

## The influence of losing or gaining access to peat on the dustbathing behaviour of laying hens

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### Abstract

This study investigated the influence of being reared with or without access to peat as well as the effects of losing or gaining substrate access on the dustbathing behaviour of young, domestic fowl (*Gallus gallus domesticus*). There were four treatments, based on the period of time chicks had access to peat during rearing: (i) always (LL), (ii) never (NN), (iii) from 0 to 6 weeks of age (LN) and (iv) from 6 weeks of age onwards (NL). Observations on the number and length of dustbaths performed were made for six days with birds aged six weeks and 50% of the birds either lost or gained access to litter. The birds then remained in the same treatment conditions until 16 weeks of age, at which point the same behavioural observations were repeated. NL birds (which had just gained access to peat) were found to be quicker than LN birds (which had just lost access to peat) to perform a dustbath during the first observation period. A significant difference was seen in the variation of the duration of the dustbathing bouts; both LL and NL birds varied less in the lengths of their bouts than NN and LN birds over both observation periods. Hence, early rearing environment had less effect on birds' dustbathing behaviour than current access or lack of access to litter. The irregular dustbathing pattern exhibited by birds that dustbathe without litter could be a sign of frustration; an indication that dustbathing without litter — unlike dustbathing in litter — does not provide the required feedback.

**Keywords:** animal welfare, domestic chicken, dustbathing, *Gallus gallus domesticus*, litter, rearing

### Introduction

In their natural habitat, birds are able to experience many different types of resources and can choose the one most appropriate for any particular activity. A suitable substrate for dustbathing consists of small, dry particles that pass between the feathers, thus helping in the removal of excess lipids from the plumage (Liere & Siard 1991). The majority of laying hens are housed indoors and have limited (if any) possibilities to choose between different substrates and birds housed in conventional cages have no access to litter whatsoever. Birds that are housed without litter will, nevertheless, be seen performing typical dustbathing movements (so-called sham dustbathing), even if this has no way of improving plumage condition. The welfare of birds that perform sham dustbathing has been called into question and studies have shown that birds accustomed to dustbathing in litter demonstrate signs of frustration (Zimmerman *et al* 2000) as well as an increased stress response (Vestergaard *et al* 1997) when deprived of litter. In fact, birds with even extremely limited previous exposure to litter have been found to be highly motivated in terms of gaining access to

peat to perform dustbathing (Wichman & Keeling 2008). This leads us to surmise that performing dustbathing in litter is extremely important to the welfare of birds.

Dustbathing is characterised by a complex sequence of behaviours. It consists of several specific movements that develop separately, becoming a functional unit (Kruijt 1964). Several studies have found that the type of litter or access to litter has an influence on the dustbathing behaviour of birds, as those which dustbathed on wire had more interruptions to their bouts (Larsen *et al* 2000), dustbathed less often (Nørgaard-Nielsen 1997) and had longer bouts (Vestergaard *et al* 1990). In addition, Larsen *et al* (2000) found that birds housed with access to sand carried out more side rubs than birds housed on wire and Vestergaard *et al* (1990) also found that birds housed in a rich environment, with access to sand, grass and perches, had a higher frequency of head and side rubs than birds housed on wire. For a review, see Olsson and Keeling (2005).

The development of dustbathing behaviour tends to occur during the first three weeks in chicks, by which time the structure of the dustbathing sequence is essentially the same as in adults (Larsen *et al* 2000).

Since behavioural development is generally considered to be shaped both genetically and environmentally, it might be expected for a chick to need feedback from the litter to acquire a 'normal' pattern of dustbathing. This requirement for appropriate feedback could be assumed to be at its greatest during the period of time that dustbathing behaviour is developing, ie early in life. Development of other behaviours, such as perching and pecking at food, has been shown to be negatively affected if they are not learnt during a sensitive phase. For example, chicks that do not have access to perches during the first eight weeks of life are less flexible in their perch use as adults (Gunnarsson *et al* 2000) and chicks which do not learn to direct their foraging (ground) pecks at earth or litter material when they first start to search for food can misdirect their pecks and develop feather pecking (Blokhuis & van der Haar 1989). In practice, it has been observed that it is common for birds housed in modified cages to perform their dustbathing on the wire floor instead of in the litter box provided (Lindberg & Nicol 1997; Olsson & Keeling 2002; Wall 2003). Most of these birds would have been reared in conventional cages and the lack of use of litter, even when it was provided in the modified cages, could be a consequence of being reared without litter. In support of this, it has also been found that birds which had been floor reared with litter had a slightly higher dustbathing activity (Blokhuis *et al* 2007) and used the litter more (Wall 2003) when housed in modified cages, than birds that had been reared in cages without litter. In accordance with this, an experimental study (Johnsen *et al* 1998) showed that rearing conditions can have long-term effects on birds' subsequent dustbathing behaviour. Chicks were reared on wire, straw and sand until five weeks of age and then kept on sand and straw. Observations of the birds' dustbathing behaviour at five–six and 40–41 weeks of age revealed that wire-reared birds dustbathed less at both ages. In contrast to this, Hogan (1999) found that the performance of the separate dustbathing movements appears innate and develops in a similar way in birds, independently of whether or not they have had access to litter or not during rearing. Similarly, Nicol *et al* (2001) found that adult behaviour was generally flexible and influenced mostly by the current access to a particular substrate.

Thus, the question still remains as to whether the rearing environment has long-term effects on birds' dustbathing behaviour and, in particular, whether there is a risk that being reared without litter could affect the birds' utilisation of a suitable dustbathing substrate when access to litter is provided at a later stage. Therefore, the aim of this study was to investigate, under controlled conditions, the effect of access to litter during rearing on dustbathing behaviour if access to litter is provided after most of the development of dustbathing behaviour has occurred. The hypothesis was that chicks reared without peat (a preferred dustbathing litter) would show an altered dustbathing pattern, even after access to litter, compared to birds reared with peat. Similarly, it was also our aim to compare how dustbathing behaviour would change in hens that had lost access to litter after being reared with the opportunity to perform functional dustbaths. In

order to allow for comparison, control treatments were instigated whereby birds that had developed their dustbathing behaviour, both with or without litter, remained with the same treatment for the duration of the timeperiod.

## Materials and methods

### Animals and housing

Forty-eight, day-old chicks of the laying strain LSL (Lohmann Selected Leghorn) were introduced into the experimental farm in Skara, Sweden. They were placed in 12 pens with four chicks to a pen. The pens were 1.5 × 1.5 m (length × width) with two perches at heights of 30 and 50 cm. For the first two weeks, chicks were kept directly on the floor, which was cleaned twice daily, and after two weeks they were then kept on wire netting (mesh size 1.9 × 1.9 cm). Each pen contained a 120 × 40 × 16 cm (length × width × height) wooden box, with one side removed to facilitate entry by young chicks during the first five weeks. In six of the pens, the box contained peat (litter) and, in the other six, it contained a sheet of corrugated paper (non litter). Water and chicken food (Pullfor Fenix, Lantmännen, Stockholm, Sweden) was provided *ad libitum* and the design of the feeder prevented chicks from walking or lying in the food. A heating lamp was provided for the first five weeks and the light schedule was a 12:12h light:dark cycle. The birds were marked individually with a numbered leg ring. At the conclusion of the study, the birds were used in another study before being euthanised in accordance with Swedish animal welfare legislation. The study was approved by the Swedish animal research ethics committee.

### Treatments and filming

Birds in the original two treatments (litter and non litter) were regrouped such that two new additional treatments were created when the birds were five, six or seven weeks old. As only four pens could be filmed at any given time, chicks from four pens (two with litter and two without litter) were regrouped at the same time and moved to four filming pens when the chicks were five-weeks old. Following this, the next four pens went through the same procedure when the chicks were six-weeks old and the last four pens at seven weeks. The filming pens were similar to the home pens, but were placed in a separate room. In two of the filming pens, the wooden box contained peat and in the other two pens the wooden box contained corrugated paper. The new treatments and groups were created by placing one of the four chicks from one home pen in each filming pen so that all birds in the new group were unfamiliar, but were still in groups of four individuals (Figure 1). Half of the birds remained on the same substrate that they had previously been reared on while the other half changed substrate. Thus, there were now four treatments; birds that had been kept on peat all their life (litter-litter, LL), birds that had changed from litter to non litter (LN), birds that had changed from non litter to litter (NL) or birds that had never experienced litter (non litter-non litter, NN).

Chicks were marked for identification with green paint (Rosinco® permanent friendly marker, Filipstad, Sweden)

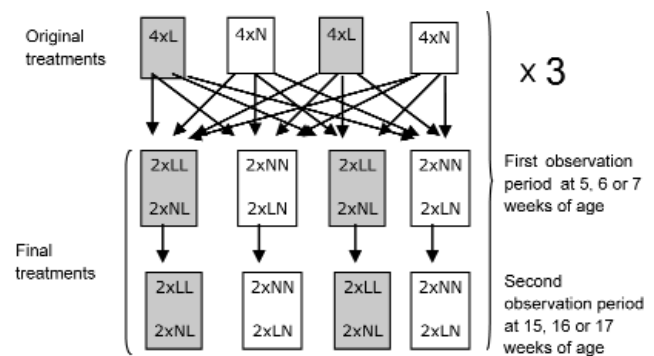
on either the head, back, wings or tail so that they could be individually identified on the video recording. No negative effects, such as increased pecking, were observed as a result of the marking. The birds were filmed for six consecutive days and filming began as soon as the four chicks had been released into the pen in the morning of day one. The light schedule was the same as in the home pens (from 0700 to 1900h) and the pen was filmed from above during the light period with a time-lapse function (24 h of real time was translated into three hours on the videotape). The room temperature was kept at approximately 18°C. When the birds had been filmed for six days (first observation period) they were returned to the home pens, but remained in the same (new) groups. To investigate the extent to which dustbathing behaviour changed over a longer period of time in the new treatments, filming was repeated when the birds were 16, 17 and 18 weeks of age (second observation period). For this second period of filming there was no regrouping. Birds remained in the same group with the same treatment as for the first filming. A number of birds died or were culled during the study as a result of Marek's disease and only healthy animals were included in the observations. In total, 41 birds (10 LL, 10 NL, 11 LN and 10 NN) were observed during the first observation period and 35 birds (9 LL, 8 NL, 8 NL and 10 NN) in the second.

### Behavioural observations from the video recordings

Behavioural observations were carried out from the video recordings. These observations were carried out 'blind' in so far as the person observing the video was unaware of whether the birds had been reared with or without litter for the first 5–7 weeks but could, of course, observe whether or not the bird had current access to litter. The duration of each dustbathing bout, the number of bouts and where in the pen dustbathing occurred were noted for the whole of the first and second observation periods (144 h in total). A dustbathing bout was considered to have begun when the bird performed its first vertical wing shake and to have finished when the chick stood up and did a body shake. Alternatively, if no body shake was performed the bout was considered to be over when the bird stood up, on the condition that it did not lie down again to perform more dustbathing within five minutes.

In addition to the general dustbathing observations described above, the details of the dustbathing were observed for one dustbathing bout performed by each bird in each observation period. The dustbath chosen for this was the first dustbath each bird performed after being moved to the filming pen. This was chosen in order to maximise the amount of data since certain birds only performed one dustbathing bout. Scans were made every 30 s after the bird had performed its first vertical wing shake until it had finished the bout. Behaviours chosen for the detailed observations were tossing behaviours (combined vertical wing shakes and leg scratching) and side rubs, as these have been shown previously to differ between different litter materials (Liere 1992). Lying still and whether or not the bird performed a body shake at the end of the bout were also observed. Other

**Figure 1**



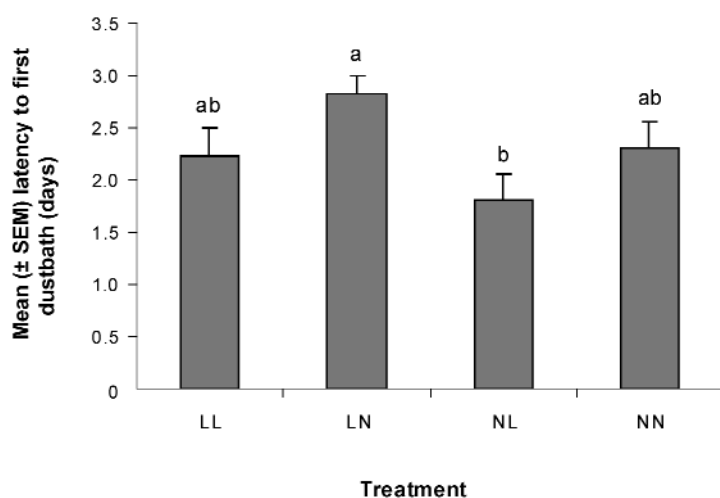
Flowchart of the reassignment of birds to the different treatments. L = litter, N = no litter (paper) during the original treatments. The procedure of moving birds from the original treatment pens to the final treatment pens is represented by the top row of arrows. The four birds in the original treatment pens were each moved to a new final treatment pen. Thus, all birds were unfamiliar to each other in the final treatment pens. LL = birds that stayed on litter, LN = birds that lost access to litter, NL = birds that gained access to litter and NN = birds that never had access to litter. The bottom row of arrows demonstrates that the birds stayed in their same treatment pens between the first and second observation periods. The whole process was replicated three times.

details in the dustbathing behaviour, such as whether a bird pecked in the litter or did a head rub in the litter were sometimes difficult to distinguish reliably from the video recordings and therefore were not included in the analyses.

### Statistical analyses

Data were analysed with statistical software R-2.5.1® (Ihaka & Gentleman 1996). Since data were not normally distributed, generalised estimating equations for linear regression models assuming asymptotic normality were used. Using the 'gee' R function, the effect levels and standard errors were estimated with the maximum likelihood method. These standard errors were then 'sandwiched' using group as a cluster variable where it was assumed that observations within group were correlated. For the variable 'latency to first dustbath' only data from the first observation period were included. For the other variables, both observation periods were included by modelling the influence of the first observation period on the second; thereby incorporating the pairing of observations at both observation periods for each individual. The model also included the interaction between treatments and the two observation periods. We used the LL treatment as intercept (reference) in most models which is why many results are presented in reference to LL. For the analyses of the detailed observations of specific components within dustbathing bouts, the number of scans that a bird was performing a specific movement was divided by the total number of scans carried out during that bout. This was done to control for the fact that the length of the dustbathing bout varied. In this way, percentages of scans of different

Figure 2



Mean ( $\pm$  SEM) latency (in days) to first dustbath performed during the first observation period for the four treatments: Litter-Litter (LL), Litter-Non litter (LN), Non litter-Litter (NL) and Non litter-Non litter (NN). Treatments designated with only an a or b are significantly different.

**Table 1** Mean ( $\pm$  SEM) duration (s) of dustbathing bouts for the four treatments in the first and second observation period.

Treatment	1st observation period	2nd observation period
LL	994 ( $\pm$ 78)	1,878 ( $\pm$ 152)
LN	1,263 ( $\pm$ 88)	1,620 ( $\pm$ 239)
NL	1,077 ( $\pm$ 87)	2,111 ( $\pm$ 206)
NN	1,150 ( $\pm$ 90)	1,754 ( $\pm$ 239)
<b>Total</b>	<b>1,139 (<math>\pm</math> 45)</b>	<b>1,778 (<math>\pm</math> 120)</b>

movements were able to be compared, irrespective of the total duration of the dustbathing bout. The variables 'latency to first dustbath', 'number of bouts', 'side rubs', 'tossings' and 'lying still' were analysed using a Poisson distribution with a log link. 'Duration' was analysed using a gamma distribution with a log link. 'Body shake' was analysed using a binomial distribution with a logit link in a maximum likelihood procedure (not sandwiched). Birds were grouped after both treatment and group. For each of these specific groups, the proportion of birds which finished their bout with a body shake was calculated for each observation period and then these proportions were fitted into the model.

## Results

In total, 257 dustbathing bouts were observed over both observation periods. One hundred and sixty two of these were carried out in the first observation period and 95 in the second. Forty-one birds were included in the first observation period but, as one of these did not perform any dustbathing, detailed analyses only exist for 40 birds. In the second period all 35 birds dustbathed.

## Location of dustbathing

During the first observation period, all dustbathing by chicks from the LL treatment was carried out in the wooden box with peat. Most of the bouts carried out by chicks in the other treatments were also performed in the wooden box, but 9% of NN, 8% of the NL and 4% of the LN bouts were carried out on the wire floor. During the second observation period, all dustbathing was carried out in the wooden box and there were no bouts seen on the wire floor.

## Latency to first dustbath

There was a significant difference between treatments with LN birds (which lost access to peat), waiting longer to dustbathe in the first observation period than birds in the NL treatment ( $z = 3.09$ ,  $P = 0.002$ ) and, also, a tendency for LN birds to wait longer to dustbathe than LL birds ( $z = 1.8$ ,  $P = 0.07$ , Figure 2).

## Number of dustbathing bouts

During the first observation period, birds performed 3.9 ( $\pm$  0.3) dustbathing bouts compared to 2.7 ( $\pm$  0.3) in the second. This difference was not significant and neither was there a significant difference between the treatments.

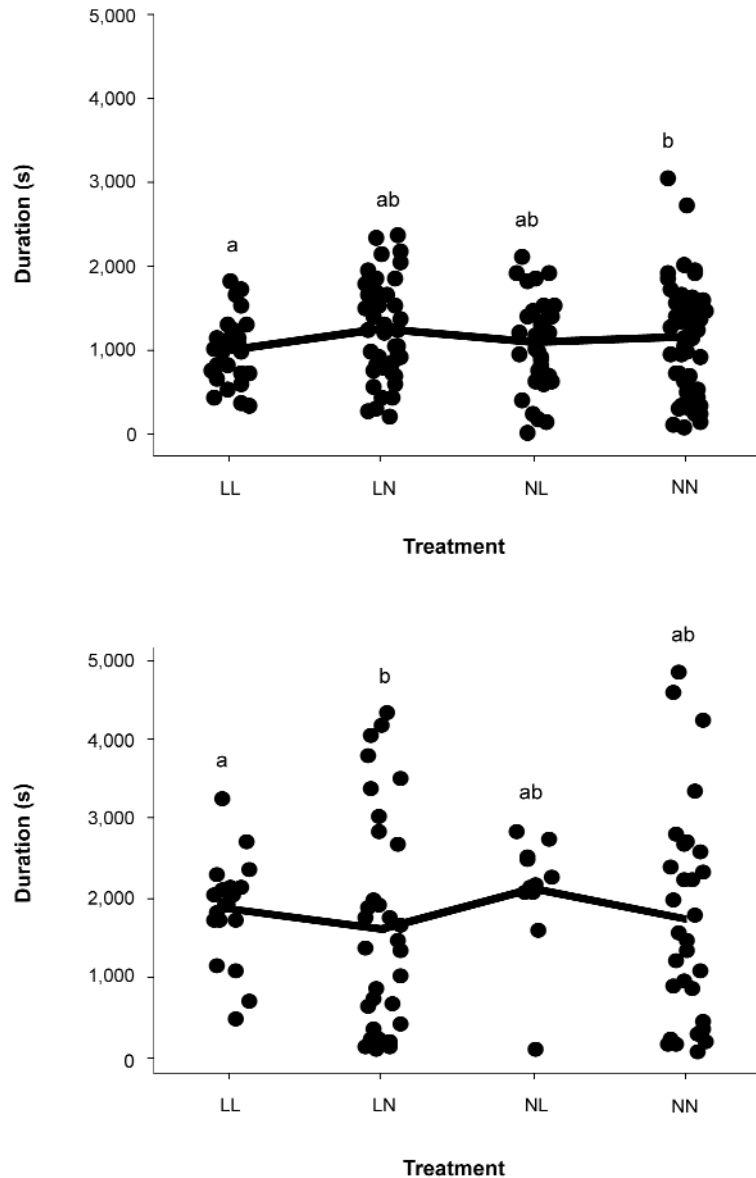
## Duration of dustbathing bout

The length of the dustbathing bout increased from 19 min (1,139 [ $\pm$  45] s) during the first observation period to 30 min (1,778 [ $\pm$  120] s) during the second ( $W = 36.7$ ,  $P < 0.001$ ) but there were no general treatment effects. There was, however, a significant interaction between treatment and observation period for birds in the LN treatment regarding the mean length of the dustbathing bout ( $W = 4.1$ ,  $P = 0.042$ ). In the first observation period, LN birds had the longest mean duration and in the second their dustbaths had the shortest (Table 1).

Significant differences were found between treatments in the variability (based on estimated standard errors)

**Figure 3**

Duration of dustbathing bouts where each dot represents one dustbathing bout. The line indicates mean values, first observation period (upper) and second observation period (lower) for the four treatments. Treatments designated with only an a or b differ significantly from each other.

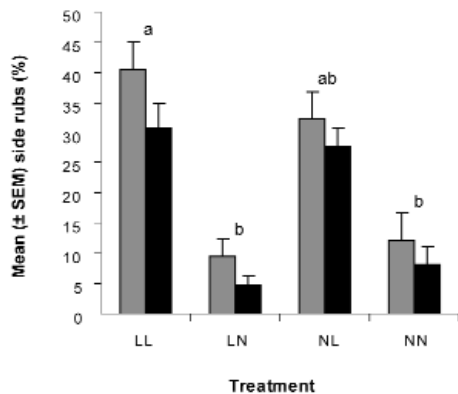


around the duration of the dustbathing bouts (Figure 3). An analysis of the overall difference through a comparison of LL and NL treatments versus NN and LN over both observation periods saw a significant difference in variance; there was a smaller variation in duration in the LL and NL treatments than in the NN and LN treatments ( $W = 17.9$ ,  $P < 0.001$ ). In the first observation period, there was a tendency for LL to differ from LN ( $W = 3.49$ ,  $P = 0.06$ ) and LL differed significantly from NN ( $W = 7.09$ ,  $P = 0.008$ ). No differences were seen in the variation around the duration of dustbathing bouts

between birds in the LL and NL treatments nor between birds in the LN and NN treatments (Figure 3, upper). In the second observation period, there was now a significantly smaller variation in the duration of the dustbathing bout for LL than for LN ( $W = 9.3$ ,  $P = 0.002$ ) but the difference between LL and NN was no longer significant ( $W = 2.4$ ,  $P = 0.11$ ) (Figure 3, lower).

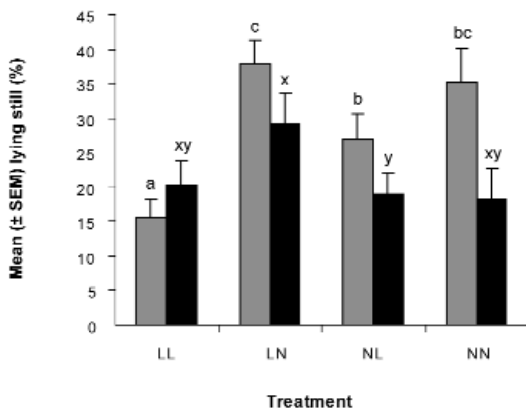
Nevertheless, when the variation in dustbathing duration was investigated on an individual basis for the NN and LN birds, no obvious pattern emerged. Birds performed dustbaths of all durations, ie short, medium and long bouts.

Figure 4



Mean ( $\pm$  SEM) percentage side rubs during the first dustbathing bout performed in the first observation period (grey bars) and second observation period (black bars) for the four treatments. The LL treatment differed significantly from the LN and NN treatment over both observation periods as indicated by the labels a and b.

Figure 5



Mean ( $\pm$  SEM) percentage lying still during the first dustbathing bout performed in the first observation period (grey bars) and second observation period (black bars) for the four treatments. The LL birds were significantly different from the three other treatments and LN and NL birds were significantly different from each other in the first observation period as indicated by the letters a, b and c. During the second observation period the LN treatment was significantly different from the NL treatment as indicated by the letters x and y.

**Table 2** Percentage of birds that finish their dustbathing bout with a body shake.

Treatment	1st observation period	2nd observation period
LL	50	100
LN	18	63
NL	70	100
NN	20	70

## Detailed observations of specific components within dustbathing bouts

### Side rubs

The percentage of side rubs performed by birds during their first dustbathing bout in the first and second observation periods was seen to differ between treatments, with the birds in the LL and NL treatments both having a higher proportion of side rubs than birds from the NN and LN treatments (Figure 4). Due to outliers, it was not possible to examine the difference between treatments for each observation period separately but, overall, the LL treatment differed significantly from the NN ( $z = -4.6$ ,  $P < 0.001$ ) and the LN ( $z = -5.7$ ,  $P < 0.001$ ) treatments. There was no difference between LL and NL.

### Tossings

The mean percentage tossings performed during the first dustbathing bout in the first observation period was 19.8 ( $\pm$  1.6)% and 29.8 ( $\pm$  2.1)% for the second. This difference was not significant. Neither were there significant differences between treatments in the first or second observation periods.

### Lying still

In the first observation period, a significant difference was seen in the percentage of scans where the bird was lying still during a dustbathing bout (Figure 5). LL birds performed significantly less lying still than LN ( $z = 4.5$ ,  $P < 0.001$ ), NN ( $z = 3.7$ ,  $P < 0.001$ ) and NL birds ( $z = 2.5$ ,  $P = 0.01$ ). A significant difference was also observed between LN and NL birds ( $z = 2.2$ ,  $P = 0.03$ ) with more lying still seen in LN compared to NL birds. In the second observation period, the LN treatment differed significantly from the NL treatment ( $z = -2.1$ ,  $P = 0.04$ ) and had a tendency to be different to the LL ( $z = -1.7$ ,  $P = 0.09$ ) and NN treatments ( $z = -1.7$ ,  $P = 0.08$ ).

### Body shake

During both observation periods there was a tendency for birds from the LN treatment to finish their dustbathing bout with a body shake less often than LL birds ( $z = -1.7$ ,  $P = 0.09$ ). No significant difference was found between NN and LL birds ( $z = -1.6$ ,  $P = 0.12$ ) but, as seen in Table 2, NN and LN birds showed similar percentages of birds performing a body shake. The same trend was seen for LL and NL birds.

## Discussion

There were two main findings in this study. The first one was that birds housed without litter showed a greater variation in the duration of their dustbathing than birds housed with litter, which we suggest could be an indication of frustration. The second was that chicks which lost or gained access to litter were able to change the pattern of their dustbathing behaviour to resemble that of birds which had been reared on that particular substrate. This was especially true for birds which gained access to litter, but birds that lost access to litter showed some long-term effects.

### Dustbathing performance

Birds housed without access to peat showed a larger variation in the length of their dustbathing bouts, ie they performed more extremely short and extremely long bouts than birds dustbathing in peat. This result helps clarify why, in certain studies, birds deprived of litter have had shorter bouts (Lindberg & Nicol 1997; Merrill & Nicol 2005) whereas in other studies they have had longer bouts (Vestergaard *et al* 1990). A likely explanation is that lack of proper feedback from the litter results in the bird either quickly interrupting the bout or repeatedly restarting within the same bout and thus making it a very long bout (Liere 1992). This shows that the birds change their behaviour according to the feedback they receive from the litter. Merrill and Nicol (2005) suggested that short bouts could be a sign of frustration, but an exceptionally long bout may equally well be problematic with the bird caught in a loop of starting the behavioural sequence again and again in the absence of sufficient feedback for it to move on to the next step in the sequence. Although the birds' behaviour suggests that they are frustrated when they do not get access to litter, it would be beneficial to seek further validation of this via other measures of frustration.

The fact that bout duration is more variable for birds without litter is in keeping with the finding that there were differences between treatments in the proportion of the different movements performed during a dustbathing bout. A bout performed in peat contained more side rubs than a bout performed without litter, which is also in agreement with Vestergaard *et al* (1990) and Larsen *et al* (2000). Liere (1992) suggested that birds dustbathing without functional litter perform fewer side rubs because litter does not get in-between the feathers and in contact with the skin, therefore the rubbing behaviour phase is not reached. However, despite the differences between treatments in the percentage side rubs performed in a bout, there was no difference between treatments in the percentage of tossing performed. More tossing of litter might have been expected if birds without access to litter did not proceed to the rubbing phase but continued to attempt to get litter material into the feathers. The results show that it is lying behaviour that differs between treatments. In the first observation period, the NN and LN birds and, to some extent NL birds, spent a greater proportion of their dustbathing bout lying still compared to LL birds. One explanation for this could be that instead of performing more tossing behaviour when there was insufficient feedback, the birds interrupted their bout and did not perform any particular dustbathing movements. This lying behaviour did not seem to change as quickly in response to gaining or losing access to litter as the other movements since NL birds were different to LL birds in the first observation period but similar in the second whereas LN birds still showed significantly more lying behaviour in the second observation period.

### Losing or gaining access to litter

The reason the mean duration between birds dustbathing with or without litter did not differ is attributable to the fact that LN and NN birds performed both very short and very long dustbaths. Nevertheless, LN birds seemed to have been affected by the change of treatment as an interaction was seen between the two observation periods. These chicks showed the longest mean duration during the first observation period and the shortest during the second. A possible explanation for this may have been that the LN birds extended their dustbaths when they lost access to peat and did not get the feedback they were used to but, by the second observation period, when they were used to being without litter, they were more likely to interrupt and shorten their bout compared to the other treatments. It is worth noting that the LN treatment was also the one that was atypical in that it had a sustained, high level of lying still during a dustbathing bout which we argue could be interpreted as these birds experiencing the current feedback as abnormal compared to what they had experienced previously. The reason why the NN birds decreased their lying time and tended to be different from the LN birds during the second observation could be that these birds had never experienced any other type of feedback than that provided by sham dustbathing.

The finding that birds which had just lost access to peat waited the longest before dustbathing whereas birds that gained access were the quickest to dustbathe, supports the notion that birds do not readily perform sham dustbathing if they are used to performing dustbathing in litter and that dustbathing in peat is attractive even to birds unused to it (Wichman & Keeling 2008). In our study, the mean latency to the first dustbath for birds which had lost access to litter was approximately one day longer than for the other treatments, whereas it has previously been observed that older birds can wait several weeks before they begin to sham dustbathe (Hogan *et al* 1991). This may imply that our birds were still rather flexible in their dustbathing behaviour. Supporting this is the fact that the mean duration of a dustbathing bout increased significantly from the first to the second observation period. In the second observation period, the length of the dustbathing bout (30 min) had almost the same duration as has been observed in adult birds housed under semi-natural conditions (Vestergaard 1982). Thus, we suggest that the dustbathing behaviour had yet to reach adult levels in the first observation period when birds were around six-weeks old. This would support Vestergaard and Hogan (1992) who found that adult levels had been reached by around two months of age. However, we had two treatments that remained on the same substrate (LL and NN) and these were assumed to act as controls, reflecting the way in which dustbathing in birds would have changed in any case over time as the birds matured.

The above arguments may lead us to conclude that one of the main reasons behind our finding that birds can change their dustbathing behaviour — although it is questionable whether the birds that lost access to peat did so completely within the timespan of this study — was the fact that we

were using birds whose dustbathing behaviour was not yet fully established. This cannot be excluded, but studies have shown that essentially all the different movements in the dustbathing sequence develop during the first three weeks (Larsen *et al* 2000). Furthermore, birds reared on wire until five weeks of age, prior to being given access to sand, showed long-term effects in their dustbathing behaviour despite the early age of change (Johnsen *et al* 1998). Therefore, it is likely that factors other than age can influence whether or not birds will be flexible in terms of their dustbathing behaviour. For example, it has been found that dustbathing behaviour is dependent on the type of litter in which it is performed (Liere & Siard 1991) and it has been pointed out that this should be taken into consideration when comparing dustbathing behaviour (Petherick & Duncan 1989). If we, as an example, compare the treatments in our study (peat and paper) with those of Johnsen *et al* (1998) (sand and wire), it could be that the difference between peat and paper, both of which have a hard surface underneath, were not as large as between sand and wire; one of which has a hard surface underneath whereas the other does not. In addition, it is possible that the paper treatment could have provided some small degree of feedback from particles on the surface, in comparison with wire, and might not be considered as pure sham dustbathing. Although an alternative explanation could be that peat has such good qualities as a dustbathing litter that birds were more readily able to change their dustbathing behaviour when they got access to peat compared to when they got access to sand.

#### Choice of dustbathing location

When birds dustbathed in filming pens, the majority of dustbaths were carried out in the wooden box. LL birds dustbathed only in the peat, but a small proportion were performed on the wire in the other three treatments during the first observation period. The fact that all dustbaths performed by LL birds were carried out in the peat is probably an indication that the chicks reared with peat also dustbathed in this litter prior to the start of observations and had developed their dustbathing behaviour on this substrate. The fact that some of the birds which had lost access to litter (LN) dustbathed on the wire floor, could be a sign that they tried different surfaces in search of a replacement for the litter. Some of the dustbaths performed by the NN and LN birds may have been performed on the wire because paper was not sufficiently rewarding to be chosen on every occasion. However, only a small number of dustbaths were carried out on the wire during the first observation period. During the second observation period, when the birds' dustbathing behaviour was fully established, all dustbathing was carried out in the wooden box, irrespective of whether or not it contained litter. This demonstrates clearly that birds prefer to dustbathe on a stable surface rather than on wire. Similarly, Merrill *et al* (2006) found that chicks prefer to dustbathe on Astroturf® over wire. But, even if birds prefer dustbathing on a stable surface, the irregular pattern of the dustbathing when there is no litter in the wooden box, indicates that it may not replace dustbathing in a functional substrate.

#### Conclusions and animal welfare implications

In summary, the results of this study suggest that, even if birds spend their first weeks without access to litter, on being introduced to functional substrate birds will use this to dustbathe, altering the pattern of their dustbathing behaviour accordingly. This implies that if birds are seen to perform sham dustbathing on the wire floor in modified cages, the quality of the litter in the dustbath is not optimal and/or it is not sufficiently accessible. Furthermore, the fact that chicks performing dustbathing without litter show an irregular dustbathing behaviour and, if they have previous experience of litter, lie still for a greater proportion of their dustbathing time, may be an indication that these birds are frustrated due to lack of feedback from loose material. This would imply that dustbathing without litter is not able to replace functional dustbathing and, thus, even if chicks do not need to be reared with litter in order to later acquire a normal dustbathing behaviour, they would benefit anyway from having access to litter also as young birds.

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