Cage hygiene, laying location, and egg quality: The effects of linings and litter provision in furnished cages for laying hens

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ABSTRACT This study investigates the influence of litter provision and linings used for nests and pecking and scratching areas on cage hygiene, laying location, and egg quality. Research was carried out in furnished cages, each housing 60 beak-trimmed ISA Brown hens. Four different treatments were compared in a factorial arrangement, including 2 different nest linings (artificial turf vs. plastic mesh), either used alone or combined with the use of litter (wheat bran) spread over the rubber mat in the pecking and scratching area (PSA). An additional treatment, using artificial turf mat in the PSA and nests (as commonly used in commercial flocks), was used to compare the effect of PSA lining in the other treatments. We observed laying location, the number of dirty and broken eggs, the microbiological contamination of eggshells according to laying location, and general cage hygiene. The use of nests for laying decreased when they were lined with plastic mesh. Eggs laid outside the nest were of lower quality than those laid inside it, and this was particularly true for eggs laid in the PSA. Although hygiene was low on artificial turf mats, eggs laid on PSA covered with a rubber mat were dirtier and had a higher count of mesophilic bacteria on the eggshell than those laid on PSA covered with an artificial turf mat. Rubber mats in PSA were rapidly destroyed and proved to be unsuitable. The provision of litter had no effect on cage hygiene but substantially increased wear on mats. This study shows nest lining and litter provision methods to be key factors that need to be taken into account to encourage the use of nest boxes for laying, and hence, to ensure good egg quality. Further research into new linings for PSA is needed for the future improvement of egg-laying conditions.

Key words: laying hen, furnished cage, litter, egg, hygiene

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INTRODUCTION

The welfare of laying hens housed in conventional cages has been placed under intense scrutiny since the 1980s. This type of cage has been widely criticized due to the restricted available space and bare environment, which are incompatible with a hen's need to express certain behavior patterns (Blokhuis et al., 2007). To improve the welfare of laying hens, the European Union Council directive 1999/74/EC will ban the use of conventional cages from the January 1, 2012, onwards. Egg production will therefore only be allowed in noncage systems, or in cages furnished with a nest, perches, and litter.

Although initial models of furnished cages were not much larger than conventional cages, recent versions house between 8 and 80 hens. Furnished cages thus provide varying amounts of space for locomotion and comfort behaviors and allow a certain amount of foraging, dust bathing, nesting, and perching behavior (Lay et al., 2011). Substantial research into the improvement of cage furnishing aims to provide the best possible welfare conditions for laying hens, and hence attain production performance and egg quality comparable to those obtained in conventional cages. Examples include studies of cage height and group size (Albentosa et al., 2007), facility location (Shimmura et al., 2009), nest design (Wall et al., 2002), and perch arrangements (Wall and Tauson, 2007).

Hens have a strong need to forage, despite ad libitum provision of feed in the feed trough (Bubier, 1996). Results of a previous experiment, where small amounts of feed were regularly distributed as litter on an artificial turf mat, confirmed that floor lining is necessary for pecking and scratching in furnished cages and that the provision of litter further favored these behaviors (Guinebretière et al., 2011). Nevertheless, the rapid scattering of feed provided as litter material through foraging and dust-bathing activities would make fre-

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quent redistribution necessary, potentially increasing variable costs.

Another major problem we encountered in our previous experiment was the deterioration of artificial turf mats in the pecking and scratching area (**PSA**). At the end of the laying period, mats were heavily worn in places where litter had been distributed. This could be due to the high appeal of feed when used as litter. Wheat bran used as litter material may be less attractive to hens and can be used in an automatic distribution system. It is of greater economic interest than complete feed, as well as being a raw material that makes it possible to feed animals without the risk of toxic residues. Wheat bran also provides more fiber within the diet, which could improve the welfare of laying hens, as shown by Hetland et al. (2003).

Most hens prefer to lay eggs in the enclosed nest area of a furnished cage rather than in the main cage area (Abrahamsson and Tauson, 1997; Appleby et al., 2002; Cooper and Appleby, 2003). As specified in the EU directive, "nest means a separate space for egg laying, the floor components of which may not include wire mesh" (European Commission, 1999). The choice and use of nests by laying hens are both very complex and can be influenced by many factors, such as surface, color, and lining. To date, cage manufacturers have mainly proposed artificial turf mat for nest lining. However, results in one of our previous experiments (Huneau-Salaün et al., 2011a) mirrored those reported in another study involving this lining (Merrill et al., 2006), showing that even perforated artificial turf mats were soiled by feces. Cleaning programs carried out before population renewal seem to be difficult in poultry houses equipped with furnished cages due to the time-consuming removal of linings, such as artificial turf mats, from the cages for individual cleaning.

It is therefore evident that research into new lining materials is crucial to attain a high level of environmental hygiene in furnished cages. Some manufacturers propose plastic mesh as an alternative to artificial turf mat in the nest, a choice that could indeed prove to be a more hygienic lining.

Hygiene in furnished cages has become a major concern. The use of linings in nest and pecking areas leads to problems of cleaning techniques and dirty eggs. In the PSA, the lining must be able to retain litter for foraging and dust-bathing behaviors. Here, the relatively simple cleaning of rubber matting could make it an alternative to artificial turf mat. The use of feed as litter is a further source of concern; very few breeders use it, mainly due to the costs incurred. Yet, European directives impose the provision of litter in furnished cages from 2012 forward. An experiment was therefore set up to assess the influence of new linings and litter. Plastic mesh was used to line the nests whereas the PSA was equipped with rubber mats. Wheat bran was used as a new litter material. Furnished cages with different linings containing 60 hens were evaluated for hygiene levels to evaluate the effect on egg dirtiness and bacterial contamination of eggshells. Moreover, any preference shown by hens for these new linings could have an effect on laying location in the cage. This study was a part of a global project investigating several physical, behavioral, and physiological parameters relevant to the production and welfare of laying hens in furnished cages.

MATERIALS AND METHODS

Birds and Husbandry

Beak-trimmed ISA Brown chicks (*Gallus gallus domesticus*) were floor-reared on wood shavings following standard management practices. At 18 wk of age, pullets (n = 4,320) were transferred into furnished cages within an experimental building and kept until they were 76 wk old. Food (2,700 kcal of ME/kg, 17.0% CP, 3.7% Ca, and 0.3% available P) was automatically distributed 3 times per day at 0700 h, 1500 h, and 1900 h (160 g/hen per day). Therefore, hens always had access to the feed for ad libitum consumption and water was available ad libitum too. Egg collection was carried out on a daily basis. Light was provided from 0600 h to 2200 h. Average light intensity values in front of the nest boxes of the cages and in front of the PSA were 2.0 and 9.6 lx, respectively.

Housing and Experimental Scheme

Four different treatments (T1–T4) were compared in a factorial arrangement, including 2 nest linings, with or without provision of litter within the PSA. An additional treatment (T0) was created with a different PSA lining to all the other treatments tested (Table 1). This experiment involved a total of 72 identical cages (MEC, Zucami Poultry Equipment, Berlain-Navarra, Spain). The cages were 3,660 mm wide, 1,260 mm deep, and 455 mm high at the rear of the cage. Each cage housed 60 hens, providing a total area of 768 cm^2 per bird, including 67 cm^2 for the nest and 67 cm^2 for the PSA (Figure 1). Nests and PSA were accessible around the clock to the hens. The cage floor was 14% slopped, enabling eggs to roll to the automatic egg belt. Each hen had access to 12 cm of food trough and 15 cm of circular plastic and metallic perches. There were 6 claw-shortening systems (abrasive strips) in each cage. A nest was placed on one side of the cage and secluded by a yellow curtain made of plastic strips. Nests in T0, T1, and T3 treatment cages were lined with a perforated artificial turf mat, whereas those in T2 and T4 treatment cages were lined with plastic mesh (Figure 2). The PSA was located opposite the nest and consisted of a rubber mat for all 4 treatments. Wheat bran litter was automatically distributed on the PSA mats for T1 and T2 cages on an hourly basis from 1100 h to 1800 h (100 g/d per cage). Sixteen cages per treatment (T1–T4) were randomly distributed within three 3-tier battery cages. Eight cages were furnished with perforated artificial turf mats in the PSA and used as

Table 1. Experimental treatments

Item	T0	T1	T2	T3	T4
PSA[1] lining	Artificial turf	Rubber	Rubber	Rubber	Rubber
Nest lining	Artificial turf	Artificial turf	Plastic mesh	Artificial turf	Plastic mesh
Litter provision	No	Yes	Yes	No	No
Number of cages	8	16	16	16	16

[1]PSA = pecking and scratching area.

T0 treatment, i.e., to assess the influence of the rubber mat in the PSA area. These cages also had perforated artificial turf mat in the nests and no litter distribution was carried out, as tested in a previous experiment (Guinebretière et al., 2011).

Laying Location and External Condition of Eggs

When the hens were 21, 23, 28, 36, 47, 65, and 71 wk of age, data was recorded for eggs laid over 2 consecutive days in each of 7 cages in T0, 9 cages in T2 and T4, and 10 cages in T1 and T3 (different samples sizes for practical reasons). Cages were selected randomly when hens reached 21 wk of age, and they were used throughout all following measures. The eggs from each cage were visually examined to record the number of broken and dirty eggs. These data were classified according to where the eggs were laid; that is, in the nest, in the PSA, or elsewhere in the cage. The percentages of broken and dirty eggs were calculated per cage and per laying location.

Eggshell Contamination

Between wk 66 and 69 of the hens' lifespan, 60 eggs in the nest and 60 eggs in the PSA were randomly sampled per treatment (eliminating dirty and broken eggs). The eggs were kept for a maximum of 48 h in ambient conditions before being analyzed by the ISAE laboratory (Institut en Santé Agro-Environnement, Rennes, France) according to the method developed by Protais et al. (2003). The eggs were pooled in batches of 3 and analyzed to obtain the mesophilic aerobic bacterial count on the eggshell. The bacterial count was log-transformed before statistical analysis and expressed as $\log_{10}$ cfu/eggshell for statistical analysis. The recovery of 0 cfu corresponded to <3.8 $\log_{10}$ cfu/eggshell. As this value could not be used directly for statistical analysis, the value of 3.8 $\log_{10}$ cfu/eggshell was used, as previously suggested by Knape et al. (2002).

Cage Hygiene

Once animals had been removed for slaughter, hygiene was evaluated at several locations within each cage, namely wire and mats in the PSA and nests, as well as wire below perches and in the rest of the cage. Each location was evaluated on a scale of 0 to 3 points, 3 being the cleanest condition. The degree of wear in PSA linings was assessed (scored as torn or intact) for all the T0 cages (8 cages with artificial turf mats) and for 59 cages with rubber mats, of which 32 (T1, T2) had wheat bran litter and 27 (T3, T4) did not.

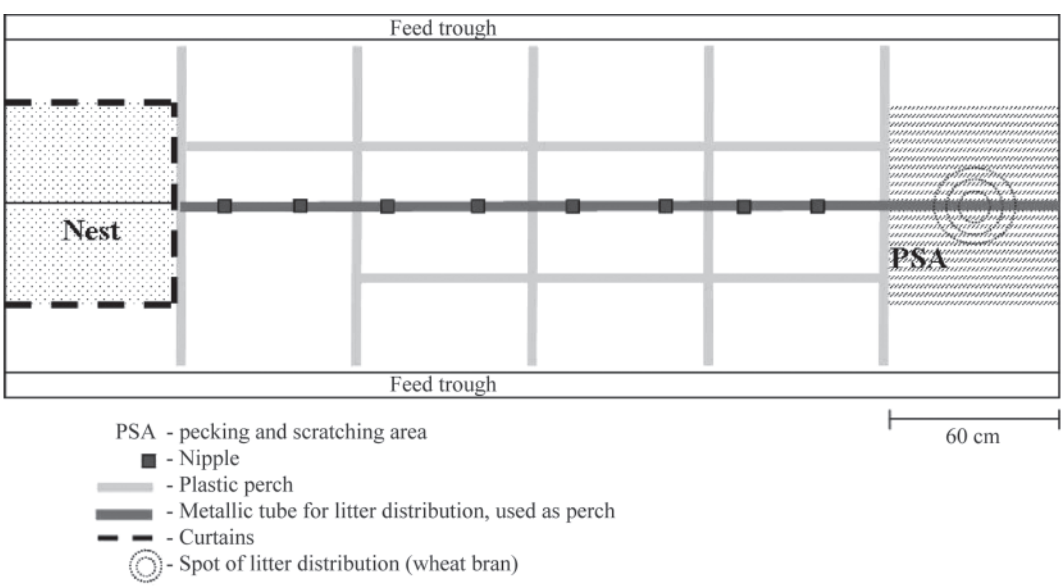

Figure 1. Furnished cage for 60 laying hens.

a

b

c

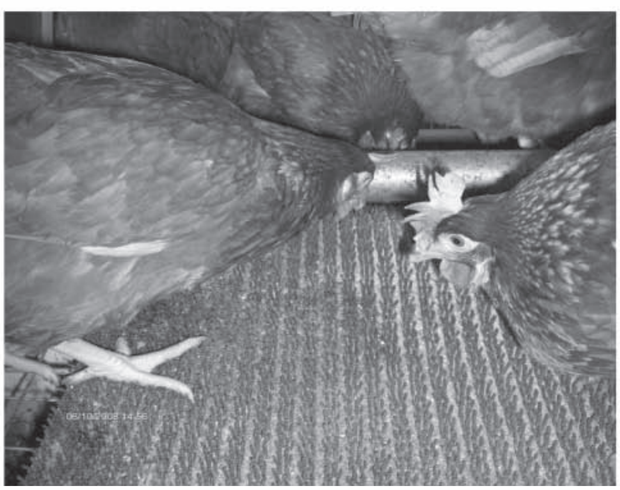

Figure 2. Linings: plastic mesh (a), rubber mat (b), and artificial turf mat (c).

Statistical Analysis

Individual cages were used as the experimental unit. Two separate analyses were carried out. The effects of nest lining and of litter provision were studied in treatments T1 to T4, and these factors were integrated as fixed effects within an ANOVA model. Analysis of variance modeling was used to test the effect of PSA lining by comparing T3 and T0 treatments.

Repeated measures ANOVA was performed on egg quality and laying location data (proc MIXED in SAS 9.1 (SAS Institute Inc., 1999) Interactions of time × fixed effects were systematically tested but were not significant and were not shown. All the laying location and egg quality data were expressed as percentages and transformed to arcsine square root for the statistical analyses, but the back-transformed means are presented in Tables 2, 3, and 4. Eggshell contamination data and cage hygiene data did not comply with parametric hypotheses for the ANOVA analysis and were therefore compared using nonparametric Kruskal-Wallis tests on the ranks. Chi square was employed to test the effect of PSA linings and of litter distribution on the degree of wear of PSA linings.

Table 2. Overall frequencies of dirty and broken eggs according to laying location in the cage (results on 7 controls between 21 and 71 wk of age)

Item	Nest	PSA[1]	Rest of the cage
Dirty eggs	1.1[c]	32.4[a]	6.4[b]
Broken eggs	0.4[c]	1.2[b]	5.7[a]

[a–c]For a given trait, frequencies with different letters differ at $P < 0.01$ (*t*-test for proportions).

[1]PSA = pecking and scratching area.

RESULTS

Global Results

A large majority of eggs (87.3%) were laid inside the nest over the entire laying period (increasing from 84.4% at 21 wk of age to 91.1% at 65 wk of age), whereas 7.8% of eggs were laid in the PSA and only 4.9% of eggs were laid elsewhere in the cage. In total, 3.8% of dirty eggs were observed with high variability between treatments. However, the percentage of broken eggs remained low in all treatments (0.8%). Dirtiness and breakage were more frequently observed in eggs laid in the PSA and elsewhere in the cage than in those laid inside the nest, whatever the linings in the PSA (Table 2). Eggs laid in the PSA also showed higher mean eggshell contamination than those laid inside the

Table 3. Cage hygiene scores in the nest and in the PSA after the removal of animals at 76 wk of age (mean ± SD; n = 72 cages)[1]

Item	Lining	Wire	*P*-value (Wilcoxon)
Nest	1.6 ± 0.8	2.8 ± 0.3	0.01
PSA[2]	1.8 ± 0.3	2.1 ± 0.2	0.01

[1]For each location, scores on a scale of 0 to 3, where 3 indicates the cleanest condition.

[2]PSA = pecking and scratching area.

Table 4. Laying location and percentage of dirty and broken eggs according to pecking and scratching area (PSA) lining (results on 7 controls between 21 and 71 wk of age)[1]

	Artificial turf mat (T0)		Rubber mat (T3)		Statistical significance	
Item	LSM[2]	SE	LSM	SE	F-value[3]	P-value
Eggs in the nest (%)	90.2	0.04	90.5	0.04	0.13	0.71
Eggs in PSA (%)	5.6	0.04	5.7	0.04	0.01	0.93
Eggs elsewhere (%)	3.8	0.02	3.1	0.01	2.30	0.13
Dirty eggs (%)	0.8	0.02	2.9	0.02	3.86	0.01
Broken eggs (%)	0.5	0.01	0.2	0.01	29.7	0.06

[1]Values shown are back transformation of means to the original scale.

[2]LSM = least squares means.

[3]Number of degrees of freedom for PSA lining df = 1, for the residual df = 61.

nests (PSA: 5.0 ± 0.4 $\log_{10}$ cfu/eggshell vs. nest: 4.8 ± 0.4 $\log_{10}$ cfu/eggshell; $P = 0.01$). All cage linings (i.e., for nest and PSA) were more heavily soiled than wired areas due to droppings stuck in the mats (Table 3). In the middle part of the cage, wire under perches had inferior hygiene (1.5 ± 0.4) than the rest of the wired area (2.4 ± 0.4; $P < 0.01$). Indeed, droppings accumulated on the wire floor under the perches because hens were perched during the night and droppings under the perches were not trampled through the floor by bird walking.

PSA Lining

The effect of PSA lining was assessed through the comparison of T0 with T3 treatment. The PSA lining had no effect on the total frequency of broken eggs in the cage nor egg-laying location (Table 4). At the end of the laying period, artificial turf mats in the PSA were dirtier than rubber mats (Table 5; $P = 0.03$). However, a large decrease was observed in the frequency of dirty eggs found on artificial turf mat-lined PSA: 4.9% of eggs were dirty in comparison with 38.3% of the eggs laid in PSA covered with a rubber mat (Figure 3; $P = 0.01$).

The increased number of dirty eggs in rubber-lined PSA had an effect on the overall production: The total percentage of dirty eggs in T0 cages was lower (artificial turf mat) compared with T3 cages (rubber mats; Table 4; $P < 0.01$).

Eggshell contamination of eggs laid in the PSA was also lower with an artificial turf mat (Table 5; $P = 0.01$). At the end of the laying period, 46% of rubber mats were torn, either in the middle or under the litter distribution screw, whereas all 8 artificial turf mats were intact.

Nest Lining

In the nest, artificial turf mats were dirtier than plastic mesh (Table 6; $P = 0.01$). However, this did not have any particular effect on egg cleanliness or eggshell contamination: Similar frequencies of dirty eggs and contamination were observed in eggs laid in nests with both types of lining (Tables 6 and 7).

Artificial turf mat made the nest attractive to hens for laying, whereas plastic mesh lining was associated with higher laying rates in the PSA and elsewhere in the cage (Table 7; $P = 0.01$). Consequently, the overall frequency of dirty eggs per cage was higher in cages

Table 5. Cage hygiene and bacterial eggshell contamination according to pecking and scratching area (PSA) lining[1]

Item	Artificial turf mat (T0)	Rubber mat (T1–T4)	P-value[2]
Hygiene score			
Nest mat	0.9 ± 0.4	1.0 ± 0.4	0.38
Nest wire	2.7 ± 0.2	2.8 ± 0.3	0.60
PSA mat	1.5 ± 0.5	1.8 ± 0.2	0.03
PSA wire floor	2.3 ± 0.3	2.0 ± 0.2	0.09
Rest of the cage: wire	2.1 ± 0.3	2.4 ± 0.42	0.30
Wire floor under the perches	1.7 ± 0.3	1.4 ± 0.3	0.13
Wear of the mat in the PSA			
Intact	8	7	
Torn	0	6	
Eggshell bacterial load (log cfu/eggshell)			
Eggs laid in the nest	4.8 ± 0.3	4.8 ± 0.5	0.39
Eggs laid in the PSA	4.8 ± 0.3	5.0 ± 0.4	0.01

[1]For each location, hygiene scores on a scale of 0 to 3, where 3 indicates the cleanest condition.

[2]P-value = probabilities of Wilcoxon test for hygiene score and eggshell bacterial load and probabilities of χ^2-test for mat in the PSA (df = 2).

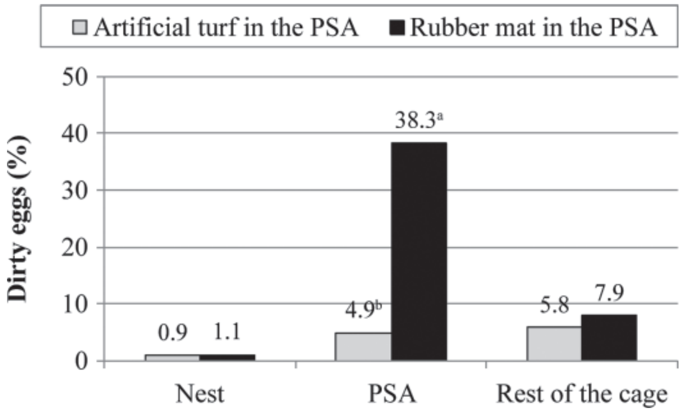

Figure 3. Overall frequencies of dirty eggs according to laying location and lining in the pecking and scratching area (PSA; results over 7 controls between 21 and 71 wk of age). For a given trait, frequencies with different letters differ at $P < 0.01$ (t-test for proportions).

with plastic mesh-lined nests due to the higher number of eggs laid in the PSA, which were considerably dirtier than those laid in the nest. Finally, nest lining did not affect the low percentage of broken eggs.

Litter Provision

The quantity of wheat bran delivered per day was 100 g/cage per day, representing less than 1% of feed intake per hen; thus, feed intake at the trough was not affected by litter provision (data not shown). However, the distribution of wheat bran as litter in the PSA increased the number of eggs laid there and inversely resulted in a decreased proportion of eggs laying in nests (Table 7; $P = 0.01$). The lower use of nests with distribution of litter was associated with an increased frequency of dirty eggs per cage because eggs laid in the PSA were far more soiled than those laid in the nest. Nevertheless, eggshell contamination of eggs laid in the PSA was comparable, with and without litter provision (Table 6). Again, litter provision did not affect the low percentage of broken eggs (Table 7).

Finally, cage hygiene was not affected by litter provision (Table 6). However, 97% of PSA rubber mats in cages with wheat bran distribution were torn at the distribution spot, versus 49% without litter distribution (Table 6; $P < 0.01$).

DISCUSSION

This study identifies nest lining as a key factor in increasing the attractiveness of nests for laying, and thereby, ensuring high egg quality. Hens showed little interest in laying in nests lined with plastic mesh, and this had a strong effect on egg quality. The use of artificial turf instead of plastic mesh as the nest lining increases nest use and consequently reduces the number of dirty eggs. Second, our study demonstrated that the distribution of wheat bran as litter for furnished cages attracted hens to the PSA for laying. Moreover, lining the PSA with a rubber mat turned out to be unsuitable from a zootechnical point of view because eggs laid on this mat were heavily soiled by droppings, resulting in lower eggshell microbiological quality.

The results showed that a high percentage of eggs were laid inside the nests (more than 84%). Nests were secluded, with low luminosity, and linings were placed on the metallic wire (artificial turf mat or plastic mesh). The combination of these factors probably increased their attractiveness, as already shown by other authors (Freire et al., 1997; Cooper and Appleby, 2003; Weeks and Nicol, 2006).

Eggs laid outside the nests were predominantly laid in the PSA rather than in the rest of the cage. This confirmed observations made in a previous experiment led by the authors using similar cages, where 80.4% of eggs laid outside the nest were laid in the PSA (Guinebretière et al., 2011). The PSA were accessible around

Table 6. Cage hygiene and bacterial eggshell contamination according to nest lining and litter provision[1]

	Nest lining			Litter provision		
Item	Artificial turf mat (T1, T3)	Plastic mesh (T2, T4)	P-value[2]	No (T3, T4)	Yes (T1, T2)	P-value
Hygiene score						
Nest lining	0.9 ± 0.4	2.4 ± 0.4	0.01	1.7 ± 0.8	1.6 ± 0.9	0.79
Nest wire floor	2.8 ± 0.3	2.8 ± 0.3	0.51	2.8 ± 0.3	2.8 ± 0.3	0.62
PSA[3] mat	1.8 ± 0.2	1.8 ± 0.3	0.86	1.8 ± 0.3	1.9 ± 0.2	0.28
PSA wire	2.0 ± 0.2	2.0 ± 0.1	0.75	2.0 ± 0.1	2.0 ± 0.2	0.66
Rest of the cage: wire	2.4 ± 0.4	2.3 ± 0.4	0.74	2.4 ± 0.4	2.3 ± 0.3	0.63
Wire floor under the perches	1.6 ± 0.4	1.5 ± 0.4	0.54	1.5 ± 0.4	1.6 ± 0.4	0.44
Wear of the mat in the PSA						
Intact	10	5	0.11	14	1	0.01
Torn	19	25		13	31	
Eggshell bacterial load (log cfu/eggshell)						
Eggs laid in the nest	4.8 ± 0.3	4.8 ± 0.4	0.53	4.8 ± 0.4	4.8 ± 0.2	0.84
Eggs laid in the PSA	5.0 ± 0.4	5.0 ± 0.4	0.97	4.9 ± 0.4	5.0 ± 0.4	0.37

[1]For each location, hygiene scores on a scale of 0 to 3, where 3 indicates the cleanest condition.

[2]P-value = probabilities of Wilcoxon test for hygiene score and eggshell bacterial load and probabilities of χ^2-test for mat in the PSA (df = 2).

[3]PSA = pecking and scratching area.

Table 7. Laying location and percentage of dirty and broken eggs according to nest lining and litter provision (results on 7 controls between 21 and 71 wk of age)[1]

	Nest lining				Litter provision				Statistical significance			
	Artificial turf mat (T1, T3)		Plastic mesh (T2, T4)		No (T3, T4)		Yes (T1, T2)		Nest		Litter	
Item	LSM[2]	SE[2]	LSM	SE	LSM	SE	LSM	SE	F[3]	P	F	P
Eggs in the nest (%)	90.7	0.02	84.1	0.02	88.4	0.02	86.8	0.02	117.2	0.01	4.7	0.01
Eggs in PSA[4] (%)	5.3	0.02	9.7	0.02	6.6	0.03	8.1	0.02	61.5	0.01	5.0	0.01
Eggs elsewhere (%)	3.3	0.01	5.3	0.01	4.3	0.01	4.3	0.01	34.8	0.01	0.1	0.99
Dirty eggs (%)	2.7	0.02	4.7	0.02	3.3	0.02	4.0	0.02	47.2	0.01	1.3	0.05
Broken eggs (%)	0.3	0.01	0.4	0.01	0.3	0.01	0.4	0.01	0.4	0.25	0.1	0.27

[1]Values shown are back transformation of means to the original scale.

[2]LSM = least squares means.

[3]Number of degrees of freedom for Nest and Litter provision df = 1, for the residual df = 247.

[4]PSA = pecking and scratching area.

the clock to the hens and were each covered by lining, as were the nests. Moreover, the location of PSA at the other end of the cage probably led to less disturbance than in the rest of the cage, like in the nesting area. Even if luminosity was higher in the PSA, the quietness and the lining covering the wire may have made it more attractive to lay there than elsewhere in the cage.

The type of lining inside the nest influenced laying location: hens preferred artificial turf mat to plastic mesh for laying purposes. This more frequent use of nests for laying when they are lined with artificial turf mat has already been described in previous experiments, comparing with wire mesh or coated wire mesh (Hughes, 1992; Cox et al., 2005). Wire mesh or plastic mesh can present more drafts than artificial turf mat with smaller perforations. Moreover, softer texture of artificial turf mat can be preferred by hens, as it closer to the texture of loose material for nest building and laying. Indeed, Reed and Nicol (1992) suggested that substrates that are textured and peckable are attractive for the hens to lay. A strip of artificial turf in the nest may be found more peckable by the hens than a plastic wire, and so, more suitable for laying. Finally, color of artificial turf mat can be preferred to clear color of plastic mesh tested here.

Wheat bran provision in the PSA influenced laying location by attracting hens away from the nest and toward the PSA. The presence of wheat bran certainly made the PSA more attractive to the hens. It has already been shown that the presence of loose material is attractive for nest building and laying (Duncan and Kite, 1989). Nevertheless, a previous study conducted in similar cages showed that litter provision (feed) also provided opportunities for behavioral expressions (dust-bathing, foraging) without affecting zootechnical performances (Guinebretière et al., 2011).

In terms of percentages of broken and dirty eggs, the results of the T0 treatment were similar to those observed in a previous experiment with identical cage furnishings, i.e., artificial turf mats in nests and PSA and no litter distribution. In T0 cages, 1.4% of eggs were dirty and 0.93% broken (all confounded areas) compared with 1% and 0.8%, respectively, in the previous experiment (Huneau-Salaün et al., 2011b).

Eggs laid outside the nest for T0 were more frequently found to be broken and dirty, as widely reported by other authors (Appleby et al., 2002; Wall and Tauson, 2002; Guesdon and Faure, 2004). These incidences have also been observed in our previous experiment: 0.6 and 1.1% of eggs laid inside the nest were broken and dirty, respectively, versus 5.2 and 4.8% of eggs laid outside the nest were broken and dirty, respectively, ($P < 0.05$; Huneau-Salaün et al., 2011b). Although nests were always free access, behavioral observations showed that they were only occupied during the daily laying period (especially during 4 h after lights were turned on), hence decreasing the risk of hens breaking the eggs or soiling them with droppings. Perches outside the nest could prevent eggs from rolling out of the cage and increase the risk of blocked eggs being soiled by the hens. Finally, the presence of perches might impair the birds' ability to efficiently trample the manure down through the cage floor.

Although cage floor hygiene in furnished cages is generally inferior to that of conventional cages (Wall and Tauson, 2007), it does not appear to have any influence on the number of dirty eggs (LayWel, 2006; Wall and Tauson, 2007; Wall et al., 2008). However, it has been shown in some cases that the housing system may have a slight effect on the level of bacterial eggshell contamination (Wall and Tauson, 2002, 2007; Mallet et al., 2006, 2010; Wall et al., 2008), but this effect is not always observed (De Reu et al., 2005). The eggshell bacterial load could have an effect on shelf-life and food safety as penetration of the shell by bacteria present on its surface may lead to actual contamination of the egg (Harry, 1963; De Buck et al., 2004).

In general, bacterial eggshell contamination is higher in noncage systems than in furnished cages (De Reu et al., 2009). In this experiment, mean bacterial load on the shells of eggs laid inside the nest was similar to that reported in other experiments on furnished cages (Mallet et al., 2006; De Reu et al., 2009; Huneau-Salaün et al., 2011b). The bacterial load on the shells of eggs laid inside the nest was slightly lower than for eggs laid elsewhere, which could be explained by the lower occupation of the nest during the day (observed by the authors) and by the presence of a curtain that could provide some protection against dust and bacteria.

Because the incidence of broken eggs was so low, it did not matter if the mat in the PSA was rubber or turf or if the nest lining was turf or plastic mesh relative to broken eggs. In contrast, artificial turf in the nest and the PSA was more beneficial in reducing dirty eggs as compared with plastic mesh in the nest and rubber mats for the PSA.

Following the removal of hens from the cages, variable difficulty was encountered when cleaning the different linings. Artificial turf mats in PSA or in the nest were dirty: drops were trapped and accumulated within the blades of these mats (even for perforated versions). In commercial conditions, this obliges farmers to remove all mats from the cages and clean them between 2 batches of laying hens. On the other hand, blades on artificial turf mats prevented eggs from coming into direct contact with the droppings trapped within the blades, hence not affecting their state of cleanliness or increasing eggshell contamination.

Plastic mesh in the nest remained clean because droppings could fall through. These mats could also be cleaned easily after the animal was removed, but as mentioned previously they were used less for laying than artificial turf mat, leading to a higher percentage of dirty eggs in the cage.

Rubber mats in the PSA could also be cleaned easily after the animal was removed, but eggs rapidly became dirty when they rolled on fresh droppings. This led to a dramatic rise in the frequency of dirty eggs in PSA (+ 32%) and a slight increase in eggshell contamination (+ 0.2 $\log_{10}$ cfu/eggshell). Moreover, the rubber mats tested in this experiment were destroyed in certain places, whereas artificial turf mats remained intact. The rubber mats tested, therefore, seem to be inappropriate for use as PSA lining.

Litter provision did not influence cage hygiene. However, PSA rubber mats were much more worn when wheat bran was distributed, particularly at the distribution spot where hens pecked to the extent that they made holes in the mats. This has also been observed on artificial turf mats in the previous study with feed as litter, where 88% of artificial turf mats in cages with litter were torn versus only 6% in cages without litter (A. Huneau-Salaün, Anses; personal communication). Holes in mats led to the loss of litter and were thus rarely used for later flocks, meaning that the replacement of damaged mats would incur an increase in production costs.

In conclusion, egg quality was lower in eggs laid outside the nest than those laid inside it. Increasing the use of nests for egg laying in furnished cages and improving the quality of eggs laid outside the nest are therefore necessary if we aim to produce safe eggs with high microbiological quality. The number of downgraded eggs is directly attributable to the cage system design. The choice of lining is important for cage hygiene, influencing the dirtiness of eggs and eggshell contamination.

Under the experimental conditions employed in the current study, the combination of artificial turf mats inside the nest and in the PSA with no litter distribution was the most appropriate with respect to egg quality (fewer dirty eggs and lower eggshell contamination) because of better use of nest for laying.

Even if a large proportion of eggs were laid inside the nest when wheat bran was distributed, the small increase of percentage of eggs laid in the PSA had an effect on egg quality. Litter also had a strong effect on the degree of wear of mats on which the substrate is distributed. To allow hens to express their natural behavior, the provision of litter in furnished cages will be a European requirement from 2012 onwards. Consequently, further research is needed to find the best litter-lining combination that culminates in cage hygiene, egg cleanliness, zootechnical performances, and the ethological needs of laying hens.

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