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Effect of early litter access on plumage and fearfulness in commercial laying hen pullets

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ABSTRACT

Providing access to fresh and dry litter, in addition to chick paper from day 1 of rearing may improve plumage and reduce fear responses in pullets due to stimulation of more positive and natural pecking behaviour. However, few studies have examined this relationship in commercial rearing flocks. The aim of this study was to investigate the effect of early access to fresh litter and chick paper on fear responses and plumage quality during the rearing period. Eight commercial pullet flocks (Lohman LSL Lite n = 6 and Dekalb White n = 2) were divided in two treatments; Litter; birds with access to chick paper and cardboard trays with litter from day 0–21 and Control; birds with access to chick paper from day 0 – 21. Each flock was visited at weeks 2, 7 and 14 of age. Data collection included scoring plumage condition using two methods, (1) a whole flock assessment using transect walk with indicators on a binary scale and (2) detailed individual scoring of 30 birds/treatment/age, in addition to two behavioural tests of fear-related behaviour, (1) Novel Object (NO) test at weeks 2, 7 and 14 and (2) Stationary person (SP) test at weeks 7 and 14. Providing fresh litter during the first three weeks of life reduced fear response towards a NO, with more Litter birds approaching the NO compared to the Control group ($P < 0.05$). There was a significant effect of age ($P < 0.0001$), with more birds approaching the NO at 7 and 14 weeks of age compared to week 2 of age ($P < 0.0001$ and $P = 0.0003$, respectively). Litter access had no effect on fear towards a SP at week 7 or 14, but more birds approached the SP at 14 weeks compared to at 7 weeks ($P < 0.006$). Furthermore, litter provision did not influence plumage quality or other health parameters at any age. Slight wear to tail feathers was detected in 65 out of 1440 birds during the individual scoring. When assessing welfare and plumage in healthy flocks with minimal welfare issues, detailed scoring of individual birds appears to be necessary, in addition to the whole flock assessment using the transect walk.

1. Introduction

The rearing period is a crucial part of laying hens' lives and is known to affect health parameters in the adult hens including immune function (Walstra et al., 2010), skeletal development (Regmi et al., 2015) and mortality risk (Schreiter et al., 2022). Furthermore, factors in the rearing environment affect behavioural development including cognitive ability such as spatial performance and spatial memory (Tahamtani et al., 2015), behavioural adaptability (Pullin et al., 2024; Skånberg et al., 2023), fearfulness (Jones, 1996; Brantsæter et al., 2017), feather pecking (Bestman et al., 2009; De Haas et al., 2014; Schreiter et al., 2022) and plumage quality in adult laying hens (Gernand et al., 2022; Schwarzer et al., 2022a; Tahamtani et al., 2016). In aviary systems, pullets are often kept confined inside the aviary tiers for the first three to five weeks to secure access to feed and water. These aviary tiers have wired floors to allow removal of waste via the manure belt underneath. Therefore, unless the tiers are covered by chick paper with build-up of down feathers, feed, dust and faecal material, pullets usually do not have access to litter in this period.

Studies have found that lack of relevant litter substrate in the first 4

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weeks of life increases plumage damage and feather pecking during both the rearing period (Janczak and Riber, 2015; De Haas et al., 2014; Mels et al., 2022) and later at the adult stage (Bestman et al., 2009; Newberry et al., 2007). Severe feather pecking remains one of the most challenging issues for laying hen farmers with regards to economics and animal welfare, due to increased feed intake (Vasdal et al., 2023) and higher mortality levels (Kittelsen et al., 2022). Feather pecking is largely accepted as redirected ground pecking (Rodenburg et al., 2013; Savory and Mann, 1997) and chicks start exploring and pecking their surroundings already at the day of hatch. Although good management and provision of environmental enrichments in the layer flocks can reduce the development of feather pecking and improve plumage (Gilani et al., 2013; Tahamtani et al., 2016), prevalence of feather pecking during rearing increases the risk of feather pecking during lay (Bestman et al., 2009; De Haas et al., 2014; Rodenburg et al., 2013). In general, it is challenging to stop feather pecking once it has started, and the focus must be on preventing the behaviour from developing.

A previous study provided commercial pullets with chick paper on the wired floor during the first 5 weeks of life and found reduced feather damage at 30 weeks of age compared to the control groups housed on wired floor the first 5 weeks (Tahamtani et al., 2016). As part of the same study, (Brantsæter et al., 2017) found that provision of chick paper during the first five weeks during rearing reduced fearfulness, measured by the stationary person test and novel object test at 30 weeks of age compared to the control birds. Thus, in addition to being associated with increased feather pecking (De Haas et al., 2014; Hüttner et al., 2023), increased fearfulness often makes the flock more challenging to manage and may increase mortality (Rodenburg et al., 2013). Therefore, reducing fearfulness and providing the foundation for appropriate foraging behaviour in early life may be vital to ensure good plumage, welfare and production throughout the hens' life.

Provision of chick paper is considered a cheap and feasible management procedure and can be applied even in large rearing facilities. However, the litter that accumulates on the chick paper is a build-up of faeces, dust and feathers, and the litter can often be hard and crusty, which is not suitable for pecking, scratching or dustbathing. Furthermore, faeces is not a preferred foraging material for laying hens (Von Waldburg-Zeil et al., 2019) and the chick paper itself can be picked apart. In pullets, several types of substrates have been tested, including sand, long straw, peat and wood-shavings and most have been found to reduce feather pecking (Blokhuis and Van Der Haar, 1989; Vestergaard et al., 1993). Furthermore, not only the type but also the quality of the substrate is known to affect the birds' use, and broilers have been shown to prefer to dustbathe in fresh, fine-grained materials such as peat (Holt et al., 2023). The same study reports that provision of fresh litter once a week stimulated both ground scratching and dustbathing. The effects of continuous access to fresh litter, in addition to chick paper provision in the first weeks has not been investigated in commercial pullets housed within aviary systems with wired floors. Based on previous literature, this combination may have additional positive effects on fearfulness and plumage during the rearing period.

Early exposure to litter allows pullets to perform natural behaviours like pecking, scratching, and dustbathing. This may both reduce frustration and give birds a more appropriate pecking substrate, thereby reducing attention towards feathers of flock mates as a target for feather pecking. The sensory and structural complexity of litter may also help birds habituate to novelty, reducing fear responses later in life. Together, these effects may make birds calmer and less prone to feather pecking and the resulting damage.

Therefore, the aim of this study was to investigate the effect of early access to fresh litter and chick paper, compared to only chick paper, on fear responses and plumage quality in aviary-housed layer pullets during the rearing period in commercial farms. We hypothesized that early-life exposure in pullets to both fresh litter and chick paper reduces fear responses to novelty and fear of humans compared with chick paper alone. We predicted that treatment birds, given a combination of fresh litter and paper, would show reduced avoidance and spend more time in proximity of the stationary person and novel object. We also expected treatments birds to have a better plumage condition at the end of the rearing period compared to the control group.

2. Material and methods

Because the study involved no experimental manipulations or invasive procedures, it was therefore exempted from approval of animal use by the Norwegian Food Safety Authority (Norwegian Regulations on Use of Animals in Research, 2015).

2.1. Animals and housing

The study comprised a total of eight commercial pullet flocks (Lohmann LSL Lite n = 6 and Dekalb White n = 2), distributed across four pullet rearers with two consecutive flocks per rearer. Each flock was visited three times (weeks 2, 7 and 14) during the rearing period (0–16 weeks of age (WOA)) from July 2024 to May 2025 to assess plumage, wounds and fearfulness. Each flock was divided into two treatments; Litter; birds with access to chick paper and cardboard trays with litter from days 0–21 and Control; birds with access to chick paper from days 0 – 21. On day 21, all birds were let out of the aviary tiers and had access to litter (wood shavings) on the floor. All other management factors were kept identical between treatments.

All flocks were comprised of white-feathered pullets with intact beaks, housed in indoor multitiered aviary systems with automatic feeding, mechanical ventilation and artificial lighting. The aviary systems had a similar layout, with 3 wired tiers above the littered floor, feed and water lines on tiers 1 and 2 and perches on tiers 1, 2 and 3. The birds were placed in tier 1 and 2 during the first 21 days. The flocks were managed according to standard practices with regards to feed, litter and lighting (KSL, 2023). All farms provided litter material (wood shavings) on the floor from day 21. The pullets arrived at the farm as day-old and were transported to egg production facilities at 16–17 WOA. The flock size ranged from 16,000 to 64,000 pullets, and animal density followed Norwegian regulations (24 animals/m^2 usable area). Average mortality in the flocks from 0 to 16 weeks was 1.57 % (flock range 0.7–2.8 %).

2.2. Treatments

The two treatments within each flock were kept in the same house and separated by wire mesh between the aviary rows. For the litter not to disappear too quickly in the Litter treatment, litter was provided in specially designed cardboard trays (Fig. 1). The trays measured 7 cm× 100 cm (height x length), and tray width ranged from 12 to 22 cm between flocks, as determined by the width between the front of the aviary and the feed line (Fig. 1). Three trays per 1000 birds were placed in the front section of the aviary. Litter made of finely ground and heat-treated rape straw (AJ Cross, AJ Energi & Strø, Aulum DK) was provided by the farmer once daily from days 1–21, with a litter depth of around 5 cm per tray after each provision. The treatment group also had chick paper provided on the wired floor (Fig. 1). The control group had an equal amount of chick paper provided on the wired floor, but no trays of litter. Apart from the litter trays, all other housing conditions and management practices were equal between treatments.

2.3. Aviary transect walk

Assessment of health and plumage was assessed using two different methods. Two observers with extensive poultry experience (GV, FMT) conducted all the assessments. Before data collection started, the 2 observers visited 2 rearing flocks to practise the transect walk and the individual assessments to achieve agreement in scoring. First, the aviary transect walk (Vasdal et al., 2022) was performed to get a whole-flock assessment of selected health indicators in both treatments.

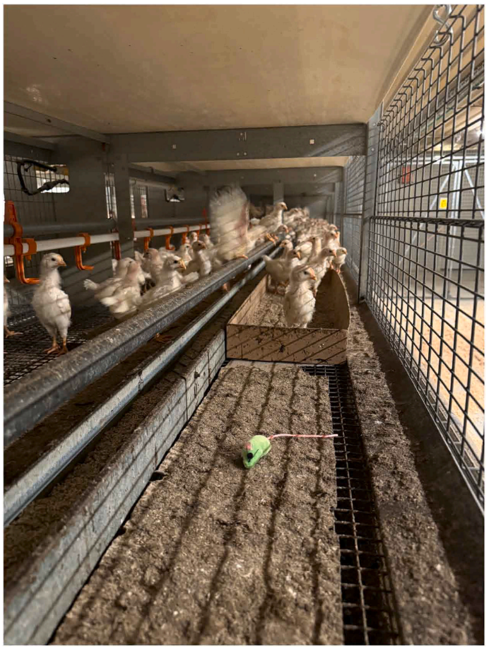

Fig. 1. The cardboard tray with litter placed in the aviary system. In front of the tray is the novel object placed on the chick paper.

Standardised transect walks were conducted along the entire length of the house. In each transect, the number of birds showing each of the 11 predefined welfare indicators was recorded (Table 1). The observer moved slowly to minimise disruption of birds and observed birds on all three tiers, on the littered floor and underneath the aviary. It should be noted that the aviary transect is developed for adult laying hens, where plumage damage is more common. However, as the present study was done in commercial flocks, the farmers preferred assessment methods that did not involve handling and potentially stressing the birds. Therefore, methods which require handling the birds in the house as in (Schwarzer et al., 2022b) or taking birds to the anteroom for individual assessment as in (Mels et al., 2022) was not an option.

Table 1
Description of 11 welfare indicator categories assessed when using the aviary transect walk (Vasdal et al., 2022).

Indicator[1]	Description
FL head	Missing feathers on the head, including the neck, $\geq$ 5 cm in diameter
FL back	Missing feathers on $\geq$ 50 % of the back, including the wings
FL breast	Missing feathers on the breast, $\geq$ 5 cm in diameter
FL tail	Missing or clearly damaged feathers on the tail, mainly shafts and rachises left
Dirty	Prominent dark staining of the back, wing, or tail feathers, covering at least 25 % of the body; not including light discoloration of feathers from dust.
Wounds head	Prominent marks on the head and neck, due to fresh or older wounds.
Wounds back	Prominent marks on the back, including the wings, due to fresh or older wounds.
Wounds tail	Prominent marks on the tail due to fresh or older wounds.
Wounds feet	Includes bumblefoot (visible dorsally), and prominent marks on the feet due to fresh or older wounds
Sick	Clear signs of impaired health, including a small and pale comb, red-watery eyes, disarranged feathers, missing or deformed body parts, clearly different (pale or yellowish) skin colour; often found in a resting position
Dead	Dead bird found when walking along a transect

[1] Hens could be classified as belonging to more than one category; **FL** – feather loss

2.4. Individual assessment

The transect method is practical to screen for more severe welfare issues in large flocks of poultry (Vasdal et al., 2023). However, to obtain more detailed scoring of individual birds, individual assessments were also included. For each age and treatment, 30 random birds were selected from predefined areas, covering all transects and tiers including underneath the aviary. Birds were not handled but each bird was observed closely to record any signs of feather loss or wounds (Table 2). This assessment is a combination of existing methods for pullets that involve handling the birds (Mels et al., 2022) and methods for laying hens including AssureWel (Blokhuis et al., 2007) and LayWel (Main et al., 2012) which are based on observations from a distance.

2.5. Novel object test

The novel object (NO) test was done in accordance with Welfare Quality® Assessment protocol for poultry and previous protocols conducted on loose housed hens (Brantsæter et al., 2017; De Haas et al., 2014). To generate a representative average for the flock, the NO test was performed in four locations per treatment at weeks 2, 7 and 14. The NOs (Katteleker, Biltema, Norway) tested were a toy mouse (week 2), a ball (week 7) and a dumbbell (week 14) (Fig. 2). During the test, the NO was placed on the chick paper (week 2) or in the litter in the corridor (weeks 7 and 14), and the researcher slowly stepped backwards 10 steps. After placement, every 10 s, the researcher counted the number of hens within bird length (approx. 25 cm) of the NO. Each of the four tests per group and per age lasted a total of two min.

2.6. Stationary person test

At weeks 7 and 14, after the novel object test, a stationary person test was done in four random locations in each treatment in accordance with the method described in (Brantsæter et al., 2017). Once the observer reached a location, they stood still and counted the number of birds within a 1-m distance every 20 s for 2 min, aided by a stopwatch. Following the SP test, the observer continued immediately with the Novel Object test. The observers always used the same type of overall for all visits, ages and tests, a dark blue disposable coverall.

2.7. Statistical analysis

Statistical analyses were performed using the software SAS 9.4 (SAS Institute, Cary, NC, USA). The data from the transect walks, NOT and SPT were averaged per treatment and per visit and frequencies were

Table 2
Description of the indicators scored on 30 random birds per treatment (based on Main et al., 2012; Vasdal et al., 2022 and Mels et al., 2022).

Indicator	Description
FL head	0: no loss, 1: slight wear, featherless area < 5 cm, 2: substantial wear, large featherless area $\geq$ 5 cm diameter
FL back	0: no loss, 1: slight wear, featherless area < 5 cm, 2: substantial wear, large featherless area $\geq$ 5 cm diameter
FL wings	0: no loss, 1: slight wear, some damaged or broken feather tips, 2: substantial wear, many damaged or broken feather tips
FL breast	0: no loss, 1: slight wear, featherless area < 5 cm, 2: substantial wear, large featherless area $\geq$ 5 cm diameter
FL tail	0: no wear, 1: slight damage, 2: substantial wear, only shafts and rachises left
Wounds feet	Wounds feet: 0: no wounds, 1: clearly visible fresh or older wound
Wounds head	Wounds head: 0: no wounds, 1: clearly visible fresh or older wound, one or more encrusted feather shafts and/or skin lesions
Wounds back	Wounds back including wings: 0: no wounds, 1: clearly visible fresh or older wound one or more encrusted feather shafts and/or skin lesions
Wounds tail	Wounds tail: 0: no wounds, 1: clearly visible fresh or older wound

FL – feather loss

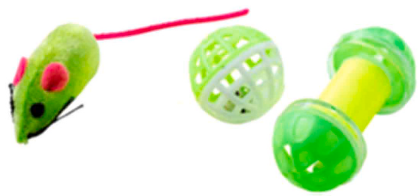

Fig. 2. The objects used in the novel object test (Katteleker, Biltema, Norway).

calculated by dividing the values by the flock size of each flock at each week of age. These data were analysed using the mixed procedure, with a Restricted Maximum Likelihood (REML) estimation method, using treatment, age and their interaction as fixed factors. In addition, flock ID nested in rearer was included in the model as a random factor. The data fit the model assumptions, e.g. normal distribution of the residuals. *Post-hoc* analyses were performed with the Tukey's HSD test.

The results of the individual assessment of 30 birds per treatment per visit revealed 100 % score 0 for feather loss of the head and back, as well as for wounds on the back, feet, and tail. The numbers of birds with scores 1 or 2 for feather loss of the tail, breast and wing as well as for wounds on the head were too few to perform formal statistical analysis. These data are, therefore, presented as descriptive statistics.

3. Results

3.1. Aviary transect walk

No cases of feather loss in any of the body parts were recorded using the transect walk method at any age. Likewise, no events of dirtiness or of wounds on the back and tail were observed. For the other variables, descriptive statistics from the data collected during transect walks are presented on Table 3. Wounds on the head were only observed at 14 weeks of age and there was no effect of treatment on their frequency ($F_{1,7} = 1.18$; $P = 0.31$). The permille of head wounds in the Control birds was 0.02 ± 0.016 (LS means ± SE) while the permille in the Litter birds was 0.015 ± 0.016 (LS means ± SE).

A total of 4 birds were recorded with wounds on the feet (2 Control, 2 Litter), all recorded in the same flock at 14 WOA. There was no effect of treatment ($F_{1,35} = 0.02$; $P = 0.87$); age ($F_{2,35} = 2.19$; $P = 0.13$) or their interaction ($F_{2,35} = 0.02$; $P = 0.97$) on the permille of wounds on the feet.

Across ages, a total of 15 sick birds were recorded (8 Control, 7 Litter), 10 of whom were observed in week 2. There was no effect of treatment ($F_{1,39} = 0.02$; $P = 0.88$); age ($F_{2,39} = 1.20$; $P = 0.31$) or their interaction ($F_{2,39} = 1.31$; $P = 0.28$) on sick birds recorded during the transect walk.

Finally, 21 birds were recorded dead during the transect walk (17 Control, 4 Litter), with 11, 7 and 3 birds in week 2, 7 and 14, respectively. There was an effect of treatment on dead birds observed during the transect ($F_{1,39} = 5.48$; $P = 0.02$), with Control birds having a higher permille of dead birds (LS Means ± SE = 0.045 ± 0.016) compared to Litter birds (LS Means ± SE = 0.012 ± 0.016). There was no significant effect of age ($F_{2,39} = 2.12$; $P = 0.13$) or the interaction between treatment and age on the frequency of dead birds ($F_{2,39} = 1.69$; $P = 0.20$).

3.2. Individual assessment

During the more detailed individual assessment of 30 birds/treatment/age, slight damage to the tail (score 1) was observed in 38 Control birds (18 and 20 birds in week 7 and 14, respectively) and 27 Litter birds (13 and 14 birds in week 7 and 14, respectively) (Fig. 3). No birds were scored with feather loss on the head or back at any age. A total of 2 Control birds were scored with slight wear of feathers on the breast in week 14, while slight wear of wing feathers was observed in 1 Control bird at week 14. Wound on the head was only observed in 1 Control bird at 14 WOA, and no birds were observed with wounds on back, tail or feet at any age using the individual assessments.

3.3. Novel object test

The results from the NOT by treatment and age are presented in Fig. 4. There was a significant effect of treatment on the number of birds approaching the novel object during the NOT ($F_{1,35} = 4.10$; $P = 0.050$), with a higher number of birds in the Litter group approaching the NO (LS means ± SE: 0.0064 ± 0.0022) compared to the Control group (LS means ± SE: 0.0048 ± 0.0022). In addition, there was a significant effect of age ($F_{2,35} = 21.94$; $P < 0.0001$), with more birds approaching the novel object at 7 and 14 weeks of age compared to week 2 of age ($P < 0.0001$ and $P = 0.0003$, respectively). In addition, there was a tendency for fewer birds to approach the novel object at week 14 of age compared to week 7 ($P = 0.09$). There was no effect of the interaction between treatment and age ($F_{2,35} = 0.05$; $P = 0.95$).

3.4. Stationary person test

In contrast, there was no effect of treatment on the number of birds approaching the observer during the SPT ($F_{1,21} = 1.14$; $P = 0.30$). There was, however, an effect of age ($F_{1,21} = 9.29$; $P = 0.006$), with more birds approaching at 14 weeks of age compared to at 7 weeks (Fig. 5).

4. Discussion

The aim of this study was to investigate the effects of early access to fresh litter on fear responses and plumage quality of aviary-housed layer

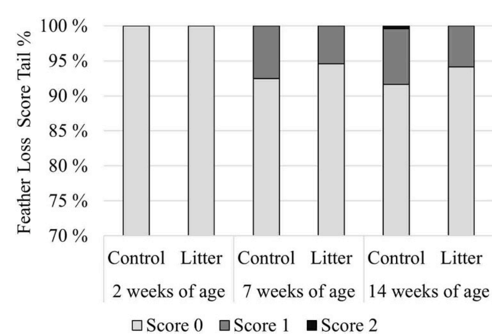

Fig. 3. Percentage of birds observed with feather loss on the tail across treatments and ages (30 random birds/treatment/age).

Table 3
Permille (mean and standard deviation) of the variables assessed during the transect walks across treatment and age.

	Control						Litter					
	2 weeks		7 weeks		14 weeks		2 weeks	7 weeks			14 weeks	
Variable	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**
Wounds head	0.00	0.00	0.00	0.00	0.02	0.04	0.00	0.00	0.00	0.00	0.02	0.04
Wounds feet	0.00	0.00	0.00	0.01	0.02	0.05	0.00	0.00	0.00	0.00	0.02	0.05
Sick	0.04	0.08	0.01	0.02	0.02	0.04	0.04	0.06	0.04	0.06	0.00	0.00
Dead	0.08	0.10	0.03	0.05	0.02	0.04	0.01	0.02	0.02	0.05	0.00	0.00

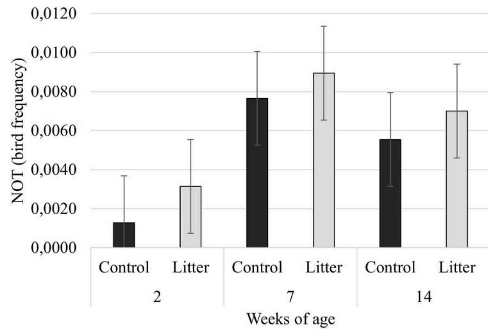

Fig. 4. Frequency of birds approaching the novel object across treatment and age (LS means ± SE).

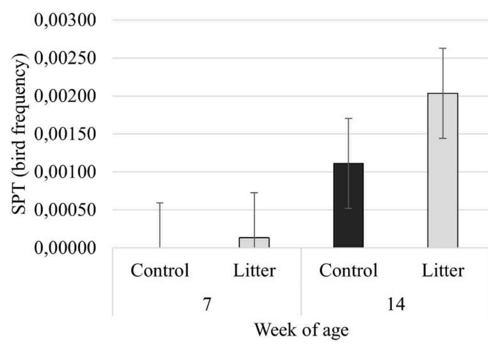

Fig. 5. Frequency of birds approaching the observer in the SPT across treatment and age (LS means ± SE).

pullets during the rearing period in commercial farms. A previous study by (De Haas et al., 2014) found that litter access during rearing reduced the risk of severe feather pecking at 40 weeks of age in ISA Brown birds. A recent study consisting of 100 flocks including brown and white hybrids on 28 rearing farms reported less feather damage at 10 weeks of age when pullets were provided litter from the first day of life (Mels et al., 2022). Therefore, we expected a better plumage condition at the end of the rearing period in our treatment birds. However, using similar indicators as (Mels et al., 2022), only 65 out of 1440 assessed birds were scored with slight wear of the tail feathers at weeks 7 and 14, and no feather loss on the head or back was observed at any age. This contrasts with the numbers reported in (Mels et al., 2022) where up to 100 % of the 15 assessed birds per flock were scored with plumage damage at 10 weeks of age, with less feather damage during rearing when birds were given access to litter from day one. It should be noted that among the 28 rearing farms in (Mels et al., 2022) there were three different hybrids, some with mixed hybrids and 17 of the farms were organic with differing management factors such as animal density and feed, which could impact feather pecking. Despite these management differences, there was less feather damage across the flocks when the pullets were provided litter from the first day. This confirms the importance of early provision of litter to direct pecking behaviour to the ground rather than towards other birds (de Jong et al., 2013) and increases foraging and natural pecking behaviour, which reduces the risk of severe feather pecking later in life (Bestman et al., 2009). In contrast to (Mels et al., 2022) we did not handle the birds during individual assessments and therefore may have underestimated minor issues. There were several reasons for not capturing birds during assessments in addition to farmer preferences; capture and handling of birds can reduce biosecurity (EFSA, 2008), it is stressful for the birds and poses a risk for sampling bias, as healthier birds might escape capture (Kjaer et al., 2011; Marchewka et al., 2015). Furthermore, when comparing plumage scoring from 2-m away with plumage scoring on captured birds, good agreement has been found in laying hens (Bright et al., 2006; Kjaer et al., 2011), indicating that reliable data can be obtained by observing birds from a distance.

In the present study, very few birds were observed with wounds on the head, back, feet or base of tail. This is far less than reported in (Mels et al., 2022), where bloody lesions were observed in 29 out of 100 flocks, with lower occurrence when pullets were raised on the floor with litter compared to aviary systems with chick paper. Lesions may be a result of direct pecking but may also be the result of injuries from the aviary system or other equipment (Guinebretière et al., 2013). (Mels et al., 2022) reported a strong correlation between feather pecking and lesions and also high variability between 100 rearing flocks for the selected animal-based indicators. In general, more knowledge is needed on these important animal-based measures in commercial rearing of layer pullets, including prevalence, variability between flocks and underlying risk factors.

Sick birds, showing clear signs of impaired health, were rare and we found no effect of treatment or age on number of sick birds observed. Across all ages, a total of 21 dead birds were observed during the transect walk, and there were fewer dead birds in the litter groups compared to control groups. However, as the study comprised around 216 000 pullets across all eight flocks, we consider 21 observed dead birds as low, and the treatment effect is considered a random finding. The low number of birds observed with wounds or sickness is in accordance with our expectations. Health status in the assessed pullet flocks was good and any bird showing signs of sickness would routinely be culled by the farmer. The visits were announced, however on several occasions the observer would enter the house before the farmer had taken the morning round, and the reported findings in this study likely reflects everyday status in the flocks.

We found a higher frequency of the treatment birds approaching the novel object during the Novel Object (NO) test compared to control birds across all ages, which is in accordance with our predictions. The NOT is a test commonly used to measure fear as the behavioural responses of approach and avoidance when animals are exposed to novelty (Forkman et al., 2007; Tahamtani et al., 2023). It is useful in on-farm settings as birds in large flocks can easily be tested in their home environment (Hüttner et al., 2023) and it works well also for young birds during rearing. Early environment is known to influence the development of fearfulness in birds and the associated activation of the HPA axis (Jones, 1996). Studies have found that enriching the rearing environment with litter access can reduce fearfulness in laying hens (Campbell et al., 2019). In the current study, more birds in both treatments approached the novel object at 7 and 14 weeks of age compared to week 2 of age, which is in accordance with previous studies showing reduced avoidance of novel object with increased age during rearing (Hocking et al., 2001). However, there was a tendency that fewer birds approached the novel object in week 14 compared to week 7, which could indicate an increased fear level from week 7–14. The NOs increased in size with the birds' age to avoid confounding the birds' body size and number of birds that could fit within a bird length of the NO. However, it was not calculated to match the birds' proportions precisely and this may have some influence on the results.

In contrast, there was no effect of treatment on the number of birds approaching the observer during the stationary person test. More birds approached the observer at 14 weeks of age compared to at 7 weeks, which is opposite to the NOT results, and might be due to the birds being habituated to humans, whereas the NOs were novel at each age. One could argue that this result is due to the increased stocking density at week 14 compared to week 7, as average bird weight will increase from around 500 g to 1000 g. However, previous studies on broilers have not found a relationship between stocking density and the outcome of fear tests in broilers (Rasmussen et al., 2024). The birds had ample space to avoid the observer if they wanted, thus, this result might reflect a reduced fearfulness towards humans as they get older.

In the present study, health and plumage damage were assessed using two different assessment methods; the aviary transect walk and individual assessment of birds. The transect walk method is commonly used to assess welfare in large flocks of animals, such as broilers (Marchewka et al., 2013), turkeys (Marchewka et al., 2019) and laying hens (Vasdal et al., 2023). For pullets the method consists of 11 predefined welfare indicators scored on a binary scale. The method is efficient as it takes around 20 min to assess the entire flock, it has good agreement with methods involving graded sampling of individual animals, and is more sensitive for less common welfare issues, such as wounds (Vasdal et al., 2022). Graded scores of individuals, on the other hand, provide a more detailed assessment of a subset of birds, including minor welfare issues such as slight wear of feathers. Indeed, in the present study, no birds were scored with feather loss using the transect method, while a few birds were scored with slight wear to the tail using the individual scoring. Thus, in situations where few welfare issues are expected, more detailed scoring methods should be applied. Feather pecking starts with minor damage to the plumage but can escalate as the birds age (De Haas et al., 2014; Schwarzer et al., 2022a) and early and sensitive scoring of plumage in rearing is therefore crucial.

This study was performed in four commercial rearing farms, and the experience of the farmers regarding the litter tray and litter provision was positive. The farmers reported that placement of the cardboard litter trays before the chicks' arrival took around 1 h, while adding fresh litter in each tray took around 10 min and was done during visual inspection of the birds. After the birds were let out to the aviary at day 21, the litter trays remained in the aviary system and were destroyed by the birds using them as pecking objects before the second visit at week 7, thus no extra cleaning or waste management was needed. Several of the rearers have continued providing litter in trays from day 1 after the study ended. However, potential effects of suboptimal factors during rearing may appear later in the hens' life. Therefore, long term studies are needed to follow the hens to the end of production to fully investigate the potential benefits of early litter access on plumage, welfare and production parameters.

There were some limitations in this study, firstly the density of 3 litter trays per 1000 birds may not have been enough to provide all birds access to litter for dustbathing and exploration. Thus, providing a higher density of trays may give a larger treatment effect. The density of trays were determined together with the rearers, with time and practical constrains as important limiting factors, but it would be interesting to investigate the effects of a higher density of litter trays. Secondly, we did not include behaviour observations in the litter trays. Observations including type of behaviour performed in the litter trays and on the chick paper, number of birds accessing the trays and potential competition will be valuable information to include in future studies. Finaly, the lack of differences in plumage between treatments could indicate that chick paper is sufficient for mitigating pecking issues, as previous studies have found a positive effect of providing chick paper on plumage (Tahamtani et al., 2016). A third treatment, wire mesh floor with no chick paper, was discussed and rejected as the aim of the study was to optimise management based on previous studies.

In conclusion, providing fresh litter and chick paper during the first three weeks of life reduced fear responses towards a novel object at weeks 2, 7 and 14, but did not reduce fear of a stationary person at weeks 7 or 14. Litter provision did not influence plumage quality or other health parameters. Plumage quality was overall good, and no birds were observed with wounds or other welfare issues in either treatment. Assessing plumage and welfare using both the transect and individual scoring of birds showed that in generally healthy flocks with minimal welfare issues, detailed scoring of minor issues must be applied. The method of litter provision, consisting of cardboard trays and daily allotment of litter, was positively received by the farmers, and should be encouraged in commercial rearing flocks.

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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CRediT authorship contribution statement

Guro Vasdal: project leader, conceptualizing, data sampling, writing. **Kathe Kittelsen**: conceptualizing, writing **Andrew Janczak**: conceptualizing, writing. **Randi O Moe**: conceptualizing, writing. **Bas Rodenburg**: conceptualizing, writing. **Sabine Gebhardt-Heinrich**: conceptualizing, writing. **Anja Riber**: conceptualizing, writing. **Fernanda Tahamtani**: conceptualizing, data sampling, statistical analyses, writing.

References

Bestman, M., Koene, P., Wagenaar, J.P., 2009. Influence of farm factors on the occurrence of feather pecking in organic reared hens and their predictability for feather pecking in the laying period. Appl. Anim. Behav. Sci. 121 (2), 120–125. https://doi.org/10.1016/J.APPLANIM.2009.09.007.

Blokhuis, H.J., Van Der Haar, J.W., 1989. Effects of floor type during rearing and of beak trimming on ground pecking and feather pecking in laying hens. Appl. Anim. Behav. Sci. 22 (3–4). https://doi.org/10.1016/0168-1591(89)90030-0.

Blokhuis, H.J., Van Fiks Niekerk, T., Bessei, W., Elson, A., Guémené, D., Kjaer, J.B., Maria Levrino, G.A., Nicol, C.J., Tauson, R., Weeks, C.A., Van De Weerd, H.A., 2007. The LayWel project: Welfare implications of changes in production systems for laying hens. Worlds Poult. Sci. J. 63. https://doi.org/10.1079/WPS2006132.

Brantsæter, M., Tahamtani, F.M., Nordgreen, J., Sandberg, E., Hansen, T.B., Rodenburg, T.B., Moe, R.O., Janczak, A.M., 2017. Access to litter during rearing and environmental enrichment during production reduce fearfulness in adult laying hens. Appl. Anim. Behav. Sci. 189, 49–56. https://doi.org/10.1016/J.APPLANIM.2017.01.008.

Bright, A., Jones, T.A., Dawkins, M.S., 2006. A non-intrusive method of assessing plumage condition in commercial flocks of laying hens. Animal Welfare 15. https://doi.org/10.1017/s0962728600030190.

Campbell, D.L.M., De Haas, E.N., Lee, C., 2019. A review of environmental enrichment for laying hens during rearing in relation to their behavioral and physiological development. Poult. Sci. 98, 9–28. https://doi.org/10.3382/ps/pey319.

De Haas, E.N., Bolhuis, J.E., de Jong, I.C., Kemp, B., Janczak, A.M., Rodenburg, T.B., 2014. Predicting feather damage in laying hens during the laying period. Is it the past or is it the present? Appl. Anim. Behav. Sci. 160 (1), 75–85. https://doi.org/10.1016/J.APPLANIM.2014.08.009.

de Jong, I.C., Gunnink, H., Rommers, J.M., Bracke, M.B.M., 2013. Effect of substrate during early rearing on floor- and feather pecking behaviour in young and adult laying hens. Eur. Poult. Sci. 77, 15–22. https://doi.org/10.1016/S0003-9098(25)01484-5.

Forkman, B., Boissy, A., Meunier-Salaün, M.C., Canali, E., Jones, R.B., 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol. Behav. 92. https://doi.org/10.1016/j.physbeh.2007.03.016.

Gernand, E., Ahlers, C., Huchler, M., Donat, K., 2022. Plumage damage and back skin lesions in laying hens with untrimmed beak depend on rearing of pullets and genetics. Br. Poult. Sci. 63 (3). https://doi.org/10.1080/00071668.2021.1975259.

Gilani, A.M., Knowles, T.G., Nicol, C.J., 2013. The effect of rearing environment on feather pecking in young and adult laying hens. Appl. Anim. Behav. Sci. 148 (1–2), 54–63. https://doi.org/10.1016/J.APPLANIM.2013.07.014.

Guinebretière, M., Huneau-Salaün, A., Huonnic, D., Michel, V., 2013. Plumage condition, body weight, mortality, and zootechnical performances: The effects of linings and litter provision in furnished cages for laying hens. Poult. Sci. 92. https://doi.org/10.3382/ps.2012-02576.

Hocking, P.M., Channing, C.E., Waddington, D., Jones, R.B., 2001. Age-related changes in fear, sociality and pecking behaviours in two strains of laying hen. Br. Poult. Sci. 42. https://doi.org/10.1080/00071660120070686.

Holt, R.V., Vas, J., Vasdal, G., Newberry, R.C., 2023. A buffet of litters – Broiler chickens behave differently according to litter type and freshness. Appl. Anim. Behav. Sci. 260, 105856. https://doi.org/10.1016/J.APPLANIM.2023.105856.

Hüttner, J., Clauß, A., Klambeck, L., Andersson, R., Kemper, N., Spindler, B., 2023. Association with Different Housing and Welfare Parameters on Results of a Novel Object Test in Laying Hen Flocks on Farm. Animals 13 (13). https://doi.org/10.3390/ani13132207.

Janczak, A.M., Riber, A.B., 2015. Review of rearing-related factors affecting the welfare of laying hens. In: In Poultry Science, 94. Oxford University Press, pp. 1454–1469. https://doi.org/10.3382/ps/pev123.

Jones, R.B., 1996. Fear and adaptability in poultry: Insights, implications and imperatives. World'S. Poult. Sci. J. 52 (2). https://doi.org/10.1079/wps19960013.

Kittelsen, K.E., Tahamtani, F., Moe, R.O., Gretarsson, P., Vasdal, G., 2022. Flock Factors Correlated with Elevated Mortality in Non-Beak Trimmed Aviary-Housed Layers. Animals 12 (24). https://doi.org/10.3390/ani12243577.

Kjaer, J.B., Glawatz, H., Scholz, B., Rettenbacher, S., Tauson, R., 2011. Reducing stress during welfare inspection: Validation of a non-intrusive version of the laywel plumage scoring system for laying hens. Br. Poult. Sci. 52. https://doi.org/10.1080/00071668.2011.554799.

Main, D.C.J., Mullan, S., Atkinson, C., Bond, A., Cooper, M., Fraser, A., Browne, W.J., 2012. Welfare outcomes assessment in laying hen farm assurance schemes. Anim. Welf. 21. https://doi.org/10.7120/09627286.21.3.389.

Marchewka, J., Estevez, I., Vezzoli, G., Ferrante, V., Makagon, M.M., 2015. The transect method: A novel approach to on-farm welfare assessment of commercial turkeys. Poult. Sci. 94. https://doi.org/10.3382/ps/peu026.

Marchewka, J., Vasdal, G., Moe, R.O., 2019. Identifying welfare issues in turkey hen and tom flocks applying the transect walk method. Poult. Sci. 98. https://doi.org/10.3382/ps/pez211.

Marchewka, J., Watanabe, T.T.N., Ferrante, V., Estevez, I., 2013. Welfare assessment in broiler farms: Transect walks versus individual scoring. Poult. Sci. 92. https://doi.org/10.3382/ps.2013-03229.

Mels, C., Niebuhr, K., Futschik, A., Rault, J.L., Waiblinger, S., 2022. Predictors for plumage damage and bloody lesions indicative of feather pecking in pullets reared in aviaries. Appl. Anim. Behav. Sci. 250. https://doi.org/10.1016/j.applanim.2022.105607.

Newberry, R.C., Keeling, L.J., Estevez, I., Bilčík, B., 2007. Behaviour when young as a predictor of severe feather pecking in adult laying hens: The redirected foraging hypothesis revisited. Appl. Anim. Behav. Sci. 107 (3–4), 262–274. https://doi.org/10.1016/J.APPLANIM.2006.10.010.

Pullin, A.N., Rufener, C.B., Millman, S.T., Tarlton, J.F., Toscano, M.J., Blatchford, R.A., Makagon, M.M., 2024. Providing elevated structures in the pullet rearing environment affects behavior during initial acclimation to a layer aviary. Poult. Sci. 103 (3), 103357. https://doi.org/10.1016/j.psj.2023.103357.

Rasmussen, S.N., Yoder, H., Erasmus, M., Riber, A.B., 2024. Influence of space availability on measures of fear in conventional broiler chickens. Appl. Anim. Behav. Sci. 273. https://doi.org/10.1016/j.applanim.2024.106216.

Regmi, P., Deland, T.S., Steibel, J.P., Robison, C.I., Haut, R.C., Orth, M.W., Karcher, D. M., 2015. Effect of rearing environment on bone growth of pullets. Poult. Sci. 94 (3), 502–511. https://doi.org/10.3382/PS/PEU041.

Rodenburg, T.B., Van Krimpen, M.M., De Jong, I.C., De Haas, E.N., Kops, M.S., Riedstra, B.J., Nordquist, R.E., Wagenaar, J.P., Bestman, M., Nicol, C.J., 2013. The prevention and control of feather pecking in laying hens: Identifying the underlying principles. World'S. Poult. Sci. J. 69 (2). https://doi.org/10.1017/S0043933913000354.

Savory, C.J., Mann, J.S., 1997. Behavioural development in groups of pen-housed pullets in relation to genetic strain, age and food form. Br. Poult. Sci. 38 (1). https://doi.org/10.1080/00071669708417938.

Schreiter, R., Krätzschmar, A., Freick, M., 2022. Effect of pullet quality on the occurrence of plumage damage, skin injuries and mortality during the laying period in commercial laying hen farms. Eur. Poult. Sci. 86, 1–13. https://doi.org/10.1399/EPS.2022.361.

Schwarzer, A., Erhard, M., Schmidt, P., Zismann, M., Louton, H., 2022a. Effects of Stocking Rate and Environmental Enrichment on the Ontogeny of Pecking Behavior of Laying Hen Pullets Confined in Aviary Compartments during the First 4 Weeks of Life. Animals 12 (19). https://doi.org/10.3390/ani12192639.

Schwarzer, A., Rauch, E., Erhard, M., Reese, S., Schmidt, P., Bergmann, S., Plattner, C., Kaesberg, A., Louton, H., 2022b. Individual plumage and integument scoring of laying hens on commercial farms: correlation with severe feather pecking and prognosis by visual scoring on flock level. Poult. Sci. 101 (10), 102093. https://doi.org/10.1016/J.PSJ.2022.102093.

Skånberg, L., Newberry, R.C., Estevez, I., Keeling, L.J., 2023. Environmental change or choice during early rearing improves behavioural adaptability in laying hen chicks. Sci. Rep. 13 (1). https://doi.org/10.1038/s41598-023-33212-0.

Tahamtani, F.M., Brantsæter, M., Nordgreen, J., Sandberg, E., Hansen, T.B., Nødtvedt, A., Rodenburg, T.B., Moe, R.O., Janczak, A.M., 2016. Effects of litter provision during early rearing and environmental enrichment during the production phase on feather pecking and feather damage in laying hens. Poult. Sci. 95 (12), 2747–2756. https://doi.org/10.3382/PS/PEW265.

Tahamtani, F.M., Kittelsen, K., Vasdal, G., 2023. Fearfulness in aviary housed laying hens – Effects of management and potential confounding effects of the Novel Object test. Appl. Anim. Behav. Sci. 260, 105794. https://doi.org/10.1016/J.APPLANIM.2022.105794.

Tahamtani, F.M., Nordgreen, J., Nordquist, R.E., Janczak, A.M., 2015. Early life in a barren environment adversely affects spatial cognition in laying hens (Gallus gallus domesticus). Front. Vet. Sci. 2. https://doi.org/10.3389/fvets.2015.00003.

Vasdal, G., Marchewka, J., Newberry, R.C., Estevez, I., Kittelsen, K., 2022. Developing a novel welfare assessment tool for loose-housed laying hens – the Aviary Transect method. Poult. Sci. 101. https://doi.org/10.1016/j.psj.2021.101533.

Vasdal, G., Newberry, R.C., Estevez, I., Kittelsen, K., Marchewka, J., 2023. The Aviary Transect - A practical welfare assessment tool to improve the management of cage-free laying hens. Poult. Sci. https://doi.org/10.1016/j.psj.2023.102659.

Vestergaard, K.S., Kruijt, J.P., Hogan, J.A., 1993. Feather pecking and chronic fear in groups of red junglefowl: their relations to dustbathing, rearing environment and social status. Anim. Behav. 45 (6), 1127–1140. https://doi.org/10.1006/ANBE.1993.1137.

Von Waldburg-Zeil, C.G., Van Staaveren, N., Harlander-Matauschek, A., 2019. Do laying hens eat and forage in excreta from other hens? Animal 13 (2), 367–373. https://doi.org/10.1017/S1751731118001143.

Walstra, I., Ten Napel, J., Kemp, B., Schipper, H., Van Den Brand, H., 2010. Early life experiences affect the adaptive capacity of rearing hens during infectious challenges. Animal 4 (10), 1688–1696. https://doi.org/10.1017/S175173111000073X.