piling.

Given the lack of a thorough description of PB, the first objective of this exploratory study was to provide a definition of PB in layer flocks applicable to identify PB in a variety of different settings and conditions (e.g., housing designs, flock sizes, breeds). The second objective was to describe key piling characteristics (i.e. number of piles, pile duration, pile size) in Swiss commercial layer flocks and relate these characteristics with potential PB influencing factors including: time of day, flock colour, flock age, environmental factors (i.e. differences in spot

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temperature and air speed), flock behaviour, as well as events preceding PB. Based on interviews (Stratmann and Winter, 2017) and personal communication (unpublished, 2017–2018) with Swiss farmers, we predicted that 1) the frequency and characteristics of PB would associate with time of day, flock colour and flock age and 2) PB would occur more frequently in barn locations with increased temperature and reduced air speed.

1.1. Ethical note

The ethical approval to conduct the study was obtained from the Veterinary Office of the Canton of Bern, Switzerland (approval number BE97/17). The animals were treated in compliance with the Swiss regulations regarding the treatment of animals involved in research.

2. Material and methods

2.1. Flocks

Observational data was collected between October 2017 and April 2018 on 13 commercial laying hen flocks throughout Switzerland. The average flock size was 4936 birds (min. 1`200, max. 15`750, Table 1). Five flocks consisted of white hybrids, five flocks consisted of brown hybrids, and three flocks consisted of white + brown (mixed) hybrids (Table 1). The flocks were of various genetic lines. Further flock details are included in Table 1. All barns were equipped with commercial (12) or self-made (1) aviary systems and provided access to a winter garden and free range. Flocks were selected based on a phone interview with the farmer following a previous survey conducted by our group that identified farmers with a history of self-identified smothering problems (Stratmann and Winter, 2017).

2.2. Definition of piling behaviour

The definition of PB was based on the only description available (Campbell et al., 2016) and our own recorded observations prior to video analysis. To define PB, we summarised the Campbell findings and applied them to a set of our own recorded video material. Thereby, we first identified piles by scanning the video material and then defining characteristics signalling start and ending of the piles. Based on this effort, PB was defined as three or more mostly immobile (maximal movement duration <5 s) hens standing in closest possible proximity (overlapping of body outlines) with most hens facing in the same direction. A piling event started when all conditions were met and ended when one of the conditions was absent. The definition of PB was tested by reassessing 25% of the video material of seven randomly selected farms. The analysis revealed that piles with a duration of >4.5 min could be detected reliably by one observer (95% of all piles were correctly re-identified). Thus, during video analysis piles shorter than 4.5 min were not considered.

2.3. Data collection

2.3.1. Video recordings

To record PB, a custom-built recording system (10-channel MULTI-EYE 3 GreenWatch Network Video recorder, artec technologies AG, Diepholz, Germany) was used with six infrared-sensitive, high-resolution, wide-angled video cameras (SNO-L6083RP, Samsung, South Korea) that were installed on each farm. Cameras were installed in four barn corners and two winter garden corners and directed towards the litter area. Differences in aisle width (distance between the aviary and barn wall) were measured manually (Laser Measure Bosch GLM 50 C Professional, Robert Bosch GmbH, Gerlingen, Germany) and camera heights resulted in observation areas varying between approximately 4 m^2 and 10 m^2 per corner. For each observed area, the pile number was counted per flock for one day at 20 weeks of age (w) and at 30 w within

Table 1
Overview of commercial Swiss flocks included in the study with information on hybrid, flock colour and flock size as well as mean and SD of number of piles, pile duration and pile size.

Flock	Genetic Line	Flock size	Flock age in weeks	Pile duration in min (mean ± SD)	Pile size (mean ± SD)	Pile number (>4.5 min)	Number of recorded corners
1	SN± BN	5000	20	12.1 ± 9.2	20.2 ± 9.4	43	8
			30	12.5 ± 19.9	25.4 ± 17.1	52	8
2	LSL ± LB	1100	20	8.1 ± 3.5	22.1 ± 10.5	14	5
			30	11.3 ± 3.2	24.8 ± 11.8	8	8
3	LB	1200	20	51.6 ± 37.8	13.4 ± 4.9	12	7
			30	18.2 ± 24.5	16.3 ± 8.0	14	8
4	SN	2023	20	16.9 ± 14.2	27.9 ± 21.0	27	8
			30	11.2 ± 10.1	25.8 ± 25.2	34	8
5	LSL	6800	20	11.5 ± 6.7	19.1 ± 8.6	31	6
			30	9.8 ± 5.2	20.0 ± 10.7	33	6
6	NW ± NB	2400	20	17.6 ± 22.1	28.1 ± 24.7	14	8
			30	12.9 ± 8.1	28.7 ± 13.9	14	8
7	LSL	8000	20	11.8 ± 4.0	28.9 ± 35.9	8	4
			30	9.9 ± 5.4	25.5 ± 21.2	35	6
8	LB	2000	20	NA	NA	0	8
			30	27.9 ± 36.0	28.2 ± 15.1	11	8
9	LB	5400	20	19.0 ± 16.4	23.8 ± 19.9	20	6
			30	19.1 ± 19.8	22.0 ± 33.6	20	6
10	BN	3000	20	7.8	13.0	1	4
			30	17.9 ± 15.3	16.2 ± 11.0	9	4
11	LSL	15,750	20	19.2 ± 20.0	58.0 ± 35.9	42	4
			30	12.6 ± 12.1	34.2 ± 39.9	73	6
12	LSL	9000	20	13.9 ± 11.8	49.3 ± 33.9	41	4
			30	17.9 ± 16.1	49.4 ± 39.8	59	8
13	LB	2500	20	23.5 ± 28.5	27.9 ± 14.5	9	8
			30	22.2 ± 21.3	18.3 ± 13.0	11	8
Total							
Mean ± SD	–	4936 ± 4167	–	15.26 ± 16.8	30.86 ± 29.0	24.4 ± 18.6	6.6 ± 1.6
Median	–	3000	–	9.83	22.00	17	7.5

White hybrids: Super Nick (SN), Lohmann Selected Leghorn (LSL), Novogen White (NV) Brown hybrids: Brown Nick (BN), Lohmann Brown (LB), Novogen Brown (NB).

three time windows per day: 0–5 hours, >5–10 hours and >10–15 hours after barn lights on. For each detected piling event, events preceding PB, the duration and pile size were assessed. Preceding events were described as visually assessable changes to the hen's environment or behaviour occurring at the time and location where the piling event started. Pile duration was calculated as the duration between start and end time of the piling event. Pile size was assessed by counting the number of combs or tail tips of the hens at the visually estimated largest size (peak) of the pile. All videos were analysed by a single observer (JW). To assess intra-observer reliability, 67 piles (>10% of all observed piles ($n = 635$)) of seven flocks were reassessed in a standardized way resulting in an agreement of >94% of re-identified piles. Differences between assessed and reassessed piles occurred for the pile start (mean-difference: 33 ± (SD) 55 s, median difference: 12 s) and end time (41 ± 88 s, median difference: 10 s). The visually estimated time point for the largest pile size showed larger differences (100 ± 147 s, median difference: 60 s), which was expected due to the constant joining and leaving of animals.

2.3.2. Environmental factors

To assess the influence of environmental factors on the occurrence of PB, air speed, average corner temperature, corner temperature distribution and environmental noise were measured along with the video recordings at 20 w and 30 w. The air speed was recorded with an anemometer (testo 405 i - Thermo-Anemometer, Testo AG, Mönchaltorf, Switzerland) held at hen height in each barn corner at two locations 2 m apart for 60 s on two days per flock age prior to the first video observation and the average calculated for each corner. The temperature was automatically recorded every minute (HOBO U12–013, onsetcomp, Bourne, U.S.) in one corner at the front and back of the barn. The average temperature was calculated for three periods: 0–5 hours, >5–10 h, and >10–15 h per day. Furthermore, thermal differences within barn corners were measured at hen height at two days per corner using a thermal camera (FLIR C2 Compact Thermal Camera, FLIR Systems, Inc., Wilsonville, U.S.) prior to video observation. The noise in the barn was recorded over the complete light period with two audio recorders (ZOOM H6 audio recorder, Zoom Corp., Chiyoda-ku, Japan) installed at the front and back inside the barn. Audio recordings were intended to serve as an additional response if sudden fear reactions leading to PB were observed by the recorded video.

2.3.3. Flock behaviour

To assess the influence of flock behavioural characteristics on the occurrence of PB, a novel object and a stationary person test were conducted (Graml et al., 2008). Both tests were conducted in each flock by the same examiner (JW) in each corner of the barn once at 20 w and 30 w. The novel object test was conducted for 60 s and consisted of a remotely controlled flashlight, which created a flickering light spot (diameter: 10 cm) on the litter area. The stationary person test was conducted with the examiner wearing a blue overall and blue disposal shoe covers, standing inert with hands folded in front of his waist for 90 s. In both tests, the latency was measured until the first and third hen approached the test objects. In the novel object test, a hen was counted when it entered the flashlight spot with its head. In the stationary person test, a hen was counted when its head was positioned less than one hen distance to the stationary person.

2.3.4. Statistical analyses

The effects of the explanatory variables flock colour (i.e. brown, white and mixed), flock age (i.e. 20 w and 30 w) and time of day (i.e. 0–5 h, >5–10 h and >10–15 h) on piling characteristics (i.e. pile number, pile duration, and pile size) as the response variables were analysed. Pile duration was analysed using a linear mixed-effects model whereas pile number and pile size were analysed with generalized linear mixed-effects models using the R-package lme4 (Bates et al., 2015). Model assumptions for linear mixed-effects models were controlled by visually checking residual QQ-plots. The package DHARMa (Hartig, 2018) was used for residual testing of generalized linear mixed-effects models. For each model, a pile characteristic served as a single response variable for which each explanatory variable mentioned above as well as the interactions between flock colour and time of day and flock colour and age were taken into consideration as fixed effects. Due to the explorative nature of the study, factors like flock and barn size (i.e. aisle width) could not be varied in a controlled way, thus these variables were included in each model as covariates. The included random factors were time of day nested in flock age, and corner nested in flock. Model selection was performed using Akaike Information Criterion (AICc) differences between models (corrected for small sample sizes). Thereby, models with substantial support (AICc<2 differences (Burnham and Anderson, 2004, p.271)) and AICc weights) were identified by applying the R-package AICcmodavg (Mazerolle, 2020). The model performance optimizer bobyqa was applied including a maximal number of 100`000 iterations. Prior to analysis, data was cleaned by using the packages tidyr (Wickham and Henry, 2020) and dplyr (Wickham et al., 2020) and checked for outliers, collinearity and interactions (Zuur et al., 2010). To assess the relation of piling characteristics and environmental factors (i.e. barn corner temperature differences and air speed) and flock behaviour (i.e. novel object test, stationary person test), Spearman correlation coefficients were calculated using the R-package corrplot (Wei, 2013). Preceding events of PB were categorized and summarised descriptively. Data was visualised by applying ggplot2 (Wickham, 2010) and summarised using the R-package psych (Revelle, 2015). All calculations were done in R Version 4.0.2 (R Core Team, 2020) using the user interface RStudio (RStudio Team, 2020). In the results section, mean values are reported with associated standard deviations.

3. Results

3.1. Observed piling behaviour

Piling was considered to occur when three or more mostly immobile hens were standing in closest possible proximity (overlapping of body outlines) with their heads facing mostly into the same direction. Independent of events preceding PB, hens constantly joined and left piles. Piles formed mostly (>95%) at the endings of barn aisles (solid walls or mesh wire). Hens often walked over or squeezed under piling hens despite having plenty of space available to distribute more evenly throughout the barn. After being restrained with several body parts (head, sternum, and wings) for several seconds, birds in the pile fell in a state of immobilisation with reduced responsiveness to external stimulation (e.g. other approaching hens). Piling hens did not vocalize, and some hens intermittently closed their eyes, while others kept their eyes open. Hens awoke from the immobilisation suddenly, but, if still squeezed in the pile, could fall into immobilisation again. Piles dispersed mostly without any obvious reason with being only occasionally dispersed by humans. Piles could immediately form again in the same corners after the dispersion of previous piles.

3.2. Descriptive analysis of piling behaviour

In total, 635 piling events (>4.5 min) were detected and characterized in all flocks during the observation period. On average piles lasted 15.26 min ± 16.82 (median 9.8 min, min. 4.51 min, max. 145.1 min) and had a size of 31 animals ± 29 (min. 22, max. 293, Table 1). The identified events preceding PB were grouped into six categories: single hens engaged in activities that were broad in nature including sitting, standing while orienting towards a wall (Fig. 3) and sometimes pecking at objects (77.9% of piling events), mass movements of hens (7.6%), sunlight spots (2.6%), persons entering the barn (1.7%), light spots from lamps (0.9%), and aggression between hens (0.31%). No discernible preceding event could be found for 8.7% of piling events.

In this study, only one likely smothering event (Fig. 4) was video recorded. In that example, brown hens ($n = 20$) piled in a barn corner on a slatted floor between a wooden perch and a concrete wall (approx. 40 cm space) for approximately 2 h (128.3 min) in the 0−5 h observation window at 20 w. In this pile, hens climbed on top of each other or crawled under the pile. When the pile dispersed, one hen, showing no signs of life (e.g., movement, body tonus) appeared with head and legs oriented towards the floor and barn wall. A high density of hens, already being present before the video recordings began, impaired the view and, therefore, made it unclear if the hen died before the pile started or in the pile. However, based on the position of the hen and ongoing piling activity in this corner throughout the assessed day, it seems likely that PB was the cause for the death. Though the dead bird was removed by the farmer, hens continued piling in this corner. A handful of floor eggs ($n = 4$) were detected next to the smothered hen.

3.3. Regression analysis of piling characteristics

3.3.1. Pile number

The best fitting model linking pile number with explanatory factors included the interactions between flock colour and flock age and flock colour and time of day (Table 2). In mixed and white flock colours, more piles occurred at >5−10 h compared to 0−5 h and >10−15 h. Compared to white and mixed flocks, brown flocks formed prominently fewer piles at >5−10 h and >10−15 h (Fig. 1). More piles occurred in brown flocks at 30 w compared to 20 w, whereas no difference was found for white and mixed flocks (Table 3).

3.3.2. Pile duration

The best fitting model for pile duration included flock colour, flock age and time of day (Table 2). Piles were longer in brown than in white and mixed flocks, at >5−10 h and >10−15 h compared to 0−5 h, and at 20 w compared to 30 w (Table 4).

3.3.3. Pile size

The best fitting model for pile size included the interactions between flock colour and time of day, and flock colour and flock age (Table 2). In white and brown coloured flocks, piles involved more animals at 0−5 h and >5−10 h compared to >10−15 h. In mixed flocks, no time of day related changes in pile size were found (Fig. 2). The number of animals involved in piling events was higher in brown flocks at 20 w compared to 30 w without differences in mixed and white flocks (Table 3).

Table 2
Overview of most effective models (*) based on model selection for the parameters number of piles, pile duration and pile size. Model selection includes Akaike Information Criteria (corrected for small sample sizes) differences (AICc differences <2), the number of model parameters (K), and Akaike weights (AICcWt).

Dependent Variable	Fixed Effects	K	AICc	ΔAICc	AICcWt
Pile number	*Flock colour:Flock age, Flock colour:Time of day	18	1335.28	0.00	0.45
	Flock age, Flock colour: Time of day	16	1335.46	0.18	0.41
Pile duration	*Flock colour, Flock age, Time of day	13	1314.20	0.00	0.25
	Flock age, Flock colour: Time of day	17	1314.80	0.60	0.19
	Time of day, Flock colour:Flock age	15	1315.05	0.85	0.17
Pile size	*Flock colour:Flock age, Flock colour:Time of day	18	10797.83	0.00	1

3.4. Environmental factors

Air speed (mean ± SD: 0.14 m/s ± 0.2) in the observed barn corners was not related to piling characteristics (pile number: $R = 0.26$, $p = 0.039$, $n = 71$, pile duration: $R = 0.2$, $p = 0.17$, $n = 51$, pile size: $R = 0.32$, $p = 0.025$, $n = 51$). Corner temperature differed between barn corners and flocks over the day but no effect on piling characteristics was detected (pile number: $R = 0.019$, $p = 0.85$, $n = 105$; pile duration: $R = 0.078$, $p = 0.43$, $n = 105$; pile size: $R = 0.14$, $p = 0.14$, $n = 105$). Thermal image data was excluded from the analysis due to high intra-location variation which was probably due to a high sensitivity of the thermal camera. Audio recordings were not analysed as no fear reactions leading to PB could be observed during video analysis.

3.5. Flock behaviour tests

No relation was found between the average flock latencies to approach the novel object and average piling characteristics (pile number: R= -0.06, $p = 0.79$, $n = 22$; pile duration: R= -0.12, $p = 0.61$, $n = 22$; pile size: R= -0.017, $p = 0.94$, $n = 22$). Similarly, no relation was found between the average flock latencies to approach the stationary person and average piling characteristics (pile number: $R = 0.26$, $p = 0.26$, $n = 24$; pile duration: R= -0.29, $p = 0.23$, $n = 24$; pile size: $R = 0.22$, $p = 0.36$, $n = 24$).

Fewer hens approached the stationary person test at 30 w (one hen = 53.6%, three hens = 29.3%, $n = 41$ tests) than at 20 w (one hen = 85.3%, three hens = 60.9%, $n = 41$ tests). Hens in white coloured flocks had increased latencies to approach the stationary person (55.7 s ± 28.4, $n = 41$ tests) than hens in brown coloured (24.64 s ± 39.2, $n = 41$ tests) and mixed flocks (32.1 s ± 35.49, $n = 41$ tests).

4. Discussion

The current study is the first investigating PB that includes preceding events, characteristics (i.e. number of piles, pile duration and pile size), and related factors in commercial Swiss laying hen flocks. We observed variation in the number of piles as well as pile size that were related to the interaction of flock colour and age, and flock colour and time of day. Furthermore, pile duration was related to flock colour, age, and time of day. No relation was found between PB and environmental factors and flock-level behaviour responses during controlled tests. Piling behaviour was mostly preceded by single hen activities such as sitting and standing, which could have elicited PB by various mechanisms. It is likely that a myriad of influencing factors are responsible for the observed variations which may or may not be consistent across farms and hybrids and possibly synergistic as well as contradictory. We nonetheless offer potential explanations based on the findings of our exploratory work which we hope can provide a foundation for future, hypothesis driven efforts.

4.1. Diurnal patterns

Piling behaviour occurred more frequently at midday and in the afternoon and was markedly lower in the morning for white and mixed flocks. Time of day was also related to piling duration with longer durations observed at midday and afternoon. In addition, pile size was smaller in brown and white flocks in the afternoon compared to the morning and midday. In other words, piles were longer and more frequent at >5 – 10 h after lights on (i.e. midday) with a decrease in the number of brown and white birds involved in the final five hours of lighted time. The time-of-day variations in piling characteristics supports our prediction that PB does not randomly occur throughout the day but peaks at certain times.

A potential explanation for increased piling frequency and duration at midday and afternoon could be that hens use the litter area more frequently at these times of day to perform behaviours such as dust bathing (Vestergaard, 1982) or foraging (Carmichael et al., 1999). The

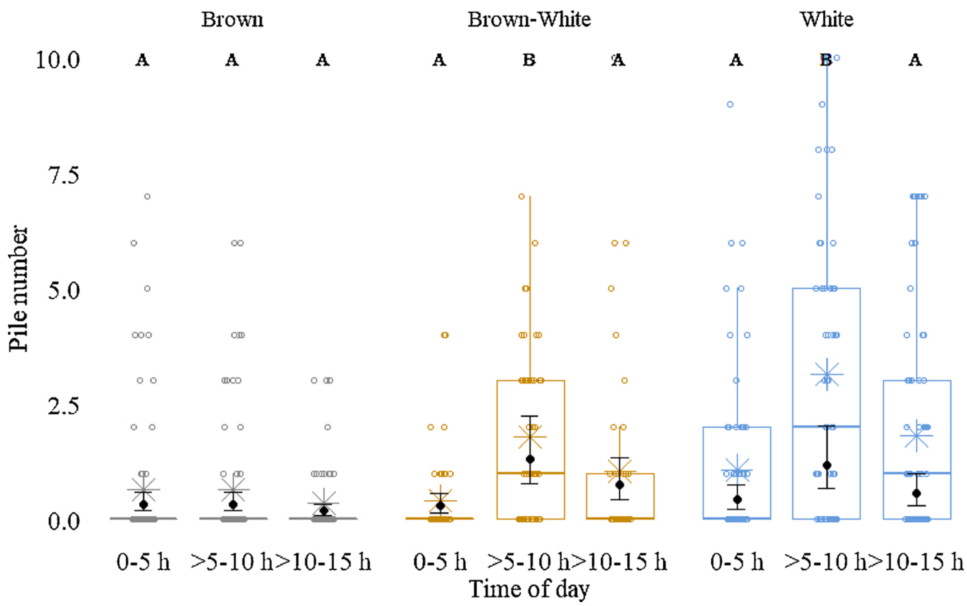

Fig. 1. Pile number depending on flock colour and time of day. Dots represent raw data points. Boxes show raw data medians, upper and lower interquartile range, upper and lower whisker. Asterisks represent raw data means. Interval plots show model predictive values, lower, and upper 95% confidence intervals. Dissimilar letters (A/B) mark outcome differences between variable categories based on the predicted means and confidence intervals.

Table 3
Predictive values and confidence interval limits (CL) for the most supported models of the parameters pile number and pile size.

	Pile size						Pile number				
	Time of day				Flock age		Time of day			Flock age	
	Flock colour	0–5 h	>5–10 h	>10–15 h	20 w	30 w	0–5 h	>5–10 h	>10–15 h	20 w	30 w
	Brown	22.41	26.61	15.03	26.29	19.62	0.33	0.33	0.18	0.21	0.34
Predictive values	Brown + White	27.01	24.58	24.52	22.40	27.10	0.29	1.31	0.76	0.66	0.66
	White	27.73	33.82	19.11	28.81	27.41	0.42	1.17	0.56	0.54	0.77
	Brown	16.60	19.66	11.0	19.42	14.60	0.19	0.19	0.09	0.12	0.20
Lower 95% CL	Brown + White	19.80	18.25	18.12	16.61	20.11	0.15	0.77	0.43	0.38	0.38
	White	22.56	27.63	15.58	23.52	22.39	0.23	0.68	0.31	0.31	0.45
	Brown	30.26	36.01	20.54	35.59	26.37	0.58	0.58	0.33	0.37	0.58
Upper 95% CL	Brown + White	36.86	33.10	33.18	30.20	36.51	0.57	2.23	1.33	1.15	1.13
	White	34.07	41.40	23.46	35.29	33.56	0.75	2.02	0.98	0.94	1.34

Table 4
Predictive values and confidence interval limits (CL) for the most supported model parameters for pile duration (in min).

	Pile duration							
	Flock colour			Flock age		Time of day		
	Brown	Brown + White	White	20 w	30 w	0–5 h	>5–10 h	>10–15 h
Predictive values	16.81	9.73	10.55	12.29	10.50	9.49	11.65	11.81
Lower 95% CL	14.19	8.40	9.59	11.18	9.69	8.35	10.72	10.55
Upper 95% CL	19.91	11.29	11.61	13.50	11.38	10.79	12.67	13.22

increased number of hens in the litter area would have made piling more likely and longer due to higher animal densities and difficulties for hens to leave the area (and piles). However, piles were smaller in the afternoon which could be explained by hens prioritising other behaviours (e.g., foraging) and showing lower motivation for joining piles at this time of day. For example, we frequently observed hens, though being close to piles, walking by, indicating that some hens are less motivated to join piles at all. Alternatively, their motivation to join piles could vary, for example, in relation to time of day. Future studies should investigate whether a hen's motivation to join piles would explain variation of piling characteristics in relation to time of day. Another explanation for a lower pile size in the afternoon could be an increase in transitions between the litter area and the aviary system (Rufener et al., 2018). Increased transitions could have resulted in hens constantly joining and leaving piles which, thereby, could have reduced the number of piling birds at any point in time.

The lower frequency and shorter durations of piles in the morning period could be explained by hens being occupied with other behaviours performed outside the litter and wintergarden areas where our observations were focused. Nesting behaviour (Carmichael et al., 1999; Villanueva et al., 2017) and feeding (Ballard and Biellier, 1975) are two prime candidates. Nonetheless, piles still did occur in the morning hours and involved a greater number of hens compared to the afternoon. Bigger piles might have resulted from hens being attracted to other hens performing these specific morning behaviours in the litter area resulting in higher animal densities. For example, it is well known that hens

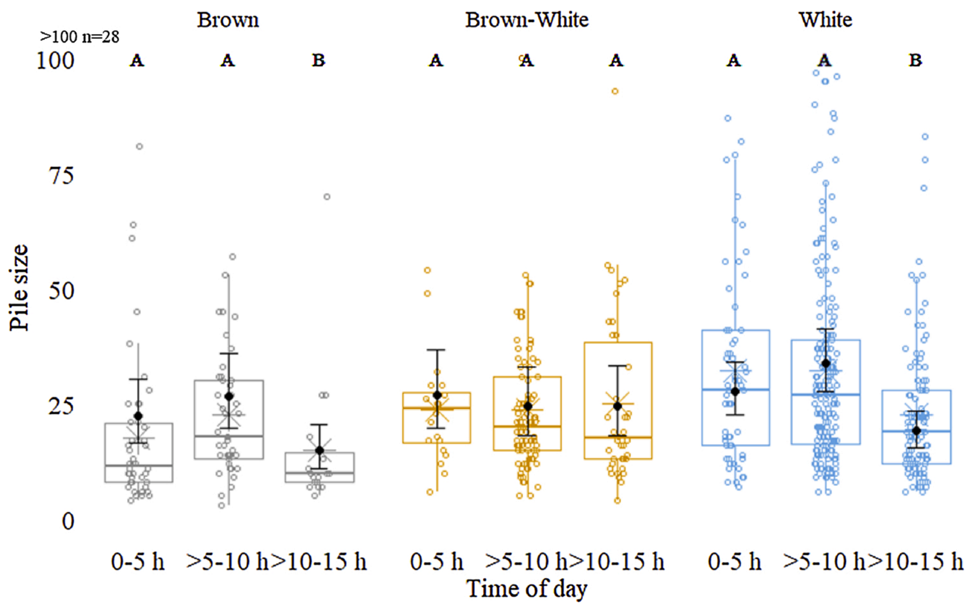

Fig. 2. Pile size depending on the interaction of time of day and flock colour. Dots represent raw data points. Boxes show raw data medians, upper and lower interquartile range, upper and lower whisker. Asterisks represent raw data means. Interval plots show model predictive values, lower, and upper 95% confidence intervals. Dissimilar letters (A/B) mark outcome differences between variable categories based on the predicted means and confidence intervals.

Fig. 3. Sequence of a pile formation in a white layer flock (9000 birds) at >5-10 h, at 20 w, in the left front corner of the barn.

mainly perform egg laying behaviour in the morning (Villanueva et al., 2017) and frequently join other hens for nesting behaviour (Riber, 2012a, 2012b, 2010; Tahamtani et al., 2018). Floor eggs were frequently recorded in the same locations in corners and along walls after piles dissolved indicating that either hens involved in a pile laid eggs or hens that laid their eggs in the corners were the stimulus themselves and elicited PB. Particularly at a young age (including those of the current study) when hens are still developing appropriate nest-seeking and egg laying behaviour, the attraction of hens to these locations could be a factor.

In conclusion, PB did not randomly occur throughout the day but varied with high and low periods. Knowing specific times of the day that are prone for PB to occur should be acknowledged in further studies and may help to develop measures to prevent PB, particularly if associated with the occurrence or absence of other behaviours and underlying motivations. To confirm variation in time of occurrence, future research should increase the sample size of investigated flocks and use a greater number of observed time periods. Furthermore, piling characteristics should be related to other factors, as, for example, variation of animal densities in the litter area over time of day.

4.2. Flock age and colour

We observed prominently longer pile durations at 20 w compared to 30 w. Furthermore, brown flocks piled less frequently but with more hens involved per pile at 20 w compared to 30 w whereas the frequency and size of piles did not vary between flock ages in white and mixed flocks. Remarkably, brown flocks had pile durations twice as long in contrast to white and mixed flocks. The observed differences in piling characteristics support our prediction that PB relates to flock age and may be related to flock colour.

A possible explanation for longer pile durations at 20 w may be that, after transfer from the rearing to the laying house, the environment remains novel and stimulates exploration behaviour in the litter area resulting in higher animal densities (Carmichael et al., 1999). Similar to that argued above for observed time of day patterns, higher animal

-1 min
Piling behaviour in a barn corner.

0 min
(Likely) smothered hen appears after pile dispersed.

Fig. 4. Sequence of a (likely) smothering event in a brown layer flock (1200 birds) at 0-5 h at 20 w.

densities could make it difficult for hens to leave piles resulting in longer pile durations. In contrast to white and mixed flocks, piles were less frequent but involved more animals in brown flocks which could be explained by brown hens performing more aviary related behaviours in the litter area after transfer to the new housing system. For example, the pattern of brown hybrids performing more egg laying in the litter area compared to white flocks (Singh et al., 2009; Villanueva et al., 2017) could explain greater numbers of birds in the litter and consequent longer lasting piles with more birds. Furthermore, large piles were occasionally observed in brown flocks shortly after lights on and before lights off suggesting that brown hens were either still not familiar with using perches to roost during the night time or generally experience difficulties in using the aviary tiers (Ali et al., 2019; Faure and Bryan Jones, 1982). Both behaviours, egg laying and sleeping in the litter area, increase the animal density in the litter area and thus the likelihood of longer lasting and larger piles occurring.

Age effects on pile characteristics are also likely to be influenced by the increased familiarity with their environment as hens age. Hens have a strong motivation to use perches (Newberry et al., 2001; Olsson and Keeling, 2000) and become more familiar with the laying and aviary environment over time which could explain why fewer animals were involved in piles at 30 w compared to 20 w in brown flocks. Measures applied by farmers to prevent hens from egg laying and sleeping in the litter area, such as chasing hens from the litter area to the aviary system at the onset of lay, might also have influenced age-related piling characteristics.

Further studies should target the benefits of measures to prevent egg laying and sleeping in the litter area. Easing access to nests and the aviary system in the first weeks after population, increasing the familiarity with the aviary system by earlier transfer to the laying house (e.g. 16 w), and providing similar rearing settings are some examples that could reduce piling. Future studies should also consider interactions between strain and housing design on piling characteristics in their investigations.

4.3. Environmental factors and flock behaviour tests

Despite temperature in corners and air speed being suggested as potentially contributing to PB (Campbell et al., 2016), no relation was found with piling characteristics in this study. As farmers frequently reported an increase of hen densities in warmer barn locations (e.g. sun spots) and it was often observed that hens clustered within draft protected areas (e.g. between pop holes), future studies might investigate the influence of these environmental factors on hen distributions in controlled settings. The high intra-location variation of thermal image data likely resulted from the previous presence of hens in the recorded corners and the observer walking through the barn. To exclude such effects, future studies could constantly record the hen environment.

No relationship was found on flock-level behavioural responses to the novel object and stationary person tests and piling characteristics. Concerning the novel object test, it is possible that the presented stimulus (light spot) was too intense and/or sudden compared to natural stimuli leading to a large response in all flocks and thus masking differences between flocks (i.e. a ceiling effect).

4.4. Potential behavioural mechanisms of piling

Based on our observations, PB was mostly preceded by a single hen closely sitting or standing aligned towards a barn wall and/or corner. The trigger was especially strong when the hen in question pecked at objects, though did not necessarily require discernible head movements. Comparable to PB, aggregation and simultaneous performance of behaviours is often observed in domestic fowl (Keeling et al., 2017) including nesting (Giersberg et al., 2019; Riber, 2012a, 2012b, 2010; Ringgenberg et al., 2015a, 2015b; Sherwin and Nicol, 1993), feeding (Collins et al., 2011; Collins and Sumpter, 2007), dustbathing (Duncan et al., 1998; Hoppitt et al., 2007; Vestergaard, 1982) and resting (Alvino et al., 2009; Riber et al., 2007).

One explanation for performing simultaneous behaviours is response facilitation where the behaviour of a demonstrator increases the probability that an observer follows the same behaviour (Byrne, 1994). Two types of response facilitation are stimulus or local enhancement (Nicol, 1995) where the behaviour of a hen draws the attention of observing hens towards a certain stimulus or location, respectively, and thereby elicits a certain behaviour. As an example for stimulus enhancement to explain piling, we frequently observed that, when a hen pecked at small objects (e.g. dust flakes, screws in the barn wall), nearby conspecifics joined and started to peck at the same object. Pecking at objects (or even the anticipation of objects) could explain why hens aggregated and faced towards the same directions. In local enhancement, hens follow the behaviour of other hens at a certain location. For example, hens could have been more likely to stand or sit in corners due to companions showing this location-specific behaviour. Overall, response facilitation mechanisms seem one suitable explanation for hens joining behaviours of other hens resulting in piling.

Another mechanism which could explain the aggregation of hens is the tendency to move together when performing certain behaviours (Keeling et al., 2017; Keeling and Duncan, 1991). For example, hens move closer together when they are preening and standing compared to walking or ground pecking (Keeling and Duncan, 1991). We frequently observed small intra-animal distances and high densities in the litter area when hens dustbathed which could have increased the risk for PB.

Lastly, the attention of hens to environmental stimuli or locations could also be explained by individualized (and independent) attraction towards a stimulus. Especially when the stimulus is strong and widely visible, the attraction of hens could be explained without response facilitation or other social behaviour mechanisms. For example, we frequently observed that hens approached sunlight spots in the winter garden and barns resulting in high densities at lighted locations. Furthermore, we often observed hens following farm personnel through the litter area resulting in dense clusters along barn exits when the personnel left. In both examples, hens seemed to respond to the stimulus (i.e. light, personnel) itself than being facilitated to respond by their companions` behaviour towards it.

Our observation that PB occurred frequently along walls and in corners despite open spaces elsewhere in the litter area could be explained by the anti-predation theory. Hens are more likely to perform risk-related behaviours in more protected areas. For example, domestic fowl are frequently observed to rest along walls (Buijs et al., 2010; Newberry and Hall, 1990) and even prefer this location within the nest box (Ringgenberg et al., 2015a) which might be experienced as more protected than the uncovered litter area.

Taken together, hens joining other hens standing and sitting aligned towards a wall might be response facilitated, elicited by external environmental stimuli, or explained by the tendency of hens to move together when performing simultaneous behaviours. The high occurrence of piles along the walls and in corners could be explained by the anti-predation theory.

5. Smothering

Across all observed piling events, we monitored only one (likely) smothering event. The rarity of smothering indicates that PB does not often lead to smothering. In the observed incident, the number of piling hens was rather few, suggesting that piles of a small size can lead to smothering. Though the pile duration was exceptionally long (approx. two hours), we have monitored several long-lasting piling events without bird losses. Future studies should investigate a relationship between piling characteristics and smothering.

The lost hen appeared on the floor, directed towards a concrete wall between a perch and the barn wall after the pile dispersed. It is perceivable that hens on the bottom of the pile are of higher risk to smother compared to hens on the pile surface. The lost hen could have experienced pressure from other hens on top of the pile which could have compromised her respiration. Furthermore, the lost hen could have experienced difficulties to leave the pile due to other hens, the perch, and wall blocking her way out. Therefore, the position of a hen in, and the housing environment around a pile could also be related to smothering.

The smothering event occurred in the morning (0−5 h) and floor eggs were detected next to the smothered hen which could indicate a relationship between hens laying floor eggs, PB and smothering. For example, it is perceivable that the lost hen nested in the corner when being joined by other hens. However, more smothering observations are necessary to confirm a relationship between floor nesting, PB and smothering as well as which piling characteristics and locations in a pile and housing environments contribute to smothering.

6. Conclusion

In this study, PB was mostly preceded by single hen activities and related to time of day, flock colour and flock age. Contrary to our expectations, PB was not related to environmental factors (spot temperature and air speed) and flock behaviour responses to two behaviour tests. Our study provides a working definition of PB which could serve as a basis for future studies investigating PB. We also use findings of our exploratory work to present theories explaining the causes of PB which should be evaluated in future research. Future studies should take behaviour analyses (and time budgets) of focal individuals at different ages into account. Studies addressing prevention measures of PB should focus on management strategies (e.g. walking of birds, harmonisation of rearing and housing conditions). Only one likely smothering event was observed indicating that most piles do not lead to smothering.

Declaration of Competing Interest

The authors report no declarations of interest.

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