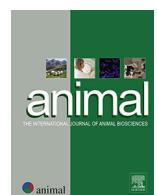




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### Gregarious nesting in relation to floor eggs in broiler breeders

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#### ABSTRACT

Gregarious nesting has often been observed in laying hens, where hens prefer to visit a nest already occupied by other hens over empty nests. This may result in overcrowding of the nests which is considered a welfare issue and, moreover, can increase the economic issue of floor eggs. This study aimed to describe gregarious nesting and spatial behavior in broiler breeders and how this relates to genetic background, fearfulness and mating behavior. Five commercially available genetic lines of broiler breeders were housed in 21 pens of 550 females and 50 males (six pens for lines 1 and 2, five pens for line 3 and two pens for lines 4 and 5) during the ages 20–60 weeks. Every 10 weeks, the plumage condition and wounds were assessed of 50 random hens per pen. Avoidance distance and novel object tests were performed to assess fearfulness at four time points. Distribution of eggs over nests was observed for 6 weeks at the onset of egg production at 26 weeks of age, and use of space was recorded at four time points, while (floor) egg production was noted daily per pen. We found differences between genetic lines over time in plumage condition and prevalence of wounds. Fear of humans was highest at the earliest age tested and did not correlate with general fearfulness as assessed by the novel object test. The distribution of eggs over nests was related to genetic background and was more uneven at the earliest age compared to later ages, and a more uneven distribution was correlated with an increased percentage of floor eggs. Distribution of birds over the litter area differed between the genetic lines, and less use of the litter area was correlated with an increased fear of humans and presence of wounds, suggesting an association with aggressive mating behavior. This difference in distribution of the birds could also explain the correlation between increased presence of wounds and decreased percentage of floor eggs. It is concluded that broiler breeders do show gregarious nesting, which is affected by genetic background. Both increased gregarious nesting and wounds are related to increased floor egg percentage, which should be studied further in broiler breeder research. Genetic selection for even use of the available nests and of the litter and slatted area would therefore support both broiler breeder welfare and performance.

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#### Implications

Chickens are known to show gregarious nesting behavior, which means that hens prefer to enter an occupied nest over an empty nest. Excessive gregarious nesting leads to crowded nests, and this decreases hen welfare. If the nests are too full to enter, a hen might also choose to lay her eggs outside the nest. These so-called floor eggs are of lower quality and require extra labor from the farmer to collect them. In this study, we investigate which factors are related to gregarious nesting, which may help to find strategies to avoid excessive gregarious nesting behavior in the future.

#### Introduction

Choosing to enter an occupied nest over an unoccupied nest is called gregarious nesting, which has often been observed in laying hens and can result in welfare and production problems (Appleby and McRae, 1986; Riber, 2010; Tahamtani et al., 2018). When many hens in a flock exhibit gregarious nesting, other hens can have an excessive energy expenditure when repeatedly trying to enter a nest which is overcrowded (Kite et al., 1980 as cited by Riber, 2010). Increased aggression has been observed in front of nests when multiple hens wanted to enter, as well as inside occupied nests after entering (Meijsser and Hughes, 1989; Appleby and Hughes, 1991). Gregarious nesting also has economic consequences as eggs might break if the number of eggs exceeds the egg belt capacity. Furthermore, when the nests are too full to enter, hens might lay their eggs outside the nests (also known as floor eggs). Floor eggs require manual collection and have a lower hatchability and

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saleability due to the fact that they are often dirty or broken (van den Brand et al., 2016).

Several possible causes for gregarious nesting have been suggested. It could be that many hens share their preference for nests in certain locations, mostly nests at the end of the row or in a corner (Riber, 2010; Ringgenberg et al., 2015). Corners might be attractive due to a difference in microclimate or a lower light intensity, but they are also more easily recognized (Appleby and McRae, 1986; Riber, 2010). Nests are often presented in long rows at commercial farms, so the nests at the end of the row are more easily found again than a nest in the middle of the row. However, offering heterogeneous nests, which should be easier to recognize, did not decrease the occurrence of gregarious nesting (Clausen and Riber, 2012). Gregarious nesting has also been suggested to be an anti-predator strategy (Riber, 2012) or the result of a lack of nesting experience in younger hens (Riber, 2010), but seems to be unrelated to dominance status (Tahamtani et al., 2018).

Gregarious nesting has only been described in laying hens, and it is unknown whether broiler breeders also exhibit this behavior, while studying this behavior in broiler breeders is interesting for several reasons. First, different genetic lines of broiler breeders have been selected for different combinations of goals, which might also affect unselected characteristics such as gregarious behavior (Dawkins and Layton, 2012). Furthermore, it has been shown that the mere presence of males reduced floor eggs in a small experimental study, although the exact reasons remain unknown (Rietveld-Piepers et al., 1985). It is suggested that broiler breeder males might influence spatial distribution of the females and that this in turn affects floor laying behavior. Broiler breeder males use the slatted areas less than the litter area and are known to be aggressive in their mating behavior, causing feather loss and wounds in females (de Jong and Guémené, 2011). Females have been observed spending more time on slatted areas to avoid aggressive males, and as the nests are accessed from this slatted area, this could affect nesting and floor laying behavior. Finally, fearfulness could also affect the use of raised areas and thereby nesting behavior. Less fearful laying hens have been found to make more use of raised areas and perches (Brantsæter et al., 2016), although it is unknown whether this is also the case for broiler breeders.

In this study, we aim to investigate how much gregarious nesting behavior is performed by broiler breeders and to understand the background of this behavior. This was part of a larger study with the same animals, and the results of the other part, which focused on leg health, are reported elsewhere (van den Oever et al., 2020). Therefore, we investigated possible relationships between gregarious nesting and use of space with plumage condition, presence of wounds, fearfulness and genetic background. Further, we studied whether these factors are correlated with egg production and floor egg percentage. The magnitude of gregarious nesting, presence of wounds and fearfulness were expected to differ between the different commercially available genetic lines. A more uneven distribution over the nests was expected to be related to a more uneven use of space, while wounds and fearfulness were thought to alter the relative use of slatted and litter areas. Floor eggs percentage was hypothesized to increase with a more uneven use of nests, more wounds and lower fearfulness.

## Material and methods

### Animals and housing

The experiment took place from June 2018 to March 2019 at a breeding station, where both gregarious nesting (this study) and leg health (van den Oever et al., 2020) were investigated. A total of 11 550 females were reared in 3 groups of mixed genetic lines with raised platforms, while 1050 males (despurred and toe-clipped) were reared in a separate group with raised platforms. All birds were non-beak trimmed and moved from their rearing facilities into the production house located at the same farm at the age of 20 weeks. Five commercially

available genetic lines, all fast-growing, were represented in different numbers. The chickens were assigned to 21 pens of 550 females and 50 males of the same genetic line, resulting in six pens for lines 1 and 2 (3300 females and 300 males per line), five pens for line 3 (2750 females and 250 males) and two pens for lines 4 and 5 (1100 females and 100 males per line). The position of the genetic lines in the house was randomized using a block design with 6 blocks; each line was present maximum once per block. The pens were identical in size ( $12 \times 6.5 \times 2.0$  m, length x width x height) and lay-out and were placed in four rows (see Fig. 1). The animal density was 7.7 birds/m<sup>2</sup>, which is comparable to commercial practice. The pens had wire mesh walls, which allowed the animals from different pens to see each other. The litter area ( $12 \times 3.7$  m) was covered with wood shavings, and the slatted area ( $12 \times 2.3$  m) was raised by 0.5 m and gave access to 9 bell drinkers and 10 nests. The group-nests were of a rollaway type (Vencomatic®), measuring  $1.15 \times 0.52 \times 0.53$  m. All nests had a green rubber nest floor slanting toward the back and red nest curtains with an entry point in the middle. The feeding line for the females was placed partially on the slats and partially in the litter area, while the male feeding line was positioned in the litter area.

The management of the birds was the same for the current study as well as the study focusing on leg health (van den Oever et al., 2020). The house was lit with artificial LED lighting. At 20 weeks of age, the animals had 8 h of light (07:00 to 15:00 h) at 10 lx measured at bird height. This was gradually increased to 14 h of light (02:00 to 16:00 h) at 60 lx at bird height at 27 weeks of age. The temperature was maintained at  $21 \pm 1$  °C. Food was provided at 08:30 h, giving a restricted amount according to the commercial practice schedule and ranging from 100 to 165 g per female and from 100 to 130 g per male. Bird weight was continuously monitored with hanging poultry scales. Water was provided from 08:30 to 12:30 h and from 15:30 to 16:00 h. The nests were available to the hens from 1 h before lights on until 30 min before lights off, from the day after the first egg was found (23 weeks of age). The birds were kept until the age of 60 weeks and then slaughtered for human consumption.

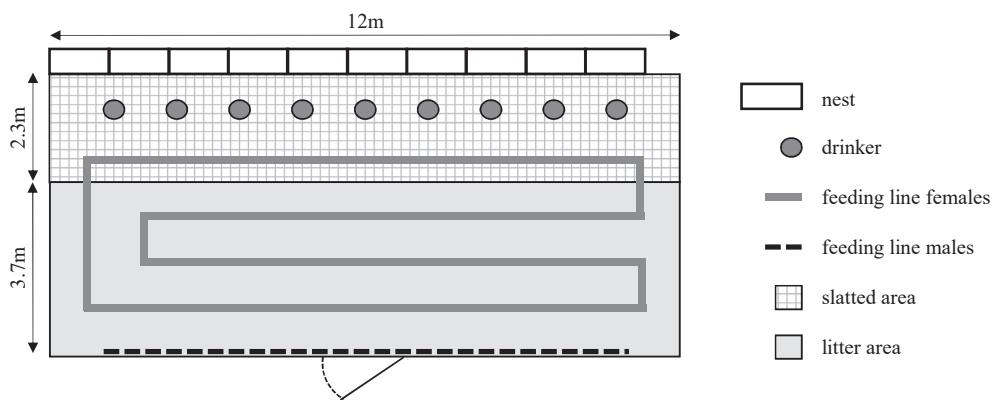
### Data collection

#### Gregarious nesting behavior and use of space

In order to assess the distribution of hens over the available nests, the number of eggs per nest was counted 1 day/week during the ages of 26–31 weeks. At 29, 38, 47 and 56 weeks of age, the spatial distribution of the birds over the pen was assessed by live observations from the passage between the rows of pens. This was done by counting the number of empty slatted areas sized  $1.15 \times 1.15$  m (total 20 areas) and litter areas sized  $1.15 \times 1.30$  m (total 20 areas). The width of the areas was chosen according to the width of the nests (1.15 m), which could be easily distinguished from a distance. The length of the areas was chosen as half of the total litter or slat length. Observations were done within a few seconds by one observer, and the birds hardly moved within this time, especially when they notice they are being observed. Assuming that an average breeder hen measures  $30 \times 15$  cm, this means that 29 birds fit into a slatted area and 33 birds into a litter area. The number of areas containing fewer than 7 birds in the slatted area or 8 birds in the litter area was noted as well, which meant that 75% of the area was empty. These measurements were repeated five times/day, starting at 03:00 h with an interval of 2 h until 13:00 h. The measurements of 09:00 h were discarded as the birds were eating and therefore all equally distributed along the feeding line.

#### Plumage condition and wounds

Approximately every 10 weeks (21, 32, 40, 50 and 59 weeks of age), a random selection of 50 hens per pen was scored for feather damage and wounds on the back and rump (adapted from the laying hen protocol of Welfare Quality®, 2009). The presence of wounds was scored on a 3-point scale: 0 for no wounds, 1 for at least one wound smaller than



**Fig. 1.** Top view of a pen used for housing the broiler breeders.

1 cm and 2 for at least one wound larger than 1 cm. Feather damage was also scored on a 3-point scale: 0 for no feather damage, 1 for areas of ruffled feathers or without feathers smaller than 5 cm in diameter and 2 for areas of ruffled feathers or without feathers larger than 5 cm in diameter.

#### Fearfulness

To investigate the relationship between fear of humans, gregarious nesting and use of space, a random selection of 20 hens per pen was subjected to an avoidance distance test at 22, 31, 41 and 52 weeks of age (Welfare Quality®, 2009). The observer walked parallel to the slatted area at a distance of 1.5 m and turned to approach the hens sitting on the edge of the slatted area with the hand in front of the body. The distance between the hand of the observer and the hen was noted (rounded to the nearest 10 cm), when the hen retreated. At 22 weeks of age, a novel object test was performed to measure general fearfulness. After placing the novel object (a colored rod) in the center of the home pen, the observer withdrew for 1.5 m and started recording. The number of hens approaching the novel object within bird's distance was noted every 10 s for 2 min in total, in order to determine the latency of 7 hens to approach the novel object and the maximum number of hens that approached the novel object. The benchmark of 7 hens was chosen after performing the test, this was the overall average number of hens approaching the novel object test.

#### Production

Production data were collected for the purpose of this study as well as the study focusing on leg health (van den Oever et al., 2020). Starting at 24 weeks of age until the end of the trial, the number of floor and nest eggs was recorded daily per pen. The number of broken nest eggs was counted as well. Floor eggs were collected three times per day, and nest eggs were collected once a day. Eggs laid on the slatted area were prevented from rolling into the litter with a 18 mm plastic tube, which allowed for separate recording of litter eggs and eggs laid on the slats. This plastic tube had to be removed at 45 weeks of age for manure management, after which no distinction could be made between floor eggs laid on the slats or in the litter.

#### Statistical analysis

Egg production percentage was calculated by dividing the total number of eggs by the number of present hens. Floor egg and broken egg percentages were calculated by dividing the number of floor or broken eggs over the total number of eggs laid per pen per week, whereas litter egg percentage was calculated by dividing the number of eggs laid in the litter over the total number of floor eggs. Number of eggs per nest

was used to calculate the distribution index using the following formula:

$$SPI = \frac{\sum_{i=1}^n |N_i - \frac{T}{n}|}{2*(T - \frac{T}{n})} = \frac{\sum_{i=1}^{10} |N_i - \frac{T}{10}|}{2*T*0.9}$$

where  $N_i$  is the number of eggs laid in each nest,  $T$  is the total number of eggs in the pen and  $n$  is the number of nests (which is 10) (adapted from Dickens, 1955). A distribution index of 0 indicates that the eggs are spread equally over all nests available and 1 indicates that all eggs are laid in one nest. The number of 75 and 100% empty slatted and litter surfaces per pen. These measurements were averaged before and after feeding and analyzed separately, to investigate differences between main laying time and the rest of the day. Percentage of empty slatted, litter and total surface was analyzed separately. The measurements of the avoidance distance test at 31 weeks of age were discarded as the majority of observations were disturbed by aggressive males. The novel object latency times and avoidance distance were analyzed as mean per pen per observation week, whereas the wound score was analyzed as the percentage of birds with wounds.

All statistical analyses were performed with SAS (version 9.4). The MIXED procedure was used to perform general linear mixed models in order to investigate differences between lines and ages. Fixed effects included line and age and their interaction; pen within line was included as a random effect. The assumptions of homogeneity of variance and normally distributed residuals were examined visually using the conditional studentized residual plots. In order to satisfy these assumptions, the percentage of broken eggs was log transformed. Pearson correlations were calculated between traits using the CORR procedure, except for correlations with percentage of broken eggs for which the Spearman's rank order correlations were calculated. Results are shown as non-transformed means with corresponding standard errors, and  $p$ -values below 0.05 were considered significant. Tukey's post hoc test was performed to investigate significant pairwise differences between test groups, which are reported in the Results section if  $P < 0.05$ . The results of pair-wise comparisons between the lines can be found in supplementary table S1.

#### Results

##### Gregarious nesting behavior and use of space

The distribution index of eggs over the nests provided was affected by line ( $F_{4,16} = 42.15$ ,  $P < 0.0001$ ) and by age ( $F_{5,80} = 9.6$ ,

$P < 0.0001$ ), but not by their interaction. Line 4 had the most uneven distribution ( $0.45 \pm 0.01$ ), line 5 had an intermediate level of distribution ( $0.29 \pm 0.02$ ) and lines 1, 2 and 3 the most even distribution ( $0.11 \pm 0.01$ ). Fig. 2 illustrates these indices by showing the percentage of eggs laid in each nest per genetic line. The distribution was more uneven at the age of 26 weeks in comparison to the following weeks.

The use of space differed between the lines, see Fig. 3. Before feeding, the total percentage of empty space was equal for all lines, but lines 1, 2 and 3 left less slatted area empty ( $F_{4,16} = 18.1, P < 0.0001$ ) and more litter area empty ( $F_{4,16} = 5.1, P = 0.008$ ) compared to lines 4 and 5. The total percentage of empty space was lower after feeding, which was caused by a lower percentage of empty slatted areas ( $F_{4,16} = 7.7, P = 0.001$ ). Also after feeding, lines 1, 2 and 3 left less slatted area and more litter area empty compared to lines 4 and 5. There was a tendency for more empty litter area with a higher percentage of wounded hens per pen at the ages of 32 and 40 weeks ( $r = 0.38, P = 0.09$  and  $r = 0.38, P = 0.09$ , respectively). Average percentage of empty slatted area per pen was negatively correlated with average avoidance distance ( $r = -0.62, P = 0.003$ ), while average percentage of empty litter area was positively correlated with average avoidance distance ( $r = 0.49, P = 0.026$ ). No correlation between nest distribution and use of space was found.

#### Plumage condition and wounds

The mean plumage score was affected by the interaction between line and age ( $F_{16,64} = 6.34, P < 0.0001$ ; see Fig. 4A). At the age of 32 weeks, lines 1 and 2 had more severe feather damage than line 4, while at the age of 60 weeks, lines 4 and 5 had more severe feather damage than lines 1, 2 and 3. The prevalence of wounds was also affected by the interaction between line and age ( $F_{16,64} = 2.49, P = 0.0051$ ; see Fig. 4B). Lines 1, 2 and 3 had the highest prevalence of wounds at 32 weeks of age after which the prevalence decreased again, while for lines 4 and 5, the prevalence increased from the age of 40 weeks onward. Within individuals (across lines), the prevalence of wounds was positively correlated with severity of feather damage ( $r = 0.31, P < 0.0001$ ). No correlation between distribution of eggs over nests and plumage condition or prevalence of wounds was found.

#### Fearfulness

The mean human avoidance distance differed per age ( $F_{2,30} = 7.99, P = 0.0017$ ) and per line ( $F_{4,16} = 4.83, P = 0.0096$ ). The mean avoidance distance was higher at the age of 22 weeks with  $85.6 \pm 1.8$  cm than at ages 40 and 52 weeks with  $61.8 \pm 1.3$  cm and  $65.0 \pm 1.2$  cm,

respectively. Line 2 had a higher avoidance distance ( $81.6 \pm 2.0$  cm) than line 4 ( $50.8 \pm 2.0$  cm). Lines 1, 3 and 5 had intermediate avoidance distances ( $72.6 \pm 1.5$  cm,  $70.1 \pm 1.9$  cm and  $59.4 \pm 1.9$  cm, respectively).

The responses to the novel object at 22 weeks of age did not differ between the lines, for both the latency of 7 hens to approach (overall average  $43.4 \pm 11.4$  s) and the maximum number of hens approaching the object (overall average  $12.3 \pm 1.7$ ). The latency of 7 hens to approach the novel object was negatively correlated with the maximum number of hens to approach per pen ( $r = -0.75, P < 0.0001$ ). Responses to the novel object test were not correlated with avoidance distance of humans. No correlation between nest distribution and any of the fear test responses was found (data not shown).

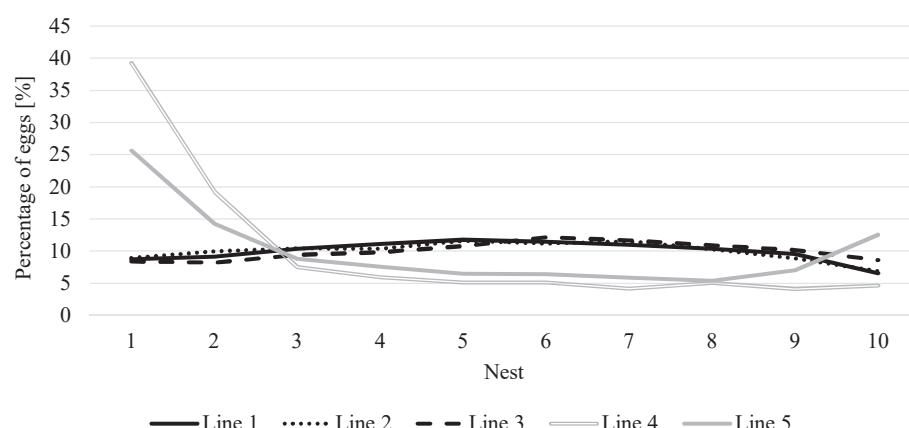
#### Production

Egg production percentage increased rapidly after the onset of lay and then declined again after the age of 30 weeks, while no clear pattern could be distinguished in the development of floor eggs. For specific results, see van den Oever et al., submitted. Most of the floor eggs were found in the litter area. The percentage of litter eggs (expressed as a percentage of floor eggs) increased significantly from  $78.3 \pm 2.0\%$  of the total number of floor eggs at 26 weeks of age to  $88.9 \pm 1.1\%$  at 30 weeks of age ( $F_{20,2840} = 15.0, P < 0.0001$ ) after which it did not increase anymore. The lines did not significantly differ in percentage of litter eggs. Average floor egg percentage was positively correlated with uneven nest distribution during the period when nest distribution was measured, e.g. 26–31 weeks of age ( $r_s = 0.28, P = 0.002$ ). Floor egg percentage had a tendency for negative correlation with average prevalence of wounds per pen ( $r = -0.40, P = 0.07$ ). No correlation between distribution of eggs over nests and percentage of broken eggs was found.

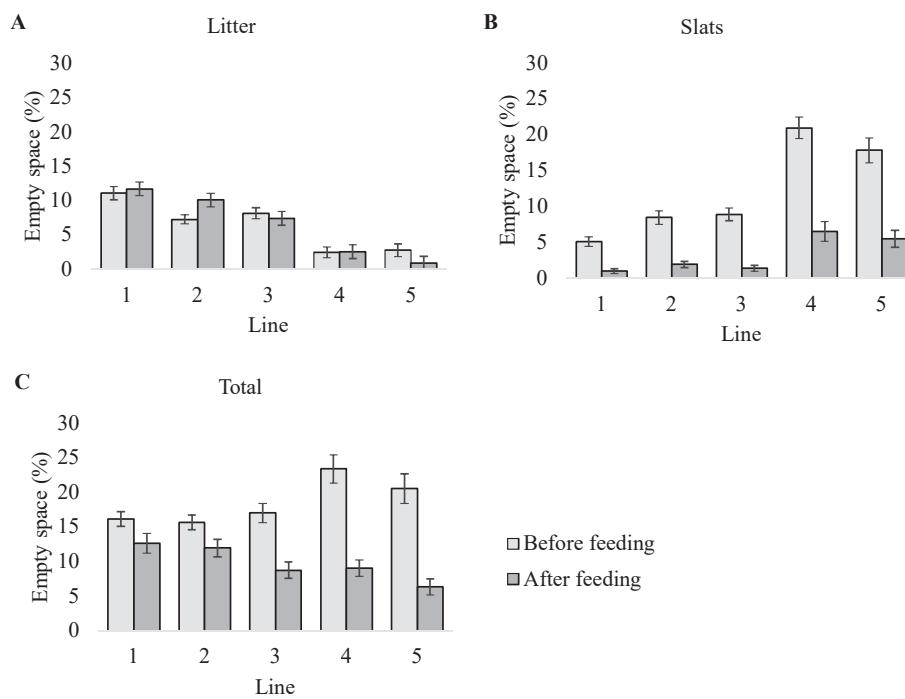
#### Discussion

##### Gregarious nesting behavior and use of space

The genetic lines of broiler breeders in this study differed in the distribution of eggs over the nests, which is most likely reflecting how much gregarious nesting behavior was shown. However, it is possible that the moment of laying could have been spread over time and thereby reducing the relationship between the number of eggs and gregarious nesting behavior. While line 4 had a very uneven distribution and line 5 also had an uneven distribution, the other lines had a very even distribution. The uneven distribution for lines 4 and 5 was caused



**Fig. 2.** The distribution of eggs (%) over the 10 nests provided per pen, specified for each genetic line of broiler breeders.



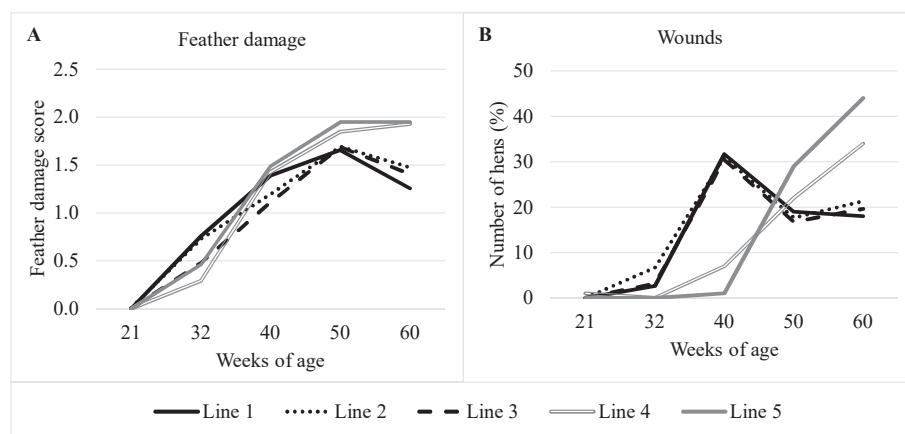
**Fig. 3.** The percentage of empty space in the litter (A), on the slats (B) and for the total (C) area specified per broiler breeder line, comparing before and after feeding.

by a higher use of the nests in the corner of the pen over the nests in the middle. This preference for nests in the corner or at the end of a row has been described previously for laying hens (Riber, 2010; Clausen and Riber, 2012; Ringgenberg et al., 2015). Since the hens in our study were all kept in the same housing and management conditions, the difference in nest distribution can probably be attributed to genetic predisposition for a trait underlying this behavior. Genetic selection against this behavior should be possible, and increasing the evenness of bird distribution over nests by genetic selection is expected to improve both broiler breeder welfare and performance. It should however be noted that possible negative genetic correlations of this selection are unknown and should be investigated in selection experiments.

Our study found a more uneven distribution over the nests at the age of 26 weeks compared to later weeks, independent of genetic line. Riber (2010) also found more gregarious nesting at the start of lay compared to 6 weeks later in laying hens. She suggested that with time, hens will

choose their own preferred nest rather than following the more experienced hens, although this theory has yet to be confirmed. In order to investigate whether lines 4 and 5 were generally more gregarious in their behavior, we studied the use of space in the rest of the pen during the day. During the morning, when egg laying takes place, we did not find differences between the lines in terms of spatial clustering. The gregarious nesting behavior was also not correlated with general spatial clustering, so this may be motivated by something else than preferring to be close to pen mates.

There was however a clear difference in proportional occupation of the litter and slatted areas between the lines. Based on the percentage of areas left empty, lines 4 and 5 used the slatted area less and the litter area more than lines 1, 2 and 3. In the afternoon, the slatted area was used more by all lines, possibly caused by fewer birds in the nest, although there was still a difference between the lines. A possible explanation for this difference in use of space can be found in the



**Fig. 4.** The development of feather damage score (A) and wound prevalence (B) over time per broiler breeder line.

correlation between incidence of wounds and a reduction in use of the litter area at ages of 32 and 40 weeks. Male broiler breeders are known to be more aggressive in their mating behavior compared to layer breeders, which can result in severe wounds on the back and flanks of the female birds (Millman et al., 2000; de Jong and Guémené, 2011). Male broiler breeders tend to spend most of their time in the litter area, only going to the slatted area to drink. It seems likely that the hens would avoid spending time in the litter area where the males are, since this increases the chance of aggressive mating (de Jong and Guémené, 2011). Genetic strains of broiler breeders are known to differ in their mating behavior (McGary et al., 2003), which may have caused the different distributions of the genetic lines in our study. Future research should try to confirm this proposed relation between mating behavior and spatial distribution, so it can be decided whether genetic selection could help improve optimal use of space in broiler breeders.

The results from the avoidance distance tests provide another explanation for a different use of space between lines. Pens, independent of genetic line, with a larger avoidance distance used the litter area less and the slatted area more. The house was set up in a way that the passages used by caretakers or researchers were between the litter areas of pens and the caretakers would enter the pens in the litter area as well. A larger avoidance distance is a sign of more fear of humans, which would explain the avoidance of the litter area where humans pass nearby and enter the pens. However, it could also be the case that an approaching human may elicit a similar response as an approaching male broiler breeder. The fear measured with this test could therefore reflect the fear of aggressive males rather than fear of humans.

#### *Plumage condition and wounds*

Feather damage increased with age, but with differences between the lines. Lines 1, 2 and 3 had more severe feather damage at the early age of 32 weeks, while lines 4 and 5 had more severe feather damage at 60 weeks of age. In chickens, feather coverage is known to be influenced by genetics, feather pecking behavior, feed and metabolism (Leeson and Walsh, 2010; Moyle et al., 2010). However, for broiler breeders, the mating activity also strongly influences the plumage condition. In our study, feather ruffling and loss were combined within one score, which does not allow us to differentiate between the genetic predisposition for feather loss and the ruffling or loss of feathers caused by the mounting of males. Interestingly, lines 1, 2 and 3 improved their plumage condition from 50 to 60 weeks of age, while lines 4 and 5 did not. This could be a sign of genetic differences in capacity of feather regeneration or sexual activity (McGary et al., 2003; Lin et al., 2013). Genetic strains differ in their mating behavior, where some strains stay continuously active in their sexual behavior, while other strains decrease sexual activity from 40 weeks of age onward (McGary et al., 2003). It is unknown whether this was also the case in our study or that the difference in wound incidence at later ages was caused by a difference in feather coverage. When feather coverage declines, the skin of the females will be wounded more easily. This is reflected in the correlation between feather damage and the incidence of wounds at the age of 60 weeks.

#### *Fearfulness*

The avoidance distance was affected by both age and genetic line. This test is used to measure fear of humans, so it is expected that the distance will decrease with age as the birds get used to the presence of humans. Line 2 was most fearful and line 4 least fearful, while the other lines had intermediate levels of fearfulness. It is known that fear of humans is a heritable trait in chickens, which could be the reason behind the differences between the genetic lines at the start of the experiment (Agnvall et al., 2014). As discussed before, fear of humans was related to decreased use of the litter area, but it does not seem to affect the distribution of hens over nests.

The genetic lines did not show any differences in response to the novel object test in terms of latency to approach the object or the maximum number of hens approaching the object. These two read out variables were positively correlated with each other, meaning that pens which would approach the object sooner would also approach with more individuals. Both of these variables are signs of reduced general fearfulness, which was not related to fear of humans in our study. Another study on different lines of laying hens found that fear of humans loaded on a different factor in the principal component analysis than the novel object test results (de Haas et al., 2014). This suggests that fear of novelty has a different origin than fear of humans, although more research is needed on this subject to gain a better understanding. Another explanation could be that a novel object test is not suitable to measure general fearfulness in broiler breeders, as they seem to show very little interest. Earlier research has shown that broilers have less marked responses compared to laying hens (Keer-Keer et al., 1996), which might make it difficult to interpret their behavior.

#### *Production*

The percentage of floor eggs was correlated with two of the studied parameters and not dependent on genetic line. A more uneven nest distribution was positively correlated with a higher percentage of floor eggs. This relation between gregariousness and floor eggs has previously been found in a study on broiler breeders (Perry et al., 1971 as cited by Riber, 2010). This is most likely due to overcrowding of the corner nests, causing hens to lay their eggs on the floor. An increased incidence of wounds was found to be correlated with a decreased percentage of floor eggs. The previously described relation between the incidence of wounds and avoidance of the litter area due to aggressive mating behavior of the males seems to be involved in decreasing the number of floor eggs. A previous study with laying hens also concluded that the presence of roosters decreased the percentage of floor eggs (Rietveld-Piepers et al., 1985). Most floor eggs were laid in the litter area and not in the slatted area, so once the hens are on the slats, the likelihood of laying an egg outside the nest decreases.

No correlation was found between the percentage of broken eggs and gregariousness of nesting behavior, which is contrary to our expectations. When the number of eggs exceeds the capacity of the egg belt, the eggs tend to pile on top of each other and this causes the eggs to break. This has also been reported in a previous study on gregarious nesting behavior of laying hens (Appleby and McRae, 1986). The unevenness of egg distribution in our study was apparently not severe enough to affect egg quality.

#### *Conclusion*

The genetic lines of broiler breeders used in this study differed in the occurrence of gregarious nesting behavior, which was correlated with the percentage of floor eggs. Genetic selection against gregarious nesting behavior could therefore improve bird welfare and performance. The genetic lines also differed in use of space, although this was not related to gregarious nesting or floor laying behavior, but was perhaps caused by differences in mating behavior. Fear of humans at an early age was related to a decreased use of litter space, although fearfulness was not related to the distribution over nests or floor egg percentage. Percentage of wounded hens, possibly due to aggressive mating behavior, was related to a decreased use of litter space and a decreased percentage of floor eggs. Most studies looking into floor eggs in broiler breeders focus on housing and management. These findings suggest that future research should focus on the effect of males on nesting behavior and methods that help to reduce gregarious nesting behavior.

## Supplementary materials

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.animal.2020.100030>.

## Ethics approval

This study is not considered to be an animal experiment under the Law on Animal Experiments, as confirmed by the local Animal Welfare Body (3 June 2018, Wageningen, The Netherlands).

## Data and model availability statement

The data that support the findings of this study are available from Cobb-vantress, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Cobb-vantress.

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## Declaration of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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