

Welfare and performance in layers following temporary exclusion from the litter area on introduction to the layer facility

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ABSTRACT When introduced to the laying facility, pullets are sometimes temporarily excluded from the litter area in order to help them locate food and water, and to prevent floor-laid eggs. This procedure is not permitted in Sweden, because it involves denying access to both litter and space, which may have a negative effect on bird welfare. The present study investigated how the welfare and performance of layers were affected by this temporary exclusion on introduction of hens to the laying facility. The study included 600 floor-reared Dekalb White layers obtained at 16 wk age and housed in 6 groups of 100 in a conventional single-tier floor-laying system. Birds were either given full access to the litter area during the whole study or were excluded from the litter area during the first 2 wk after transfer to the

laying facility. From 18 to 72 wk age, birds in both treatments had full access to the litter area. Excluding birds from the litter area for 2 wk resulted in better feather cover and reduced fearfulness, according to novel object and tonic immobility tests. Furthermore, birds initially excluded from the litter area produced eggs with a lower proportion of shell irregularities than birds with full access to the litter area throughout. No difference was found in corticosterone metabolites in droppings rate of lay, mortality, or proportion of floor-laid eggs. In conclusion, none of the parameters studied indicated that the welfare of laying hens was compromised by temporary exclusion from the litter area on introduction to the laying facility. In fact, some of the data suggested that bird welfare had improved.

Key words: pullets, welfare, performance, fencing, litter area

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INTRODUCTION

Laying hens are most often reared at one facility and then transferred, at around 15 to 18 wk age, to the laying facility. On arrival at noncage production systems, a routine sometimes adopted is to restrict access by the pullets to the litter area (Lambton et al., 2010), in order to help the birds quickly find food and water, and to minimize the number of floor-laid eggs later on. This exclusion procedure, which may last from a couple of days up to several weeks, is permitted in most European Union (EU) countries until laying maturity as per Council Directive 1999/74/EC. However, in Sweden the procedure is not permitted because it involves denying access to both litter and a substantial amount of space, which may have negative effects on bird welfare (Gunnarsson et al., 2000; Bestman et al., 2009).

Despite huge efforts to solve problems with feather pecking, this behavior still occurs in commercial layer flocks, resulting in both impaired welfare and economic losses (Rodenburg et al., 2013). Access to litter is important for layers and can reduce the risk of feather pecking behavior developing (e.g., Blokhuis and Van der Haar, 1989; Gunnarsson et al., 2000; Van de Weerd and Elson, 2006). In an epidemiological study by Lambton et al. (2010), fencing layers on a slatted floor was identified as being one of the major risk factors for occurrence of feather pecking. The impact of increased stocking density on bird welfare has been examined in a number of studies but the results are inconclusive, showing both negative and positive effects (e.g., Nicol et al., 1999, 2006; Zimmerman et al., 2006; Bestman et al., 2009).

The aim of the present study was to investigate how the welfare and performance of nonbeak-trimmed layers were affected in the long run, by depriving the birds of access to both litter and space when they were first introduced to the laying facility. The study comprised the period from 16 to 72 wk age and examined a wide range of welfare parameters, such as feather cover, pecking

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wounds, fearfulness, egg shell irregularities, behavior, and levels of corticosterone metabolites in droppings.

MATERIALS AND METHODS

Birds, Housing, and Experimental Design

A total of 600 Dekalb White layers were used in the study. They were reared in a conventional single-tier floor-laying system with a stocking density of 15 hens/m² at a commercial breeding facility (Swedfarm AB). All birds were vaccinated against infectious bronchitis, Marek's disease, and avian encephalomyelitis and, in accordance with Swedish regulations, no beak trimming was performed. At the age of 16 wk, the pullets were transferred from the rearing house to the study unit at the Swedish Livestock Research Center at Lövsta, Uppsala, where the study was performed. There, the layers were housed in 6 groups of 100 in a conventional single-tier floor-laying system, resulting in a stocking density of 7.5 hens/m². Each pen had a total area of 13.4 m² and included a litter area (1.32 × 3.56 m or 35% of total area), a raised slatted floor area (2.30 × 3.56 m) with access to 2 group nests (1.15 × 0.46 m each), 1 bell drinker, 4 round feed hoppers, and 5 rows of perches integrated into the slatted floor (Figure 1). The nests were initially closed and were opened after 12 d, when the first egg was laid. Wood shavings were used as litter material and were replaced approximately every 2 wk between 22 to 72 wk age due to high litter moisture. Although the house was artificially heated the impaired litter quality was likely a consequence of a low stocking of birds in the study house producing less heat in relation to optimal ventilation regarding humidity.

Underneath the slatted area, automatic floor scrapers were used to remove manure 5 times per wk. Eggs on the egg belt, which was located outside and behind the

nests, were collected manually once per day, and misplaced eggs in the litter area or the slatted area were collected at least twice per day. All birds received a standard commercial layer diet, mainly based on wheat and soybean meal, with a calculated content of about 16% CP, 4% Ca, and 2,700 kcal (11.3 MJ) ME/kg. Feed and water were supplied ad libitum. The birds were given 10.5 h light (0530 to 1600) per 24-h period in the beginning of the study (16 wk age). The light was then gradually increased to 16 h (0200 to 1800) per 24-h period at 22 wk age, according to the breeder guidelines. In addition to the artificial lighting, the house was also equipped with daylight inlets and both light sources were adjusted due to season of the year as well as the behavior of the birds.

Roundworm infection with *Ascaridia galli* was detected during the study and therefore the birds were dewormed with Verminator supplied in the water at 50 and 66 wk age. Each pen was equipped with a video camera (Zavio, D7110 Outdoor Dome) situated in the ceiling to record bird behavior during the study.

The study included 2 treatments each administered to 3 groups of 100 birds, each of which was regarded as a statistical unit, resulting in 3 replicates per treatment. In the study period, the birds were either given full access to the litter area throughout (open treatment) or were excluded from the litter area during the first 2 wk (16 to 18 wk age) after transfer to the laying facility (closed treatment). From 18 wk age until the end of the study at 72 wk age, birds in both treatments had full access to the litter area. The study was approved by the Uppsala Local Ethics Committee as per C358/11.

Data Recording

Production performance Egg production, laying percentage, number of floor-laid eggs (i.e., eggs laid in the litter or slatted area) and mortality were recorded daily, whereas feed intake and feed conversion ratio (FCR) were recorded on a 4-wk basis. All eggs collected during 3 d (consecutive) on 7 different occasions (at 24, 32, 40, 48, 57, 68, and 72 wk age) were assessed for the proportion of cracked and dirty eggs using an experimental candling machine. Prior to analysis, the proportion of cracked and dirty eggs was expressed as mean value for each group and age, whereas the other parameters were added up and expressed as mean value per group for the entire production period (20 to 72 wk age).

Integument scoring At 40 and 54 wk age, 30 randomly selected birds from each replicate were weighed and scored from 1 (worst) to 4 (best) with respect to feather cover on 6 body parts (neck, breast, cloaca, back, wings, and tail), pecking wounds (on comb and rear body part, including vent), bumble foot condition and keel bone deviations, using the integument scoring protocol devised by Tauson et al. (2005). Before statistical analysis, scores for feather cover on the 6 body

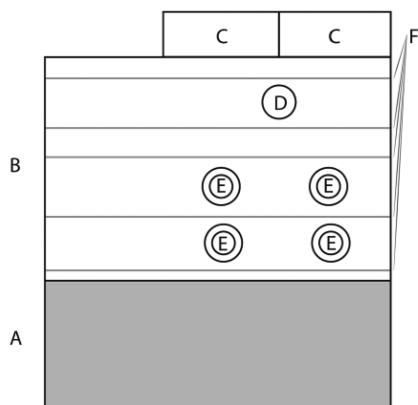


Figure 1. Schematic figure of a floor pen. Each pen had a total area of 13.4 m² and included a litter area (1.32 m × 3.56 m) (A) and a raised slatted floor area (2.30 m × 3.56 m) (B), with access to 2 group nests (1.15 × 0.46 m each) (C), 1 bell drinker (D), 4 round feed hoppers (E), and 5 rows of perches integrated into the slatted floor (F).

parts were added together, generating a total possible score of between 6 and 24.

Fearfulness Fearfulness was assessed by the novel object (NO) test and tonic immobility (TI) test. The NO test was carried out based on the description provided in the Welfare Quality (2009) assessment protocol for poultry with some adjustments due to the design of the pens used in the present study. The NO test was performed by the same operator on 12 occasions (approximately every 4 wk) between 20 to 72 wk age, and between the hours of 1400 to 1700. Before the start of each test period, the operator entered the house and walked around (outside the pens) for 10 min in order to accustom the birds to the human presence. The operator then slowly entered each pen, sat down on the edge of the raised slatted area, and waited for 3 min, again to accustom the birds to the human presence. The NO, a 20-cm long stick with multicolored bands and a diameter of 2 cm, was placed in the middle of the litter area. The operator then walked to the end of the litter area (about 1.5-m away from the NO) and immediately started to record the number of hens within 1 hen length of the NO (about 35 cm) every 10 s for a total period of 2 min. In the statistical calculations, the proportion of hens within 1 hen length of NO was calculated by dividing the mean number of hens per recording (every 10 s) by the total number of hens in each group (corrected for mortality). Mean value per group and age was then used in the statistical analysis.

The TI test was performed at 49 wk age using 10 randomly selected birds from each replicate. The birds were caught by an operator slowly walking into the pen and huddling the birds towards the nests, from where they were easily caught. The selected birds were tested on a rotational basis with 1 bird at a time from each of the 6 pens. The time from catching until start of induction was about 60 s, because testing was conducted in a separate room adjacent to the layer house. All birds were tested by the same operator between the hours of 0900 and 1800 and within 3 d (consecutive). The TI test was performed as described by Jones and Faure (1981). The bird was placed on its back in a U-shaped wooden cradle covered with a black cloth. The operator then induced TI by gently restraining the bird for 15 s with one hand over the bird's breast and the other over the head. In order for induction to be considered successful, the bird had to remain motionless for at least 10 s. After a successful induction, the operator moved out of sight of the bird and started to record the duration of TI, i.e., latency to self-righting. A maximum of 3 inductions per bird was used. Birds still in TI after 15 min were recorded as having a latency of 15 min. The mean duration of TI per replicate was used in the statistical analysis.

Bird activity During the period of exclusion from the litter area in the closed treatment, the number of 'runs' was recorded in both treatments on a group basis. A run was defined as a hen running for a minimum distance of 2 hen lengths (around 70 cm), which was determined

by analyzing video recordings. Run determinations were made for 3 periods of 10 min/d (hours 0715, 1215, and 1515) on a total of 5 d during the first week of exclusion from the litter area. The total number of runs for each period (10 min) and replicate was used in the statistical analysis.

Utilization of the litter area The proportion of hens utilizing the litter area was recorded on a total of 15 d between 18 and 62 wk age. The number of hens in the litter area was counted by studying a snapshot from the video recordings on 3 occasions per day (hours 0515, 1215, and 1515), although some recordings were made 1-h earlier or later due to the changes in the lighting schedule. The mean proportion of hens utilizing the litter area per day (corrected for mortality) was used for the statistical analysis.

Corticosterone metabolites in droppings Bird droppings were collected between the hours of 1000 and 1200 by placing 2 plastic trays beneath the slatted area of each replicate. A collection was made once per day during the first 16 d after arrival (16 to 18 wk age) and then once per week at 19, 20, 21, 22, 40 and 53 wk age. In total, 22 collections were carried out per replicate. Samples of droppings (mean weight 175 g) were placed in sealed plastic bags and frozen (-20°C) until further analysis. Fecal corticosterone metabolites (FCM) were analyzed using 2 different enzyme immunoassays (EIA) and extraction procedures; a cortisone EIA and a corticosterone EIA. For analysis of FCM by the cortisone assay, 0.5 g well-homogenized and thawed droppings was mixed with 5 mL 60% methanol and extracted as described previously by Palme et al. (2013). An aliquot was diluted 1:10 with assay buffer and 30 μL analyzed with a cortisone EIA. Details of the EIA and its validation for use in laying hens are presented in a previous publication (Rettenbacher et al., 2004). For analysis of FCM by the corticosterone assay, the samples were thawed, homogenized, and dried at 103°C for 16 to 20 h. They were then milled to a fine powder and DM content was determined after another 16 h at 103°C and used for expressing the results in nanograms/gram DM. Extraction was made according to the manufacturer's protocol (Arbor Assays, Ann Arbor, MI). In brief, 0.2 g dried sample was shaken for 30 min with 2 mL 95% ethanol. After centrifugation, the supernatant was dried down in a SpeedVac (Savant Instruments Inc., Holbrook, NY) and stored at -20°C . The extract was then dissolved in 100 μL ethanol and 400 μL assay buffer and a 50- μL portion was analyzed with a corticosterone EIA (Arbor Assays, Ann Arbor, MI). More details of the EIA and its validation for use in laying hens can be found in Alm et al. (2014).

Egg shell irregularities At 40 wk age, 60 randomly selected eggs per replicate were assessed with respect to visual irregularities in the egg shell. These were defined as: wrinkled top, pimples (small bumps), spotted (areas with thinner shell), striped (longitudinal grooves), and thin-shelled. One egg at a time was examined and categorized as being either without irregularity or having

Table 1. Effect of treatment on production performance parameters and mortality from 20 to 72 wk age. Values presented least-square means

Item	Feed intake (g/hen d)	FCR ² (g/g)	Lay (%)	Egg production (kg egg mass/HH ⁴)	Floor laid eggs ³ (%)	Mortality (%)
Treatment ¹						
Open	143 ^a	2.50 ^a	89.8	20.0	3.01	12.0
Closed	127 ^b	2.20 ^b	90.7	20.8	2.99	6.33
SEM	2.66	0.05	1.14	0.30	0.98	2.52
<i>P</i> -value	0.040	0.029	0.725	0.253	0.960	0.312

¹Open = hens with full access to the litter area; closed = hens excluded from the litter area during 2 wk after transfer to the laying facility; for both treatments n = 3.

²Feed conversion ratio.

³Eggs laid in the litter area or slatted area.

⁴Hen housed.

^{a,b}Values within columns with different superscripts are significantly different ($P < 0.05$).

one, or occasionally 2, of the irregularities categorized as mentioned previously.

Statistical Analysis

All data were processed and analyzed using SAS statistical software (SAS Institute Inc., Cary, NC, version 9.2). The software MIXED procedure was used for production performance, mortality, and traits measured repeatedly (integument scores, proportion of cracked and dirty eggs, FCM, bird activity, utilization of the litter area, and NO test) and included the fixed effect of treatment (n = 2), day/time of sampling (n = 2 to 22 depending on measurement), and their interactions. An autoregressive covariance structure was used in the analysis of integument score, the NO test, and the proportion of cracked and dirty eggs. A spatial power law covariance structure was used in the analysis of FCM, bird activity, and utilization of the litter area, in order to adjust for nonregular intervals between repeated measurements. Egg shell irregularities were analyzed with the software GLIMMIX procedure, using a logistic regression distribution, whereas TI latencies were compared by a Kruskal–Wallis test. In order to achieve a normal distribution, the NO data were log-transformed, and mortality and proportions of misplaced, cracked, and dirty eggs were subjected to arcsin transformation before analysis (Snedecor and Cochran, 1989). However, the results shown in this paper are the untransformed mean values. In pairwise comparisons, a value of $P < 0.05$ after Tukey–Kramer adjustment for multiple comparisons was considered to indicate a statistically significant difference. Pen was considered as the statistical unit, giving 3 replicates per treatment.

RESULTS

Production Performance

The production performance results showed that birds in the open treatment had higher feed intake ($P = 0.040$) and inferior FCR ($P = 0.029$) than birds in the closed treatment (Table 1). No difference be-

tween treatments was seen with respect to laying percentage, egg production, floor-laid eggs, or mortality (Table 1). The average proportion of cracked and dirty eggs was 2.1 and 15.7%, respectively, and both values increased with time (SEM = 0.20, $P < 0.001$ and SEM = 0.93, $P = 0.004$, respectively), although no effect of treatment was observed (data not shown). In a separate analysis including only the first 4-wk period in the study, no difference was found in feed intake between birds in the open (121.2 g/hen d) and closed (121.9 g/hen d) treatments (SEM = 0.23, $P = 0.196$).

Integument Scoring

Feather cover (total score) was better ($P = 0.025$) in the closed treatment compared with the open treatment (Table 2). The individual body parts with significantly better feather cover score in the closed treatment were neck, back, and wings (data not shown). Bird live weight, pecking wounds, bumble foot, and keel bone deviation did not differ between the treatments (Table 2). Feather cover decreased ($P = 0.002$) from 40 to 54 wk age, whereas condition of pecking wounds on comb and keel bone deviations improved with age ($P = 0.040$ and $P = 0.003$, respectively). There was a significant treatment \times age interaction in rear pecking wounds (Table 2). However, when applying the Tukey–Kramer adjustment for multiple comparisons, differences between treatment means were not detectable.

Fearfulness

In the NO test, the average proportion of hens within one hen length of the NO was higher (SEM = 0.25, $P = 0.001$) in the closed treatment (3.2%) than in the open treatment (1.2%) (Figure 2). A treatment \times age interaction ($P = 0.013$) revealed that the NO response of hens in the open and closed treatments was similar in the beginning and end of the study, whereas the response differed between 38 to 57 wk age. The average proportion of hens within 1 hen length of NO gradually decreased with age in both treatments.

Table 2. Effect of treatment on live body weight and integument scores at 40 and 54 wk age. Values presented are least-square means

Item	Live weight (kg)	Feather cover ³	Pecking wounds ⁴		Bumble foot ⁴	Keel bone deviations ⁴
			comb	rear		
Treatment ¹						
Open	1.715	11.1 ^b	2.86	3.03	3.56	3.60
Closed	1.746	16.2 ^a	2.86	3.34	3.51	3.56
Age ²						
40 wk	1.731	19.7 ^a	2.81 ^b	3.16	3.45	3.46 ^b
54 wk	1.730	16.8 ^b	2.91 ^a	3.21	3.60	3.70 ^a
SEM	0.007	0.728	0.016	0.091	0.051	0.021
<i>P</i> -value						
Treatment (T)	0.081	0.025	1.000	0.162	0.647	0.471
Age (A)	0.942	0.002	0.040	0.663	0.093	0.003
T × A	0.650	0.242	1.000	0.034	0.390	0.598

¹Open = hens with full access to the litter area; closed = hens excluded from the litter area during 2 wk after transfer to the laying facility, for both treatments n = 3.

²For both ages n = 6.

³Score between 6 and 24 where a higher score indicates a better condition.

⁴Score between 1 and 4 where a higher score indicates a better condition.

^{a,b}Values in columns within the sections treatment and age with different superscripts are significantly different ($P < 0.05$).

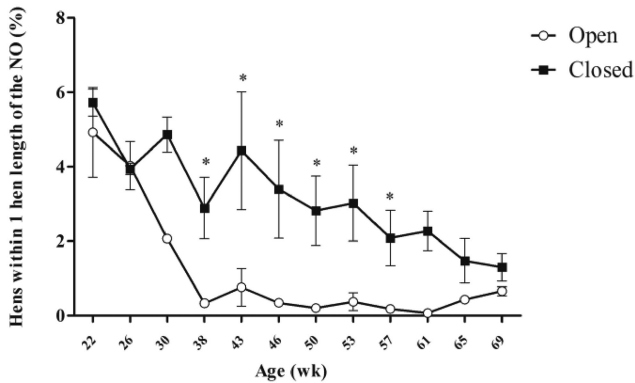


Figure 2. Percentage of hens (mean \pm SEM) within 1 hen length of the novel object recorded at different ages (22 to 69 wk age) for hens with full access to the litter area (open) and hens excluded from the litter area during the first 2 wk after transfer to the laying facility (closed). The 2 treatments differ significantly ($P < 0.05$) at ages marked with an asterisk.

In the TI test, birds in the open treatment showed longer (SEM = 1.21, $P < 0.05$) average duration of tonic immobility (11.4 min) compared with birds in the closed treatment (6.6 min).

Bird Activity

During the period 16 to 18 wk age, when birds in the closed treatment were excluded from the litter area, they performed fewer runs (SEM = 2.50, $P = 0.01$) per 10-min period (25.4) compared with birds in the open treatment (48.6). The average number of runs recorded per time interval (10 min) was 37.0 and no effect of time of day, or interaction between time of day and treatment, was seen.

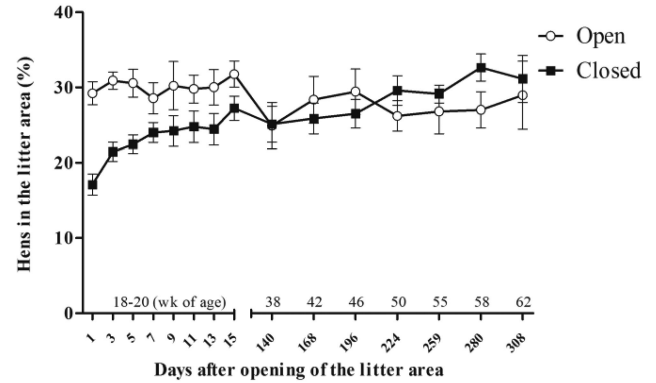


Figure 3. Percentage of hens (mean \pm SEM) utilizing the litter area recorded for hens with full access to the litter area (open) and hens excluded from the litter area during the first 2 wk after transfer to the laying facility (closed). The number of hens was recorded at 15 different times between 1 and 308 d after the pen litter area was opened.

Utilization of the Litter Area

There was no difference (SEM = 0.79, $P = 0.118$) in mean proportion of hens utilizing the litter area between the open (28.8%) and closed (25.7%) treatments. However, a numerically lower proportion of hens in the closed treatment utilized the litter area in the first days after they were given access (Figure 3). The proportion then gradually increased to the same levels as in the open treatment.

Corticosterone Metabolites in Droppings

No difference in FCM levels could be detected between the open and closed treatments with either the corticosterone EIA (129 ng/g DM vs 128 ng/g DM; SEM = 2.24, $P = 0.930$) or the cortisone EIA (120 ng/g vs. 116 ng/g; SEM = 1.74, $P = 0.317$). Over time, the corticosterone EIA only showed a significant

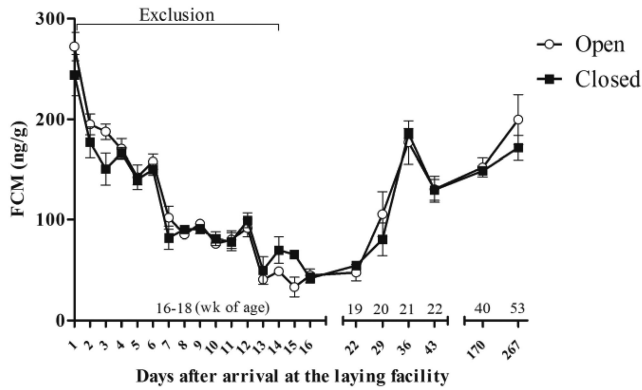


Figure 4. Concentrations (mean \pm SEM) of fecal corticosterone metabolites (FCM) measured by a cortisone enzyme immunoassay in droppings from hens with full access to the litter area (open) and hens excluded from the litter area during the first 2 wk after transfer to the laying facility (closed). FCM levels were measured 22 times between 1 and 267 d after the arrival at the laying facility.

difference ($P = 0.007$) between 2 of the days studied (d 13 and 29), whereas the cortisone EIA displayed a greater change over time (several days, $P < 0.001$) and hence a more distinct pattern. Therefore only the results of the cortisone EIA are shown in Figure 4.

Egg Shell Irregularities

The egg shell irregularity data (Table 3) revealed that the proportion of eggs with a wrinkled top was higher ($P = 0.034$) in the open compared with the closed treatment, but no significant differences were found in pimpled, spotted, striped, or thin-shelled eggs. However, when the values for all categories were added together, the eggs in the open treatment had a higher proportion ($P = 0.047$) of irregularities compared with those in the closed treatment.

DISCUSSION

In this study, hens were 1) excluded from the litter area during their first 2 wk in the laying facility, and consequently housed at a higher stocking density and without litter (closed treatment), or 2) were given full access to the litter area from the start (open treat-

ment). Fencing the layers onto the slatted floor in the closed treatment resulted in better feather cover and less fearful hens laying eggs with a lower proportion of eggshell irregularities compared with hens given full access to the litter area from the start. An interesting unanswered question is whether these differences arose from the temporary difference in bird density or from the delayed access to litter.

Litter has been identified as an important resource for layers (e.g., Gunnarsson et al., 2000; Widowski and Duncan, 2000) and denied access to litter might be a risk factor for feather pecking (de Haas et al., 2014). In the present study, the birds with postponed access to the litter area gained access to this resource (which can increase their occupancy) at the critical period of onset of lay, which could have been beneficial. However, when the period of temporary exclusion from the litter area ended, there was no difference between the treatments in terms of proportion of hens occupying the litter area, indicating that this was not the case.

Thus a more plausible explanation is that the differences between the treatments were due to the difference in densities. The better feather cover seen in layers excluded from the litter area supports previous findings by Nicol et al. (2006) of better feather cover at the end of production in hens kept at high stocking density (12 hens/m²) compared with low stocking densities (7 and 9 hens/m²). However, several studies show the opposite, i.e., that higher stocking density both during rearing (e.g., Bestman et al., 2009) and during lay (e.g., Nicol et al., 1999) decreases feather cover later on. In our study, the birds were reared at a density of 15 hens/m² and the temporary exclusion from the litter area resulted in a similar stocking density (12.2 hens/m²) for these birds during their early time in the layer facility. The hens in the other treatment were subjected to an abrupt decrease in stocking density from 15 to 7.8 hens/m². There is a general recommendation that pullets should be reared in a system similar to that in the subsequent layer facility in order to allow them to adapt more easily after transfer (Colson et al., 2008). It is also reported that a smooth transition can reduce feather pecking (Van de Weerd and Elson, 2006). Therefore in the present study the

Table 3. Effect of treatment on egg shell irregularities at 40 wk age. Values presented are means

Item	Wrinkled top (%)	Pimples (%)	Spotted (%)	Striped (%)	Thin shelled (%)	Sum ² (%)
Treatment ¹						
Open	21.1 ^a	8.88	18.3	9.44	2.22	60.0 ^a
Closed	8.89 ^b	12.2	18.9	3.89	1.11	45.0 ^b
SEM	2.92	1.27	2.17	1.93	0.43	5.42
<i>P</i> -value	0.034	0.363	0.899	0.110	0.465	0.047

¹Open = hens with full access to the litter area; closed = hens excluded from the litter area during 2 wk after transfer to the laying facility; for both treatments $n = 3$.

²The sum of all shell irregularities recorded in the study.

^{a,b}Values within columns with different superscripts are significantly different ($P < 0.05$).

possibility cannot be excluded that keeping the hens in the closed treatment at a similar density as during rearing resulted in a smoother transition between systems which may have been beneficial for the hens.

Birds can perform runs for several different reasons but the cause for the behavior was not investigated in the present study. Aggressive interactions such as chases (Estevez et al., 2002) might be one of the reasons. The hens initially restricted to the slatted floor in the present study performed fewer runs, perhaps indicating that they were less aggressive during this period. Similar results have been reported by Zimmerman et al. (2006), who found that the lowest incidence of feather pecking and aggression occurred with the highest stocking density early in lay. This led them to conclude that housing hens at a higher initial stocking density and then decreasing the density with age might be a way to reduce aggression and feather pecking. The decreased social distance between group mates in the present study might have lowered the aggression due to constant violation of personal space, in contrast to occasional violation when kept on larger space as suggested by Hughes and Wood-Gush (1977). However, the fact that fewer runs were performed by hens initially excluded from the litter area may also be attributable to lack of space to perform this behavior, and thus limiting the use of runs as criteria for aggression.

As a consequence of less available space for birds being excluded from the litter area, a hen exposed to something stressful might not spread fear to the other hens in the same way as if she had shown a flight reaction. This might explain why hens given access to the whole pen area in the present study throughout showed both longer latency of TI and higher avoidance of a NO which according to Jones (1986), for example, indicates that they were more fearful than hens excluded from the litter area for 2 wk. These hens also showed worse feather cover which is supported by other studies that have reported a correlation between fear and feather pecking (e.g., Vestergaard et al., 1993; Rodenburg et al., 2004). However, there are studies showing contradicting results. Rodenburg et al. (2010) could not correlate fear and feather pecking, in lines selected for either high or low feather pecking, and Bögelein et al. (2014), for example, show contrasting results depending on type of fear test used and age of birds. In the present study, hens in both treatments became more fearful with age, according to NO observations, as previously observed in some other studies (Anderson et al., 2004; Uitdehaag et al., 2008). However, several studies show the opposite i.e., a decreased fear with age (Hocking et al., 2001; Albentosa et al., 2003; Bögelein et al., 2014). These differences between studies suggest that the relationship between fear and feather pecking as well as the impact of age is complex, and most likely multifactorial. The interaction between treatment and age in hen response to a NO revealed that the treatments did not differ in the beginning or in the end of the study. In the present study, the hens nor-

mally utilized the whole litter area, but during the NO test and towards the end of the study in particular, most hens completely avoided the litter. This indicates that the hens became more fearful with age, rather than that they became accustomed to the NO and lost interest.

During a layer's production cycle, various irregularities in egg shells are normally seen (Wolc et al., 2012). Some of these irregularities are caused by delayed oviposition due to stress (Hughes et al., 1986; Mazzuco and Bertechini, 2014). Thus the number of eggs with misshapen shells produced by a flock can be used as an indicator of a stressful environment (Hughes et al., 1986; Solomon, 1997; Reynard and Savory, 1999). Because the proportion of eggs with large deviations in their shells is usually not very high, we opted to also record very small differences in egg shell appearance that might not have been classified as a defect in previous studies, e.g., eggs with small wrinkles at the top. We found that the occurrence of this irregularity and the total number of all shell irregularities were higher in the groups given open access to the whole pen than in those initially closed off from the litter area. The fact that access to the whole pen also resulted in hens with poorer feather cover is in line with the finding by Sherwin et al. (2010) that an increased proportion of irregular egg shells is correlated with poor feather cover and a higher concentration of FCM. If the 'wrinkled top' shell irregularity proves to be correlated to welfare or stress parameters in other studies, it might have the potential to serve as an objective and easy-to-use welfare indicator.

In the present study, FCM was measured as an indicator of adrenocortical activity (Palme, 2012). No difference in FCM concentrations between the treatments was seen with either of the 2 EIAs and a significant variation over time was only observed with the cortisone EIA. The difference between the 2 EIAs may be due to the different extraction methods used (Palme et al., 2013) and because the assays detect different groups of corticosterone metabolites (Möstl et al., 2005). Thus the cortisone EIA seems to show a higher biological sensitivity in measuring smaller differences in adrenocortical activity (Touma and Palme, 2005). The high values observed with the cortisone EIA immediately after bird arrival at the layer facility in the present study was most likely due to the stress arising from catching and transport between the rearing and laying facilities, followed by introduction to a new and unfamiliar environment, as described previously by Rettenbacher and Palme (2009). The similar levels of FCM in both treatments during the first 14 d may indicate that being excluded from the litter area itself is not stressful, although the stress from being transferred might overshadow any other effects. During this 14-d period, FCM levels decreased in both treatments, but levels increased again towards the end of the production period. This increase in FCM levels, together with the increased fearfulness and impaired feather cover, suggests that hen

welfare declined with age. This is in line with results reported by Nicol et al. (2006) of decreased feather cover and higher FCM levels at the end of the production period. In the present study, however, impaired feather cover and increased fearfulness appeared much earlier and more rapidly in hens given open access to the whole pen area compared with those initially closed off from the litter area.

Due to the large size of the flock and the pen including the litter area in a commercial layer unit, resources such as nests, feed, and water may be located at a considerable distance from the litter. Hence, one of the reasons for fencing pullets on the slatted floor on introducing them to the laying facility is to help them to find feed, water, and nests. With the small groups and limited pen size used in the present study, the layers were always rather close to feed and water, regardless of whether they were fenced onto the slatted floor or not. Feed consumption after the first 4-wk period did not differ between treatments, but over the entire experimental period hens given access to the whole pen area from the start had higher feed intake and FCR compared with hens initially excluded from the litter area. This can be linked to the significantly inferior feather cover in the former group, as this increases feed consumption in order to compensate for the body heat loss due to impaired insulation (Tauson and Svensson, 1980; Peguri and Coon, 1993).

In commercial facilities, the pullets are also restricted to the slatted floor area in order to reduce the proportion of floor-laid eggs. However, in the present study there was no difference in the proportion of floor-laid eggs between treatments. This is possibly because the conditions in a small-scale experiment differ from those in a large-scale production facility by having (for example) a relatively small distance to walls and a higher number of corners per bird in the litter area, which can affect the willingness of hens to lay their eggs in the litter.

No difference in rate of lay, egg production, proportion of cracked or dirty eggs, or mortality was seen between treatments. Numerically, mean mortality was much higher in the hens given access to the whole pen area throughout, but this was mainly caused by a single group with particularly high mortality (primarily due to pecking) in comparison with the others. The proportion of dirty eggs was relatively high, which could be correlated to the high litter moisture. Presence of pecking wounds on comb and keel bone deviations decreased with age, but did not differ between treatments. Pecking wounds on the comb are an indication of aggression, which commonly occurs early in the production phase during the establishment of pecking order (Guhl, 1968). The reason for the decrease in keel bone deviations observed in this study is unknown, but it is probably explained by random factors in the selection of birds. Otherwise, an increase in this defect normally occurs with age due to the cumulative load on the keel bone when resting on perches (e.g. Valkonen et al., 2009).

In conclusion, temporary exclusion of pullets from the litter area after transfer to the laying facility resulted in less fearful hens with better feather cover producing eggs with fewer shell irregularities than giving the pullets open access to the whole pen directly after transfer. However, there was no difference in the proportion of floor-laid eggs or in FCM levels. None of the parameters included in this study indicated that the welfare of laying hens was compromised by being excluded from the litter area for 2 wk after they were introduced to the laying facility. In fact, some of the data obtained suggested that welfare had improved.

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