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Title: Predicting feather damage in laying hens during the laying period. Is it the past or is it the present?

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Highlights belonging to Is it the past or is it present? Effects of the rearing environment and laying environment on feather damage in commercially housed laying hens.

- Severe feather pecking at five weeks of age was related to high levels of feather damage at 40 weeks of age.
- Feather damage was more severe in floor compared to aviary housing.
- An adjusted management reduced feather damage compared to standard management.
- A large group size was associated with high feather damage.
- Fear of humans at rearing and lay was associated with high feather damage.
Predicting feather damage in laying hens during the laying period. Is it the past or is it the present?

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ABSTRACT

Feather damage due to severe feather pecking (SFP) in laying hens is most severe during the laying period. However, SFP can develop at an early age and is influenced by early rearing conditions. In this study we assessed the risk factors during the rearing and laying period for feather damage at 40 weeks of age, in ISA brown and Dekalb White laying hens. Variables related to housing conditions during the rearing and laying period, and variables related to fearfulness (response to novel object, stationary person, and social isolation) and feather pecking (SFP, feather damage and feather eating) were tested to affect feather damage at 40 weeks of age. Feather damage on the neck, back and belly region was assessed on 50 hens, resulting in a total body score, and averaged per flock (based on Welfare Quality®). First, analysis was conducted by a two-way ANOVA to assess separate factors to influence feather damage at 40 weeks of age. Hereafter, the final GLM for predicting feather damage at 40 weeks of age included only variables which had P<0.1 in the two-way ANOVA. Risk factors during the rearing period were high levels of SFP at five weeks of age and elevated fear of humans (explained variance 29% and 5.3% resp.). Risk factors during the laying period were a large group size (explained variance: 1%), distance to stationary person (explained variance: 16%), floor housing compared to aviary housing (1.27±0.18 vs. 0.75±0.07, explained variance: 21%) and a standard management compared to adjusted management such as a radio, pecking blocks, round drinkers and/or roosters (0.98±0.31 vs. 0.51±0.04, explained variance: 26%). Approximately 49% of the laying flocks and 60% of the rearing flocks in this study showed high SFP or feather damage. This high incidence emphasizes the severity of the problem and the
importance of finding a solution. The results of this study may aid in providing practical solutions to this serious animal welfare problem.

Keywords

Laying hens, Feather pecking, Rearing, Laying, Litter, Genotype

1. Introduction

In laying hens, severe feather pecking (SFP) - the plucking and pecking of feathers of conspecifics that can result in feather damage and mortality (Savory, 1995) - remains a serious welfare problem in commercial enterprises despite the many studies focusing on the prevention of the behaviour (Nicol et al., 2013; Rodenburg et al., 2013). Outbreaks occur mostly during the laying period (Newberry et al., 2007; Bright, 2009) but SFP and consequent damage can develop already at an early age (Blokhuis and van der Haar, 1992; Johnsen et al., 1998; Riedstra and Groothuis, 2002; de Haas et al., 2014). The percentage of laying flocks affected by SFP ranges from 65% (Gilani et al., 2013) and 69% in free range systems (Lambton et al., 2010) and 86% organic systems (Bestman and Wagenaar, 2003). The percentage of rearing flocks affected by SFP ranges from 27% in free range systems (Gilani et al., 2013) to 54% in organic rearing systems (Bestman et al., 2009), whereas the prevalence in other systems is unknown due to the limited number of studies (de Jong et al., 2013). In approximately 90% of the cases when feather damage is observed at rearing, feather damage also occurs during the laying period (Bestman et al., 2009; Gilani et al., 2013).

Feather pecking occurs more frequently when a foraging substrate, i.e. litter, is absent (Huber-Eicher and Wechsler, 1997; Huber-Eicher and Sebo, 2001). As such, litter absence during early life has a strong influence on SFP during rearing (Blokhuis and van der Haar, 1992; Johnsen et
Absence of litter during early life does, however, not always lead to SFP or feather damage during the laying period when litter is adequately available for the adult birds (Nicol et al., 2001b; de Jong et al., 2013). Moreover, the availability of litter during lay strongly affects the occurrence of SFP at laying (Potzsch et al., 2001; Nicol et al., 2003; Lambton et al., 2010), indicating that the need of hens to forage can lead to SFP at any time when this behaviour is thwarted. Litter absence during early life may, however, result in a preference for pecking at feathers rather than pecking at litter. Under suboptimal conditions such as a high light intensity, this environmentally-induced predisposition may contribute to the development of SFP (Kjaer and Vestergaard, 1999; de Jong et al., 2013).

Feather pecking is a multi-factorial problem (Rodenburg et al., 2013). Apart from the influence of litter, increased SFP has been recorded in hens housed in large groups (Zimmerman et al., 2006; Bestman et al., 2009; Lambton et al., 2010), under high stocking densities (Nicol et al., 2006), high light intensities (Mohammed et al., 2010), fed pelleted food (van Krimpen et al., 2009; Lambton et al., 2010), and given immunological challenges (Parmentier et al., 2009). These unfavourable conditions may impose stress in the hens. An elevated sensitivity to stress can derive from high fearfulness (de Haas et al., 2012) which has been associated with SFP (Jones et al., 1995; Rodenburg et al., 2004) and, which makes animals more likely to have difficulty coping with challenges. Additionally, genetic selection for egg production may have caused strain-dependent differences in the ability to cope with challenging (social) conditions. Supporting this are the many studies indicating that commercial lines originating from a White Leghorn (WL) or Rhode Island Red (RIR) genetic background differ in levels of fearfulness, stress sensitivity, and the propensity to develop SFP (Hocking et al., 1999; Hocking et al., 2001;
Uitdehaag et al., 2008; Uitdehaag et al., 2009; Uitdehaag et al., 2011; de Haas et al., 2013; de Haas et al., 2014). We have recently shown that hens originating from a RIR line are more strongly affected by social factors such as a large group size (de Haas et al., 2013) and mixing with other genotypes (Uitdehaag et al., 2009; Uitdehaag et al., 2011) than hens from a WL line.

In this prospective longitudinal study, our aim was to assess which factors during the rearing and laying period affect feather damage at 40 weeks of age, under commercial conditions in non-cage systems in two commercial crosses originating from a WL and RIR background. In The Netherlands, non-organic laying hen farms constitute over 90% of all laying hen production (CBS, 2013a; b). Beak treatment is currently still applied in these systems to reduce damage due to SFP, but will be prohibited in The Netherlands from 2018 onwards. To limit aggravated feather damage and mortality which likely occurs in non-beak trimmed flock which exhibit SFP, it is extremely important to find a practical solution to limit the development of SFP in commercial flocks.

2. Material and Methods

All hens in this study originated from the Ter Heerdt hatchery and rearing company, Zevenaar, The Netherlands (www.broederijterheerdt.nl) and were reared by the same company on contract rearing farms. Chicks’ beaks were infrared treated and chicks were vaccinated at the day of hatching by the hatchery. This study was conducted between August 2010 and August 2012 on farms in The Netherlands and Germany. Approval of the Animal Care and Use Committee of Wageningen University was given for rearing and laying flocks (permit number for rearing flocks: DEC 2010083; permit number for laying flocks: DEC 2010042). For both rearing and laying farms, the codes of practice for maximum stocking densities were applied enabling
sufficient space per hen. For an overview of housing factors of both rearing and laying farms, see Table 1.

2.1. Rearing farms

Thirty-five flocks situated at 19 rearing farms were followed until 40 weeks of age. These flocks were a subset of a sample of 47 flocks, which we studied only during the rearing period (de Haas et al., 2014). Thirteen flocks were of an ISA brown cross, originating from a RIR background, and 22 flocks were of a Dekalb White cross, originating from a WL background. Chicks/pullets stayed on the rearing farm from one day of age until 17 weeks of age. Three flocks were kept in a level system and 32 flocks were kept in an aviary system (Table 1). The aviary system consisted of a tiered system with a litter area between the tiers. The first four to five weeks of life chicks were locked in the tier system. The level system consisted of elevated platforms above a main middle platform and a litter area around the main middle platform, for details see (de Haas et al., 2014). Chicks were housed on cardboard paper until approximately 3.5 weeks of age onwards inside the tiers or on the main platform of the level system. Housing on cardboard paper prevented the chicks falling through the mesh wire floor of the system due to their small body size. Access to the litter area was enabled for all flocks from approximately four to five weeks of age onwards. Some farmers removed the cardboard paper and left chicks inside the system for a certain number of days without providing additional floor substrate, we recorded this practice as “litter disruption”. Litter provided by the farmers was wood shavings, alfalfa or cardboard paper remnants. In case of only supplying paper remnants or small amounts of litter, we recorded this practice as “litter limitation”. All flocks were housed under artificial light. Light intensities were
measured at bird level at one, five and 10 weeks of age with a Voltcraft MS-1300 light meter (Conrad Electric 124 Benelux, Oldenzaal, The Netherlands). We recorded an increase or decrease in light intensity over ages, by comparing minimal light intensities between ages. Commercial feed was provided by one of four Dutch feed suppliers. From one until four weeks of age, chicks received starter1 mashed-diet. From week five till 10, pullets received starter2 semi-mashed diet. From week 10 till 17, a pre-lay diet in the form of mash, pellets and grains was provided. Chicks received multiple vaccinations (aerial, eye-drops and injections) in accordance to a pre-set vaccination schedule advised by the rearing company. Thirteen flocks received additional medical treatments (Table 1). In eight farms there were sound influences from a radio playing or children playing inside the chicken house.

2.2. Laying farms

At 17 weeks of age, pullets were transported to the laying farms. All laying farms were visited around 40 weeks of age. For details on laying factors see Table 1. All rearing flocks were placed at different laying farms, except for three flocks which were placed at the same laying farm. Farms either had an aviary system or a floor housing system. Both systems provided perches, a litter area and nest-boxes, with the aviary system consisted of tiers with in between tiers a litter area. The floor system consisted of a middle platform with litter area around the platform. Most farmers supplied litter when flocks arrived at the farm, although eight did not. Litter quality was assessed qualitatively. Litter was assessed as wet, when an ammonia odour and visible wet areas were detected and dry when litter was in a friable state without a strong ammonia odour or visible wet areas. Flocks were kept in one group or divided into subgroups. Seven farms had an outdoor area either in the form of a Wintergarten or an outdoor range. Multiple flocks received medical treatments. Artificial light was provided for 8-9 hours a day. Light intensity was
assessed as dark when observer visibility was less than 20 m, bright when visibility was more than 20 m. In five farms, birds were exposed to daylight, and in eight farms light cables were placed throughout the system. Feed was provided by one of 15 commercial feed producers of the farmer’s choice. Specific management was applied by certain farmers which consisted of either providing a radio to diminish disturbances by sounds, aerated concrete blocks for pecking, round bell drinkers, roosters in the flock or combinations of the aforementioned.

2.3. On-farm bird measurements

We visited the flocks at week 1, 5, 10, 15 and 40 of age. At each visit we conducted observations related to fearfulness and SFP. All data were recorded by direct observations. One researcher performed the majority of farm visits on the rearing farms, with the exception of three flocks. Another researcher performed the majority of farm visits on the laying farms, with the exception of two flocks. Beforehand, measurements of the same flocks and birds were checked for conformity and similarity between researchers to optimize inter-observer reliability. For tests and measurements conducted on different locations, we analysed the effect of location and order of tests (1st, 2nd etc.) and used the average over tests as no effects of location or order were detected.

2.3.1 Behavioural tests

Behavioural tests related to fearfulness were: a novel object test, a stationary person test, and a novel environment test. The novel environment test was only conducted during rearing.

2.3.1.1. Novel object test

The novel object test (NOT) was conducted at 1, 5, 10 and 40 weeks of age. At one and five weeks of age, the novel object was a wooden box (5*5*2cm) taped with coloured scotch tape
(green, yellow, red and white). At 10 and 40 weeks of age, a plastic PVC tube (50cm) taped with coloured scotch tape of similar colours was used (based on Welfare Quality®, 2009). The novel object was placed on the floor in the housing area, after which the observer moved away to a distance of 3 meters from the object. The NOT was repeated four times on different locations inside the chicken house i.e. at each end of the chicken house, and 1/3 and 2/3 of the end of the chicken house. At each location, birds were exposed for two min. Every 10 sec we recorded the number of birds which were within 25cm of the novel object. Afterwards we determined the time point at which at least three birds were in close proximity of the object which we used as the latency of three birds to approach. At 40 weeks of age we estimated the minimal distance in cm of the bird closest to the novel object by approximation.

2.3.1.2. Stationary person test

A stationary person test (SPT) was conducted at 1, 5, 10 and 40 weeks of age. At one and five weeks, the observer placed her right arm inside the system. At 10 and 40 weeks, the observer stood still inside the litter area of the system (based on Welfare Quality®, 2009). Birds were exposed to the human arm or stationary person for two min. The SPT was repeated four times at four different locations in the chicken house (i.e. at each end of the chicken house, and 1/3 and 2/3 of the end of the chicken house). We estimated the minimal distance of the bird closest to the stationary person in cm.

2.3.1.3. Novel environment test

A novel environment test (NET) was conducted at 1 and 5 weeks of age. The novel environment was an orange round bucket at one week of age (30 cm diameter with 30 cm height) and a white round bucket at five weeks of age (40 cm diameter with 50 cm height). The larger white bucket
was needed to prevent chicks from jumping out, which occurred in the smaller orange bucket.

Chicks were selected from random locations (n=20 at week one, n=15 at week five) and their response was recorded, individually, for one minute in this novel environment. During the NET, the observer was visually out of sight of the test subject. We recorded the latency to vocalize and the number of vocalizations.

2.3.1.4. Feather pecking observations

Severe feather pecking (SFP) behaviour was recorded at 1, 5 and 10 weeks of age. We recorded SFP for 20 min by means of behaviour sampling at two locations in the chicken house. The observation area was approximately 1 m$^2$ and contained all resources (litter, feeding trough, perches). We recorded the total number of pecks of SFP for all birds in the observation area, and took the average over both observations. Severe pecking was defined as forceful pecks and feathers pulls that resulted in a reaction from the recipient (Savory 1995). We used a variable habituation time before initial behaviour scoring. The criterion was that 80% of chicks within the observation area did not direct their attention to the observer. We used this criterion to ensure that the chicks were not distracted by the observer. During observations we also recorded if feather eating occurred (yes/no).

2.3.2. Feather damage

 Feather damage score (FS) was recorded at 5, 10, 15 and 40 weeks of age. During rearing, sample size was 20 birds per flock. During laying, sample size was 50 birds per flock. Birds were selected from random locations in the chicken house. Feather damage was assessed by scoring damage to neck, back and belly region on a three point scale (a=no damage, b=moderate damage, c=severe damage (>5cm), based on Welfare Quality®, 2009). During rearing, cuts in
the wings and tails were included as an indication of early feather damage (ab score). All areas together gave a total feather score (a=0, one ab=0.5, one b=1, one c=2). We calculated the average feather damage per flock, per body area and the proportion of hens with damage per body area and severity of damage (i.e. 0, 0.5, 1 or 2 score). The presence of severe damage in the flock during rearing i.e. hens with wounds was scored as a nominal variable. The presence of severe damage during laying was recorded if more than 10% of the measured hens had severe feather damage (score 2). During laying, it was not always possible to capture hens. When capture was not possible, assessment of feather damage was done by assessing feather damage of individual hens from a small distance (Bright et al., 2006).

2.4. Statistical analysis

Statistical analysis was performed with SAS version 9.2 (SAS 9.2, SAS Institute Inc., Cary, NC, USA), and included analysis using 1) PCA analysis, 2) a two-way ANOVA, 3) final model predicting feather damage at 40 weeks (GLM) 4) a mixed model for binomial data (GLIMMIX), 5) a Chi-Square test ($X^2$) and 6) Pearson correlations ($r$). The dependent variable feather damage at 40 weeks of age per flock conformed to the assumptions of a general linear model (normal distribution of residuals, equality of variance, and linearity) based on a GLM including cross as a class variable.

First, a PCA with an orthogonal varimax rotation was used to test for similarity between variables recorded in the behavioural tests conducted during the rearing period (see Table 2). This was done in order to remove redundancy in the data used for later analysis. Variables were retained if their eigenvalue was more than 1. As loadings of variables rely partly on the sample size (Hair et al., 1998) and our sample size was relatively small (n=35) we used a stringent cut-
off point of 0.5 to retain variables assigned to the factors. Results of the PCA were used to
generate factor scores related to fearfulness for use as a response variable in the two-way
ANOVA as described in the following.

Second, we tested the effect of each independent variable (both during rearing and laying) and its
interaction with cross on dependent variable feather damage at 40 weeks of age by a two-way
ANOVA. Independent effects were only retained in the final GLM if after inclusion of effects
they remained to have an alpha level of less than 0.1 (P < 0.1), if they were equally balanced, and
if there were no interactions with other factors (Table 3).

Third, we build the final GLM for predicting feather damage. Cross was included as it was part
of the experimental set-up. The fixed factors of the final GLM were: cross (DW/ISA), laying hen
housing system (floor/aviary) and specific management during laying (yes/no). Covariates in the
final model were PCA-factor 2 (see Table 2), SFP at five weeks of age, and minimal distance to
stationary person at 40 weeks of age and group size at laying. P-value of less than 0.05 was used
as indicating a significant effect. The variance explained by the model was interpreted by the R²
of the model, while the respective variation explained by each factor was calculated by dividing
the factor’s SUM of SQUARES by the sum of all factors’ SUM of SQUARES.

Fourth, proportions which did not fulfil the assumptions of GLM were analysed with a
GLIMMIX to test effect of cross, age and their interaction, using a binary distribution with logit
link function and a contrast statement to assess specific comparisons.
Fifth, binominal variables of severe damage at laying were related to binominal variables of severe feather damage and feather eating at rearing comparing each age (5, 10 and 15 weeks of age) separately by means of a Chi-square test. For correlations between fear test over age, Pearson correlations within cross were calculated.

3. Results

3.1 Prevalence of feather pecking at rearing and feather damage at laying

See Table 4 for percentage of flocks with severe feather damage during rearing and the laying period. During the laying period, 49% of the flocks visited had signs of severe feather damage, i.e. more than 10% of the sampled hens had moderate or severe feather damage. During rearing, between 37% and 66% of the flocks had severe damage (i.e. recordings of wounds). Average feather damage score per flock was at five weeks: 0.31 ± 0.04, ten weeks: 0.22 ± 0.03, fifteen weeks: 0.16 ± 0.02 and 40 weeks 0.86 ± 0.07. At five weeks of age, 36% of the flocks showed high SFP (more than 10 pecks/20min), 24% showed moderate SFP (between 4-10 pecks/20min), 27% showed low SFP (2 pecks or less/20min) and 12% showed no SFP.

During the laying period, more hens had feather damage to the neck and fewer hens had damage to the back compared to the rearing period (Fig. 1A). During the laying period, more hens had severe feather damage compared to the rearing period (see Fig 1B). The percentage of flocks with back damage was higher at five weeks of age compared to 15 weeks and tended to be higher at 10 compared to and 40 weeks of age (GLIMMIX: back5: 71%, back10: 17%, back15: 71%, back40: 29%, $X^2_1 = 2.8$, $P = 0.09$; back5 vs. back15 $X^2_1 = 4.32$, $P < 0.05$; back5 vs. back40 $X^2_1 = 3.2$, $P < 0.07$).
Flocks with severe damage in the laying period also had a higher occurrence of feather damage at five weeks of age compared to flocks with no damage at laying ($X^2_2 = 7.8$, $P < 0.05$, Table 4), whereas severe feather damage at 10 and 15 weeks were not associated with having severe damage at laying (10 weeks: $X^2_1 = 0.01$, NS, 15 weeks $X^2_2 = 0.45$, NS, Table 4). In flocks with severe damage at 40 weeks of age, feather eating during rearing tended to occur more often, especially at 15 weeks of age in comparison to flocks with no severe damage at 40 weeks of age ($X^2_2 = 4.8$, $P = 0.09$; Table 4).

The crosses did not differ in average feather damage at 40 weeks of age (GLM: $F_{1, 21} = 0.78$, $P = 0.39$, DW: $0.88 \pm 0.07$ vs. ISA $0.80 \pm 0.16$). The proportion of hens with damage to the back was higher for ISA than for DW flocks at 40 weeks of age, while at 10 weeks of age the proportion of hens with damage to the back was higher in DW flocks vs. ISA flocks (GLIMMIX: cross * age: $P<0.01$: 40 weeks: 0.22 vs. 0.10; 5 weeks: 0.16 vs. 0.40 ). For DW flocks average damage to the back was not related to average feather damage ($r_{back} = 0.26$, $P = 0.24$) while it was for ISA flocks ($r_{back} = 0.82$, $P = 0.0005$).

### 3.2 Principal Component Analysis

The Principal Component Analysis of the variables from the Novel Object test (NOT), Stationary Person test (SPT) and Novel Environment test (NET) performed during the rearing period resulted in four factors (see Table 2 for variance explained by each factor and contribution of variables to each factor). For factor 1 the highest loading was for the number of hens that approached the novel object and to lesser extent vocalizations in the NET, hence this factor was
named “approaching novel object”. Factor 2 consisted mainly of distance to stationary person (SP); hence this was the corresponding name for factor 2. Factor 3 consisted of latency to approach in the NOT and latency to vocalize in the NET, and as both may reflect fear responses to a novel stimulus or environment, thus this factor was named “fear of novelty”. Factor 4 consisted of vocalizations in the NET, and was named similarly (see Table 2). Only factor 2 (distance to SP) affected feather damage at laying and was therefore retained in the analysis.

3.3 Risk factors for feather pecking

The factors which affected feather damage at laying are displayed in Table 3. Factors at rearing that were associated with feather damage at 40 weeks were: sound influences on the rearing farm, SFP at five weeks of age, feather damage and feather eating at 15 weeks of age and PCA-2 distance to SP (ANOVA). Factors at laying associated with feather damage were: litter quality, outdoor access, light intensities, specific management, housing system, group size, medical treatments and minimal distance to stationary person (ANOVA). The final model, which predicted feather damage at laying included cross, SFP at five weeks of age, PCA-2, specific management, system at laying, group size at laying, and minimal distance to stationary person at 40 weeks of age (GLM). The model explained 91% of the total variance ($R^2$). Of that variance, little was explained by cross (0.7%), 34.3 % was explained by factors at rearing and 64 % was explained by factors at laying.

3.3.1. Risk factors during the rearing period

High SFP at five weeks of age resulted in more feather damage at 40 weeks of age (Fig 2, $F_{1,21}=4.62, P = 0.05$; variance explained: 29%). A high score for distance to SP tended to be
related to increased feather damage at 40 weeks of age for both crosses (Fig 3, $F_{1,21} = 3.31$, $P = 0.06; \text{variance explained: 5.3%}$).

3.3.2. Risk factors during the laying period

Average feather damage was higher when flocks were housed in a floor system compared to an aviary system (floor system vs. aviary system: 1.27 ± 0.18 vs. 0.75 ± 0.07; GLM: $F_{1,21} = 19.3$, $P < 0.001; \text{variance explained: 21%}$). Flocks in which specific management was applied had lower average feather damage than flocks in which no specific management was applied (specific management vs. standard management: 0.51 ± 0.04 vs. 0.98 ± 0.32; GLM: $F_{1,21} = 11.8$, $P < 0.001; \text{variance explained: 26%}$). A smaller group size tended to associate with lower average levels of feather damage ($\beta = -0.0004$: $F_{1,21} = 3.73$, $P = 0.07; \text{variance explained: 1%}$). A larger distance from the SP at laying was associated with more feather damage for both crosses ($\beta + 0.002$: $F_{1,21} = 5.76$, $P = 0.03; \text{variance explained: 16%}$), similar to response to SP during rearing.

3.3.3. Cross effects

None of the DW flocks approached the SP within 25cm at 40 weeks of age, while 60% of the ISA flocks did not approach the SP within 25cm ($X^2_2 = 14.9$, $P < 0.001$). At 40 weeks of age, DW hens also kept a greater distance to the novel object than ISA hens (96 ± 13 cm vs. 39 ± 8 cm: $F_{1,22} = 22.14$, $P < 0.01$) and to the stationary person (264 ± 41 cm vs. 38 ± 9 cm: $F_{1,22} = 41.4$, $P < 0.01$). Similarly, in the fear tests during rearing, distance from SP was higher for DW hens than for ISA hens (PCA-2: $F_{1,22} = 15.72$, $P < 0.001; 0.56 \pm 0.25 \text{ vs. -0.73 \pm 0.17}$ while
fewer hens approached the novel object in DW flocks compared to ISA flocks (PCA-1: $F_{1, 22} = 6.85, P = 0.02; -0.42 \pm 0.23$ vs. $0.55 \pm 0.30$). For both crosses, number of hens approaching the novel object during rearing (PCA-1) was related to latency to approach novel object at 40 weeks of age ($r = 0.54, P < 0.01$). Distance to SP during rearing (PCA-2) was related to distance to SP at laying ($r = 0.45, P = 0.03$).

**Discussion**

Is it the past or is it present? In this study we associated factors and behavioural recordings on the rearing farm and laying farm with feather damage at 40 weeks of age in laying hens housed in non-cage systems in the Netherlands.

**Rearing factors**

Factors related to severe feather pecking (SFP), feather damage and feather eating at rearing were associated with high levels of feather damage at the laying period. This is in agreement with other studies which show that SFP and feather damage during rearing can increase the risk of feather damage at laying (Nicol et al., 2001a; Bestman et al., 2009; Lambton et al., 2010; Gilani et al., 2013). Feather eating has been repeatedly associated with SFP (Harlander-Mataushek et al., 2006; Harlander-Matauschek et al., 2007; Harlander-Matauschek and Häusler, 2009) and may indicate the presence of SFP. In our study of 47 rearing flocks, we found that a disruption and limitation in litter during the first four weeks of age increased SFP at five weeks of age and feather damage during rearing (de Haas et al., 2014). As high levels of SFP at five weeks of age related to high levels of feather damage at 40 weeks of age, this relation could be indirectly affected by litter availability during early life. This suggestion is supported by other studies which show that a lack of foraging substrate at an early age yields a risk for the
development of feather damage during the production phase (De Jong et al., 2013; Nicol et al., 2001a; Bestman et al., 2009; Lambton et al., 2010; Gilani et al., 2013).

Variables related to high fearfulness (mainly birds keeping a large distance from a stationary person) in the rearing period were associated with high levels of feather damage in the laying period. High fearfulness at a young age, measured in a novel environment/social isolation test, has been associated with an elevated propensity to develop SFP at adult age (Jones et al., 1995; Rodenburg et al., 2004). Here, we see similar results at flock level, as PCA-factor 2 consisted, partly, of the response to a novel environment/social isolation. However, PCA-factor 2 loaded more strongly for distance to a stationary person. These results indicate that high fearfulness due to fear of humans, both at a young and adult age, can pose a risk for SFP. Fear of humans can have negative impact on production (Hemsworth and Barnett, 1989), while additional exposure to humans can reduce fear of humans through habituation (Barnett et al., 1994) and may reduce high fearfulness and SFP.

**Laying factors**

The housing system, group size and management on the laying farm affected feather damage. During rearing we found similar factors related to feather damage during this period (de Haas et al., 2014). Floor housing resulted in higher levels of feather damage in the hens compared to aviary housing. This has also been reported for organic laying farms (Bestman and Wagenaar, 2003). Aviary housing provides more possibilities for hens to escape from feather pecking birds (i.e. more levels and elevated perches), and local density is lower as hens’ living space is spread over multiple levels compared to only a floor level. As a large group size also tended to increase feather damage levels, the space per bird appears to have a large influence on feather damage on
a flock level. In other studies, a large group size also increased SFP (Bilcik & Keeling, 1999; Zimmerman et al., 2006; Bestman et al., 2009; Lambton et al., 2010). In a larger group, feather pecking birds can peck more victims. Additionally, transmission of SFP may have occurred because of birds attraction to ruffled plumage or damaged feathers and denuded area’s or wounds (McAdie & Keeling, 2002). Consequently this may lead to more birds being involved in SFP and creating more birds with feather damage. In a large flock, there might be a higher risk for SFP to occur and to aggravate.

In flocks where farmers applied an adjusted management in the form of supplying aerated blocks for pecking, having a radio playing, round drinkers provided or roosters in the flock, hens had lower levels of feather damage compared to flocks without such management. A radio playing may reduce fear as shown in a study indicating a fear-reducing effect of intermittent exposure to recorded sounds (Campo et al., 2005). By reducing fear these measures might have had a positive effect on SFP. For example, exposure to varied sound levels have been associated with reduced feather damage (Gilani et al., 2013). Other measures may reduce feather damage, such as roosters in a flock (Bestman and Wagenaar, 2003). Round drinkers on the other hand are often associated with higher feather damage (Green et al., 2000; Zimmerman et al., 2006) probably due to reduction of litter quality due to spilling of water as suggested by Gilani et al. (2013). In our case, round drinkers were supplied additionally to nipple drinkers, which likely reduces competition for water resources and thereby we would expect less spilling. The success of an adjusted management regime in reducing feather damage can indicate that these farmers are actively working to prevent SFP. The implementations of these various measures appear to either target feather pecking and or fearfulness. Recently a custom-made management package consisting of varying curative measures to reduce SFP was tested on problem flocks and control
flocks in a free-range system (Lambton et al., 2013). Although not specified which factors were implemented, use of a large number of different management measures led to a decrease in SFP and feather damage in problem flocks. We argue, based on our findings and those of Lambton and co-workers (Lambton et al., 2013) that management practices which facilitate foraging behaviour (such as aerated concrete blocks) and reduce fearfulness (radio playing) may further lessen the risk of SFP.

Genetic differences

In this study we compared hens of a Dekalb White (DW) and ISA Brown (ISA) cross. Feather damage in ISA hens was seen on all body regions, while for DW hens feather damage was mainly seen on the neck and belly region. Feather damage on the neck and belly region is most likely caused by aggressive pecking and vent pecking, respectively, while feather damage to the back region is most likely caused by SFP at the base of the tail (Bilick & Keeling, 1999). This result suggests that ISA hens show more forms of injurious pecking behaviour while DW hens mainly show aggressive and vent pecking. This suggestion is supported by our study of parent stock hens, where DW hens had more damage to the belly region than ISA hens (de Haas et al., 2013). We also found that the DW hens were generally more fearful than the ISA hens for a novel object and the stationary person. The founder lines of DW (i.e. White Leghorn) have shown to have higher fear-levels than hens from a Rhode Island Red background (founder lines of ISA) when tests measured responses to humans (Uitdehaag et al., 2006; Uitdehaag et al., 2008; de Haas et al., 2013; de Haas et al., 2014). A positive human animal relationship (Hemsworth et al., 1993) from an early age onwards, combined with a predictable and controllable environment may reduce stress (Koolhaas et al., 2011), which might be extra important for hens of a White leghorn background to prevent high fearfulness and SFP.
Prevalence of feather pecking during rearing and laying

Feather damage during rearing was limited to damage to the back, while during the laying period feather damage was mainly to the neck (i.e. severe damage to the neck occurred in 86% of the laying flocks). Feather damage to the neck can be attributed to SFP but also to molting, aggressive pecking and abrasion against the housing system. Under natural conditions, prior to onset of lay, hens temporarily cease eating and molt can occur (Berry, 2003). Feed-intake generally increases rapidly prior to peak of lay (Hurwitz et al., 1975). During the period of increased feed-intake, aggressive pecking can occur during feeding. Feather damage to the neck is associated with aggressive pecking (Savory, 1995). Aggressive pecking caused by frustration has been observed when feed is not readily available (i.e. when hens needed to use an operant feeder in their home cage Lindberg and Nicol, 1994). Damage to the neck feathers can also be caused by abrasion (Bilcik and Keeling, 1999) against feeders and perches mounted above feeders, especially during molt where feathers easily let go. These factors together may explain the high incidence of feather damage to the neck area at laying. Feather damage at the back region both during rearing and laying most likely indicates SFP (Bilcik and Keeling, 1999), and can be used by farmers as a criterion for SFP and requests adjustments of management.

The prevalence of feather damage during the laying period was 49% at flock level. In other studies prevalence was substantially higher: 57% (Green et al., 2000), 65% (Gilani et al., 2013), 71% (Bestman and Wagenaar, 2003), 69-86% (Lambton et al., 2010), which can be due to lack of beak treatment, age at recording, threshold level used, genetic crosses and housing systems (Nicol et al., 2013). The prevalence of SFP during the rearing period was 60% in the present study, which resembles that of several other studies: 47% (Lambton et al., 2010) and 54%
(Bestman et al., 2009). These numbers indicate that at least 49% of flocks are affected by this animal welfare problem, and thus highlights the importance of finding a solution.

**Conclusion**

In this study we aimed to find risk factors for feather damage during the laying period in laying hens housed in non-cage systems. Fears of humans and SFP during rearing are risk factors for feather damage at laying. Floor housing, a large group size and fear of humans during laying increased levels of feather damage, with ISA flocks having more hens with feather damage to the back compared to DW flocks. During laying, an adjusted management reduced feather damage. An adjusted management can be applied as a preventive measure for feather damage, especially in large flocks housed in floor systems. The overall risk of SFP can thus be reduced when management is optimized in terms of providing appropriate foraging substrates and strategies aimed at reducing fearfulness both during the rearing and laying period.

**Acknowledgements**

We acknowledge Hatchery Ter Heerdt BV (Babberich, the Netherlands) and its farmers, farmer advisors, managers and sales managers for their cooperation in this study. We thank the MSc and BSc students of Wageningen University for their help during study: Linda Persoon, Lourdes Icalla and Tomas Stevens. This study was supported by the Division of Earth and Life Sciences with financial aid from the Netherlands Organization for Scientific Research and the Ministry of Economic Affairs, Agriculture and Innovation within the program “The Value of Animal Welfare.” Andrew M. Janczak was supported by funding from the Foundation for Research Levy on Agricultural Products (FFL), the Agricultural Agreement Research Fund (JA), and Animalia.


Table 1. Overview of factors during the rearing period and laying period with corresponding sample size (n)

<table>
<thead>
<tr>
<th>Rearing factors</th>
<th>n</th>
<th>Laying factors</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Housing system</td>
<td></td>
<td>Housing system</td>
<td></td>
</tr>
<tr>
<td>Aviary system</td>
<td>32</td>
<td>Aviary system</td>
<td>28</td>
</tr>
<tr>
<td>Level system</td>
<td>3</td>
<td>Ground system</td>
<td>7</td>
</tr>
<tr>
<td>Litter type</td>
<td></td>
<td>Litter supplied</td>
<td></td>
</tr>
<tr>
<td>Wood shavings or alfalfa</td>
<td>32</td>
<td>Yes</td>
<td>27</td>
</tr>
<tr>
<td>Paper remnants</td>
<td>3</td>
<td>None</td>
<td>8</td>
</tr>
<tr>
<td>Disruption in litter supply</td>
<td></td>
<td>Litter quality</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23</td>
<td>Dry</td>
<td>23</td>
</tr>
<tr>
<td>No</td>
<td>12</td>
<td>Wet</td>
<td>12</td>
</tr>
<tr>
<td>Limitation in litter supply</td>
<td></td>
<td>Medical Treatments</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>Medical treatments given</td>
<td>18</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>No medical treatments given</td>
<td>17</td>
</tr>
<tr>
<td>Light intensity changes</td>
<td></td>
<td>Subgroup</td>
<td></td>
</tr>
<tr>
<td>Adjustments from 1 to 5 weeks</td>
<td>24</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>No adjustments from 1 to 5 weeks</td>
<td>11</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Adjustments from 5 to 10 weeks</td>
<td>27</td>
<td>Yes</td>
<td>7</td>
</tr>
<tr>
<td>No adjustments from 5 to 10 weeks</td>
<td>7</td>
<td>No</td>
<td>28</td>
</tr>
<tr>
<td>Missing data</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical Treatments</td>
<td></td>
<td>Outdoor or wintergarden</td>
<td></td>
</tr>
<tr>
<td>Medical treatment given</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No medical treatments given</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sound influences</td>
<td></td>
<td>Light intensity</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>Dark</td>
<td>8</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>Bright</td>
<td>14</td>
</tr>
<tr>
<td>Specific management</td>
<td></td>
<td>Extra light</td>
<td>13</td>
</tr>
</tbody>
</table>

Footnote:  
- Housing system: tiers with in between tiers litter area, level system: elevated platforms with litter area around main platform;  
- Aviary system: tiers and litter area between tiers, ground system with main elevated platform and litter area around the platform;  
- Litter type: provided;  
- Litter supplied or no litter supplied;  
- Disruption in litter supply indicates litter supply disruption during first 4 wks of life;  
- Limitation in litter supply indicates only paper or small amount of wood shavings;  
- Light intensity measurements were compared between ages to assess an adjustment;  
- Medical Treatments consisted of extra feed-supplements, vitamins, vaccinations or medications;  
- Radio playing or excessive noise from children playing inside the chicken house;  
- Medical treatment given or no medical treatment given;  
- Light intensity levels: dark: visibility less than 20 m or bright visibility more than 20 m;  
- Specific management: either a radio playing, aerated beton blocks, round drinkers, or roosters and a combination of the aforementioned.
Table 2. Factor loadings of a Principal Component Analysis with orthogonal varimax rotation, with interpretation of factors and variance explained by each factor of variables of the behavioural tests measured at one, five and ten weeks of age

Factors of the Principal component analysis

<table>
<thead>
<tr>
<th>Nr of factors</th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
<th>Factor 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naming of factors&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Approaching novel object</td>
<td>Distance to stationary person</td>
<td>Fear of novelty</td>
<td>Vocalizations in social isolation/novel environment</td>
</tr>
<tr>
<td>Variance explained per factor</td>
<td>38%</td>
<td>24%</td>
<td>16%</td>
<td>11%</td>
</tr>
</tbody>
</table>

**Behavioural test**

<table>
<thead>
<tr>
<th>Variable measured</th>
<th>Age at testing (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Novel object test</strong></td>
<td></td>
</tr>
<tr>
<td>Latency to approach</td>
<td>1 -0.86 -0.02 -0.02 -0.16</td>
</tr>
<tr>
<td>Latency to approach</td>
<td>5 -0.62 0.36 0.35 0.29</td>
</tr>
<tr>
<td>Latency to approach</td>
<td>10 -0.24 -0.02 0.78 0.04</td>
</tr>
<tr>
<td>Average number of hens that approached</td>
<td>1 0.91 -0.10 -0.05 -0.09</td>
</tr>
<tr>
<td>Average number of hens that approached</td>
<td>5 0.75 0.06 -0.44 -0.14</td>
</tr>
<tr>
<td>Average number of hens that approached</td>
<td>10 0.19 0.20 -0.80 -0.09</td>
</tr>
<tr>
<td><strong>Stationary person test</strong></td>
<td></td>
</tr>
<tr>
<td>Minimal distance of hens that approached</td>
<td>1 0.27 0.08 -0.29 -0.74</td>
</tr>
<tr>
<td>Minimal distance of hens that approached</td>
<td>5 -0.02 0.89 -0.08 0.06</td>
</tr>
<tr>
<td>Minimal distance of hens that approached</td>
<td>10 -0.15 0.90 0.13 -0.02</td>
</tr>
<tr>
<td><strong>Novel environment test</strong></td>
<td></td>
</tr>
<tr>
<td>Number of vocalizations</td>
<td>1 0.24 0.06 -0.10 0.84</td>
</tr>
<tr>
<td>Latency to vocalize</td>
<td>1 0.15 0.13 0.68 0.01</td>
</tr>
<tr>
<td>Number of vocalizations</td>
<td>5 0.73 0.42 0.03 0.21</td>
</tr>
<tr>
<td>Latency to vocalize</td>
<td>5 -0.46 -0.69 0.18 0.11</td>
</tr>
</tbody>
</table>

Footnote: Factor refers to respective factors resulting from the PCA-analysis, <sup>1</sup> Name given to each factor was based on the highest positive loadings (> 0.5) of the variables on these factors; for factor 1: average number of hens approaching a novel object; factor 2: minimal distance to stationary person, factor 3: both latency to approach novel object and latency to vocalize in the novel environment/social isolation test; factor 4: vocalizations in the novel environment/social isolation test.
Table 3. Overview of rearing and laying factors which showed a main effect or an interaction with cross.

<table>
<thead>
<tr>
<th></th>
<th>REARING PERIOD</th>
<th>Main effect</th>
<th>Interaction with cross</th>
</tr>
</thead>
<tbody>
<tr>
<td>Management</td>
<td></td>
<td>F  P  F</td>
<td></td>
</tr>
<tr>
<td>Sound influences (radio or children playing)</td>
<td>15.35 0.005 11.99 0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe feather pecking (SFP)</td>
<td>5.34 0.03 3.32 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average feather damage score/flock (FS)</td>
<td>4.10 0.05 4.21 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feather eating (yes/no)</td>
<td>3.96 0.02 2.11 0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCA factor based on behavioural tests of fearfulness (Table 2)</td>
<td>13.04 0.001 9.62 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>LAYING PERIOD</th>
<th>Main effect</th>
<th>Interaction with cross</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter conditions</td>
<td></td>
<td>F  P  F</td>
<td></td>
</tr>
<tr>
<td>Litter quality</td>
<td>6.77 0.003 9.52 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoor</td>
<td>0.05 0.83 9.45 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light intensity</td>
<td>3.68 0.01 2.51 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific management (radio, earated blocks, round drinkers, roosters)</td>
<td>11.76 0.00 1.50 0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housing system</td>
<td></td>
<td>F  P  F</td>
<td></td>
</tr>
<tr>
<td>Floor/ aviary</td>
<td>4.30 0.05 1.18 0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Groupsize</td>
<td>10.31 0.00 0.01 0.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical treatments</td>
<td></td>
<td>F  P  F</td>
<td></td>
</tr>
<tr>
<td>Yes/no</td>
<td>9.40 0.001 5.80 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fearfulness at lay</td>
<td></td>
<td>F  P  F</td>
<td></td>
</tr>
<tr>
<td>Minimal distance to stationary person</td>
<td>4.44 0.05 4.58 0.04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4 Percentage of severe feather damage at laying (40 weeks of age) in relation to severe feather damage and feather eating at rearing (5, 10 and 15 weeks of age)

<table>
<thead>
<tr>
<th></th>
<th>Severe Feather Damage</th>
<th>Feather Eating</th>
</tr>
</thead>
<tbody>
<tr>
<td>4FWFSF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUMBZJOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>/PTFWFSF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Table 4 Percentage of severe feather damage at laying (40 weeks of age) in relation to severe feather damage and feather eating at rearing (5, 10 and 15 weeks of age).
Figure 1a. Proportion of sampled hens per flock with feather damage to neck, back and belly at five, ten, fifteen and forty weeks of age

Figure 1b. Proportion of sampled hens per flock with no, little, moderate or severe feather damage at five, ten, fifteen and forty weeks of age

Figure 2. Correlation between severe feather pecking behaviour measured at five weeks of age with average level of feather damage per flock at five, ten, fifteen and forty weeks of age

Figure 3. Correlation between the principal component (PCA) factor 2 “distance to humans at rearing” and average level of feather damage at forty weeks of age expressed by genetic-cross
Figure 1

A

Proportion of hens/flock with feather damage

Age in weeks

B

- damage to neck
- damage to back
- damage to belly

- no damage
- little damage (0.5 score)
- moderate damage (1 score)
- severe damage (2 score)
Figure 2.

Average level of feather damage (0=min, 2-max)

Age in weeks

No
Low
Moderate
High
Figure 3.

Average level of feather damage at forty weeks of age
(min=0, max=2)

PCA factor 2 "distance to humans"