

## Stress and feather pecking in laying hens in relation to housing conditions

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**Abstract** 1. Possible association between high rates of feather pecking and increased stress were investigated in laying hens.  
2. From week 19 to week 30 after hatching, 16 groups of 11 hens (white Lohman Selected Leghorn hybrids) were kept in pens with or without long-cut straw as foraging material and provided with food in the form of pellets or mash.  
3. Stress was assessed by egg production, weight gain, tonic immobility (TI), heterophil/lymphocyte (H/L) ratio and antibody titres to sheep red blood cells (SRBC), tetanus toxoid (TT) and human serum albumin (HSA).  
4. Provision of foraging material and food form influenced feather pecking. Rates of feather pecking were highest in groups housed without straw and fed on pellets.  
5. Egg production was reduced in pens without straw but not affected by food form. Both the duration of TI and H/L ratios were influenced by provision of foraging material and food form. TI was longer and H/L ratios were increased in hens housed without straw and in those fed on pellets. Antibody titres to SRBC and TT were lower in pens without straw than with straw but not influenced by food form.  
6. In conclusion, foraging material and food form affected both feather pecking and indicators of stress, suggesting that feather pecking in laying hens is associated with stress.

### INTRODUCTION

Feather pecking in laying hens occurs both in conventional battery cages and in alternative housing systems (Appleby and Hughes, 1991). It causes animal welfare problems, as it may lead to injuries and even the death of birds (Hughes and Duncan, 1972; Allen and Perry, 1975, Huber-Eicher and Wechsler, 1997, 1998). Moreover, feather pecking may result in economic losses because of increased food consumption in defeathered birds (Lesson and Morrison, 1978; Tullett *et al.*, 1980) and increased mortality. It is therefore important to identify factors which are of relevance for the development of this abnormal behaviour. The present study investigated whether laying hens showing high rates of feather pecking are also characterised by increased stress.

In his review, Maxwell (1993) concluded that heterophil/lymphocyte (H/L) ratio is a reliable indicator of avian stress. For example, Jones (1989) reported significant increases in H/L ratios in Brown Leghorn pullets exposed to fasting or frustration when feeding and Jones *et al.* (1988) observed significant increases in H/L ratios in laying hens after implantation of minipumps delivering corticosterone. In the latter study, corticosterone infusion also led to a significant increase in the duration of tonic immobility (TI), a measure of fear (Jones, 1987), which was found to be related to feather

pecking (Blokhuys and Beuving, 1993; Vestergaard *et al.*, 1993; Jones *et al.*, 1995). As an additional measure of stress the humoral immune response to various antigens was measured in the present study. Thaxton and Siegel (1972) reported lower antibody titres to sheep red blood cells in young chickens after repeated exposure to short periods of heat stress.

In order to induce differences in feather pecking between groups of laying hens, housing conditions were varied with regard to provision of foraging material (long-cut straw) and food form (mash or pellets). Several studies have shown that feather pecking in laying hens is reduced if the birds are provided with incentives that elicit foraging behaviour (Hughes and Duncan, 1972; Blokhuys, 1986; Nørgaard-Nielsen *et al.*, 1993; Huber-Eicher and Wechsler, 1997, 1998). With respect to food form, Bearse *et al.* (1949) and Walser (1997) reported a greater tendency for feather pecking in birds fed on pellets compared to those fed on mash. Assuming that housing conditions resulting in high rates of feather pecking also lead to an increase in stress it was expected that hens housed without access to straw and hens fed on pellets would show increased H/L ratios, a reduced antibody response after immunisation, a longer duration of TI, a reduced number of attempts necessary to induce a TI

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response and lower values for weight gain and egg production.

## METHODS

### Animals and housing

A total of 176 white laying hens (Lohman Selected Leghorn hybrids) were used. They were reared by a commercial breeder and not beak-trimmed. On arrival at 18 weeks of age, they were randomly assigned to groups of 11 individuals and distributed among 16 pens of identical size (265×90 cm, height 235 cm; 4.6 ens/m<sup>2</sup>) built side by side along a corridor.

Provision of foraging material and food form were varied between pens. In 8 pens, a floor area of 100×90 cm at the front of the pens was made of slats (width 1 cm, 2.5 cm apart), while the rest of the floor was covered with long-cut straw providing foraging material. In the other 8 pens, the whole floor area was of slats. In 8 pens the hens were fed on mash and in 8 on pellets. Foraging material and food form were varied as independent factors (2×2 factorial design), resulting in 4 housing conditions (mash/straw, mash/no straw, pellets/straw and pellets/no straw). The row of 16 pens was subdivided into 4 blocks, and the 4 housing conditions were assigned at random to the 4 pens of each block. For more detail see Aerni *et al.* (2000).

### Procedures

All hens were marked individually with coloured leg rings. The feeding troughs were refilled manually every 2nd day. In order to keep the layer of long-cut straw dry and attractive to the hens, it was replaced or added to whenever necessary. The pens were entered to collect eggs.

To avoid unnecessary pain, all injuries caused by feather pecking were treated with tar. This effectively prevented other hens from pecking at the wounds. Open wounds were found in 13 animals. No animal died during the experiment. However, 2 hens were removed (week 20 and 25) because of persistent aggression from pen mates and toe-pecking, respectively. The experiment was subject to the authorisation procedure prescribed by Swiss Animal Welfare Legislation (application No. 91/96).

### Feather pecking

Methods of data collection on feather pecking are described in Aerni *et al.* (2000).

### Egg production and weight gain

Eggs were collected daily; total egg production in weeks 19 to 25 was calculated for each pen as a percentage of the maximum number of eggs that would have been produced if every hen had laid 1 egg per d. Body weight was recorded individually when the hens were 19 and 27 weeks old.

### Heterophil/lymphocyte (H/L) ratios

At 26 weeks of age, one drop of blood was taken from a small puncture in the comb of each hen for determination of the H/L ratio. Blood samples from all hens were taken between 13.00 and 17.00 h on 2 consecutive days (2 blocks of pens per d, selected at random). The blood was smeared on to a glass slide using a cover glass technique (Campbell, 1988). The smears were stained using a Diff-Quik staining kit (Dade AG, Switzerland). One hundred leucocytes, including granular (heterophils, eosinophils, basophils) and nongranular (lymphocytes, monocytes), were counted once on each slide using a light microscope and 1,000× magnification. The H/L ratios were determined by dividing the number of heterophils by that of lymphocytes.

### Tonic immobility (TI)

At 27 weeks of age, the TI reactions of each of 96 hens (6 birds per pen) were quantified. Data collection was carried out on 4 consecutive days between 09.00 and 16.00 h (4 pens per d representing the 4 housing conditions, 1 in each block). TI was induced by placing the bird on its back with the head hanging in a U-shaped wooden cradle (Jones and Faure, 1981) and restraining it by holding 1 hand on its sternum for 45 s. When the experimenter removed her hand, a stopwatch was started. The experimenter then retreated 1 m, moving out of sight of the hen. The behaviour of the bird was recorded by a video camera and observed on a television screen. The following variables were recorded: the number of attempts necessary (45 s periods of restraint) to obtain TI lasting at least 10 s, and the duration of TI, that is, the latency until self righting. If a hen did not show a righting response within 15 min after induction of TI, a maximum score of 900 s was given.

### Immunisation

At 28 weeks of age, blood samples (1 ml) were obtained from the right wing veins of all hens to determine baseline antibody concentrations before immunisation. The procedure was carried out on 4 consecutive days (4 pens per d representing the 4 housing conditions, 1 in each block). At 29 weeks of age, an antigen cocktail (0.25 ml/bird, containing 30 mg human serum albumin (HSA) and 8 IU tetanus toxoid (TT) was injected intramuscularly into the left breast muscle. Concomitantly, 0.1 ml of 20% (V/V) of sheep red blood cells (SRBC in phosphate buffer saline (PBS)) was injected into the right breast muscle. Immunisation was performed on 4 consecutive days in the same sequence as used for preimmunisation bleeding.

### Antibody titres

Blood samples were collected from the left wing veins at 5, 8, 11 and 14 d post-immunisation. To

minimise handling of individual birds, animals from only 1 of 4 equally treated pens were bled after each of the above post-immunisation intervals. Again, on a given day of bleeding, the groups representing the 4 housing conditions were derived from separate blocks. Blood samples were centrifuged (1500 rpm for 5 min), sera were collected, stored frozen and all assays were run simultaneously. Antibody titres to TT and HSA antigens were determined using enzyme-linked immunosorbent assay (ELISA), while antibody titers to SRBC were quantified using indirect hemagglutination assay.

For determination of antibody titres to TT, ELISA plates (immunoplates, CS-Starwell, 441 653, Nunk, Roskilde, Denmark) were coated with 100 µl per well of TT (20 LF antigen from Berna, Swiss Serum and Vaccine Institute, Bern, Switzerland) overnight at room temperature, using a humid chamber. The antigen was diluted 1:1000 in sodium carbonate-bicarbonate buffer (pH 9.6). Plates were washed 3 times with washing solution containing 0.9% sodium chloride and 0.25% Tween-20 using an ELISA washer. One hundred µl of a 1/20 dilution of each serum sample (pre-immunisation or post-immunisation sample) in ELISA buffer containing 0.25 M sodium chloride, 20 mM Tris pH 7.5 and 0.125% Tween-20 were added to TT-coated and negative control wells. After washing as above, 100 µl of 2000-fold diluted goat anti-chicken IgG (Fc-portion-specific) conjugated with horse radish peroxidase (Bethyl, Montgomery, TX) was added to the wells. Following a further 1 h incubation at room temperature and washing, 100 µl of phosphatase substrate (*p*-nitrophenyl phosphate) was added to each well. After 10 to 15 min, 50 µl of 3 M H<sub>2</sub>SO<sub>4</sub> was added to terminate the reactions. The optical densities of the wells were read at 490 nm using an ELISA reader and converted into arbitrary units, using a calibration curve run on the same plate. For the latter, a high-titred reference chicken serum was used throughout. The ELISA for determining

HSA-specific antibodies was performed likewise with the following modifications: The plates were coated with HSA (10 µg/ml). The sera were tested at a 50 fold (pre-immunisation samples) or at a 500 fold dilution (post-immunisation samples).

For determination of SRBC antibodies 10 µl of SRBC (10% V/V in PBS) was added to wells of sterile 96-well round bottomed microtiter plates (Dynatech, Embrach, Switzerland) containing 10 µl of 2-fold diluted (PBS, 10 mM phosphate pH 7.4) serum sample. The plates were shaken for 1 min, incubated for 2 h at 37°C and incubated for 20 min at 4°C. SRBC were washed twice with PBS, followed by resuspension in 100 µl of PBS. Then, 50 µl was transferred to a new plate. 100 µl 300-fold diluted rabbit anti-chicken IgY (heavy and light chain-specific, Jackson ImmunoResearch Laboratories, West Grove, PA) was added to these wells. The plates were incubated at 37°C for 2 h. Agglutination titres were expressed as the natural logarithms of the reciprocal of the highest dilution showing 50% agglutination.

### Statistical analysis

The pens were treated as independent units ( $n=16$ ) in all analyses. Mean values for the behavioural, immunological and production variables were calculated for each pen. The analyses were performed using Systat and Microsoft Excel. All statistical tests are 2-tailed with an alpha level of 0.05.

A 2-way analysis of variance (ANOVA) with foraging material and food form as main factors was used. Data on weight gain were subjected to square root transformation to achieve a normal distribution of the residuals. However, untransformed data are given in the Table. Post-immunisation titres for each individual bird were adjusted by subtracting the respective pre-immunisation titre, and antibody titres were converted to appropriate natural logarithms. Because

**Table.** Effects of foraging material and food form on rates of feather pecking interactions (per 11 hens per 60 min), weight gain (g), duration of tonic immobility (TI), number of attempts necessary to induce a TI response and heterophil/lymphocyte (H/L) ratios. Means as well as minimum and maximum values (in parentheses) of 4 pens each per housing condition are given. *P* values derived from ANOVA

	Housing conditions				<i>P</i> values		
	Pellets/straw	Mash/straw	Pellets/no straw	Mash/no straw	Foraging material	Food form	Interaction
Feather pecking <sup>1</sup>	5.5	3.4	80.0	11.9	<0.0001	<0.0001	<0.0001
Weight gain	(3.1, 10.6) 197.9 (153, 272)	(2.5, 4.0) 204.4 (173, 236)	(65.5, 97.2) 194.2 (143, 260)	(6.6, 24.6) 194.7 (143, 268)	NS	NS	NS
TI duration	2.3 (1.2, 3.7)	2.0 (1.6, 2.3)	6.8 (4.8, 7.8)	4.3 (3.7, 5.4)	<0.0001	<0.02	<0.04
TI induction	1.3 (1.0, 1.5)	1.3 (1.0, 1.5)	1.2 (1.2, 1.3)	1.2 (1.2, 1.3)	NS	NS	NS
H/L ratio	1.8 (1.7, 1.9)	1.6 (1.5, 1.7)	2.9 (2.9, 3.1)	2.6 (2.5, 2.7)	<0.0001	<0.001	NS

<sup>1</sup>Data from Aerni *et al.* (2000)

blood samples of only 1 group per housing condition were collected on a given day post-immunisation, the length of the post-immunisation interval could not be included as a factor in the analysis of variance for the antibody titres.

## RESULTS

Provision of foraging material and food form both had significant effects on rate of feather pecking (Table, Aerni *et al.*, 2000), which was highest in hens housed without straw and fed on pellets.

Weight gain was not significantly influenced by the housing condition factors (Table), whereas hens provided with long-cut straw laid significantly more eggs ( $F_{(1,12)}=8.55$ ,  $P=0.01$ ; Figure 1). Food form had no statistically significant effect on egg production ( $F_{(1,12)}=0.00$ ,  $P=0.98$ ), and there was no significant interaction between foraging material and food form ( $F_{(1,12)}=0.13$ ,  $P=0.72$ ).

Duration of TI was significantly higher in hens housed without straw than with it and in those fed on pellets compared to those fed on mash (Table). There was also a significant interaction between foraging material and food form. On the other hand, the number of attempts necessary to obtain a TI reaction was not significantly influenced by these 2 factors (Table).

H/L ratios were significantly higher in groups housed without straw than with it and in hens fed on pellets than in those fed on mash (Table). The interaction between the 2 factors was not statistically significant.

Before immunisation, no antibody titres to SRBC were detected by indirect hemagglutination. After immunisation, antibody titres were significantly higher in hens housed with straw than without it ( $F_{(1,12)}=11.93$ ,  $P<0.005$ ; Figure 2a). The effect of the food form ( $F_{(1,12)}=0.10$ ,  $P=0.76$ ) and the foraging material by food form interaction ( $F_{(1,12)}=0.15$ ,

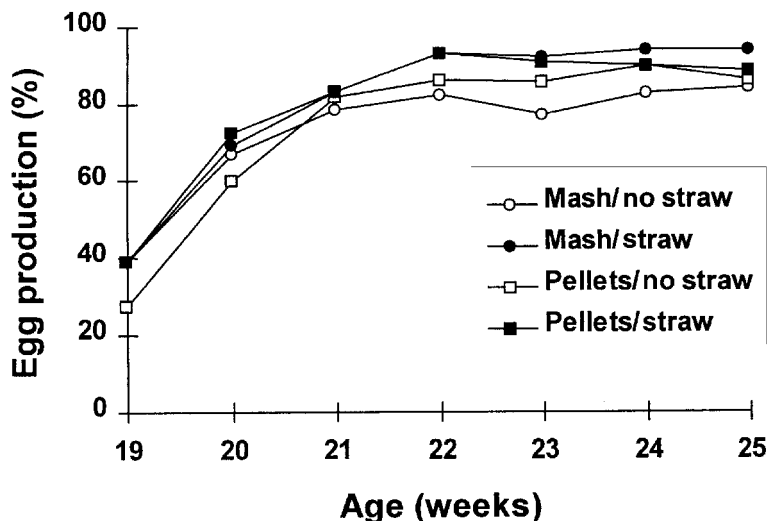
$P=0.71$ ) were not statistically significant. In all housing conditions, the peak antibody response was recorded at or before day 5 post-immunisation.

Tetanus toxoid was a recall antigen (an antigen eliciting a secondary rather than a primary immune response) because most hens showed titres prior to immunisation. These were not influenced by the factors varied between the pens (foraging material:  $F_{(1,12)}=0.12$ ,  $P=0.74$ , food form:  $F_{(1,12)}=0.33$ ,  $P=0.57$ ; interaction:  $F_{(1,12)}=0.00$ ,  $P=0.96$ ). After immunisation, the increase in antibody titres to TT antigen was larger in hens housed with straw than without it ( $F_{(1,12)}=29.51$ ,  $P<0.001$ ; Figure 2b), whereas the effects of food form ( $F_{(1,12)}=0.55$ ,  $P=0.47$ ) and the interaction between the 2 factors ( $F_{(1,12)}=0.23$ ,  $P=0.64$ ) were not statistically significant. Maximal increases were observed on day 8 or 11 post-immunisation.

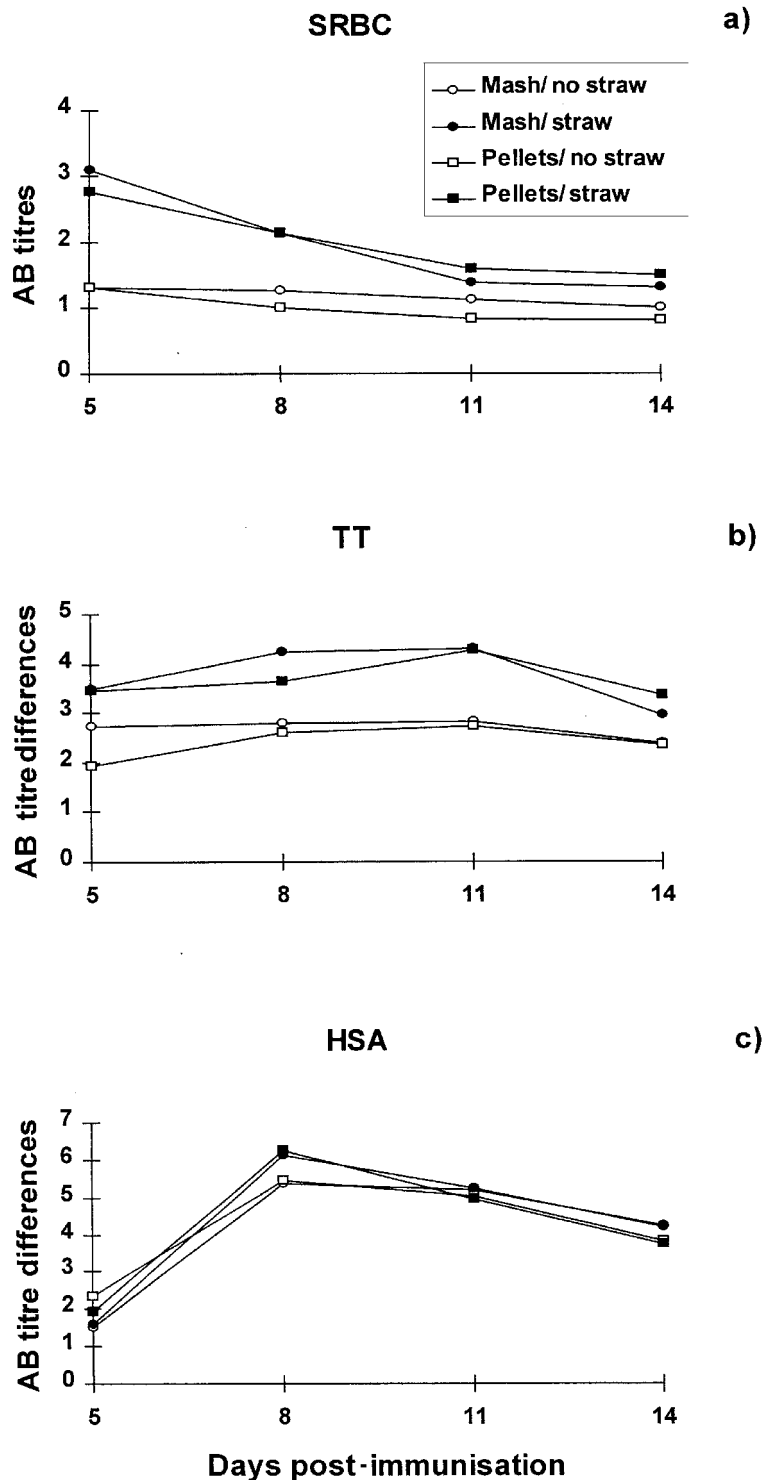
Antibodies to HSA were also detected before immunisation. However, these titres were low and not significantly affected by housing conditions (foraging material:  $F_{(1,12)}=0.23$ ,  $P=0.64$ , food form:  $F_{(1,12)}=1.07$ ,  $P=0.32$ ; interaction:  $F_{(1,12)}=0.00$ ,  $P=0.94$ ). Following immunisation, there was a strong increase in the anti-HSA titres (Figure 2c). Neither foraging material ( $F_{(1,12)}=0.02$ ,  $P=0.89$ ) nor food form ( $F_{(1,12)}=0.00$ ,  $P=1.00$ ) had a significant effect on the increase in titre to HSA, and there was also no significant interaction between these 2 factors ( $F_{(1,12)}=0.01$ ,  $P=0.92$ ). Titre differences were maximal on day 8 post-immunisation.

## DISCUSSION

In the present study, several variables believed to be related to stress were significantly affected by the 2 factors varied in the housing conditions: hens kept without straw and hens fed on pellets showed prolonged TI duration and higher H/L ratios when compared to hens with access to straw and hens fed on mash; the humoral immune response to SRBC



**Figure 1.** Egg production (percentage of the maximum number of eggs that would have been produced if every hen had laid 1 egg per d) in weeks 19 to 25. Average values of 4 groups each of hens housed in 4 different housing condition are presented.



**Figure 2.** Kinetics of humoral immune response in hens housed under 4 different conditions. Mean antibody (AB) titres to (a) sheep red blood cells (SRBC), (b) tetanus toxoid (TT) and (c) human serum albumin (HSA) are presented on a log scale to the base  $e$ . On a given day, blood samples of 11 hens were taken per housing condition.

and TT was impaired when no straw was provided and; egg production in weeks 19 to 25 was significantly reduced in pens without straw. The differences observed in the effects of the housing conditions on the different measures of stress may indicate that different aspects of stress were reflected by different variables.

In accordance with Jones *et al.* (1988), hens characterised by high H/L ratios also showed longer duration of TI. In their study, H/L ratios were

elevated because of corticosterone infusion. The duration of TI therefore seems to be a sensitive behavioural indicator of stress in laying hens. On the other hand, we found no statistically significant effects of the housing conditions on the number of attempts necessary to induce TI.

To relate stress variables to the humoral immune response, 3 antigens were administered at the same time and in the absence of an adjuvant. No antibodies to SRBC were observed prior to

immunisation, whereas low antibody titres to HSA and TT were already present. The latter 2 antigens have therefore to be considered as recall antigens, inducing a secondary immune response. The significantly reduced titres to SRBC and TT found in hens kept without access to straw are consistent with the hypothesis that housing conditions characterised by increased stress induce an immunosuppression. An influence of stress on an antigen-specific immune response has also been found in other studies (Thaxton and Siegel, 1970, 1972; Thaxton, 1978). In contrast, antibody titres to HSA were not influenced by the housing conditions. It should, however, be noted that the immune response to HSA in our study was very strong. The high degree of immunogenicity of this antigen may have masked the influence of stress and it may be more informative to use weaker antigens to investigate the effect of stress on antibody responses. The possibility that only primary immune responses are influenced is remote because the antibody response to TT was influenced by housing condition and TT antibody titres were already observed prior to immunisation, possibly due to exposure to cross-reactive environmental antigens.

The anti-SRBC titres in this study tended to be lower than titres reported in other papers (Siegel and Gross, 1980; Davis and Glick, 1988; Munns and Lamont, 1991). Some of these differences may be due to the genetic background of the birds or the chosen route of administration. The antigens in this study were given im and not iv (Siegel and Gross, 1980; Davis and Glick, 1988; Munns and Lamont, 1991). Van der Zijpp *et al.* (1986) showed lower titres to SRBC with the im than the iv route of administration. Antibody response to SRBC reached a peak at or before day 5 post-immunisation and then declined rapidly. The decrease was more rapid than would be expected, based on the half-life of IgG antibodies in mammals and humans. To our knowledge, no reliable figures concerning the half-life of IgG antibodies in chickens are available.

Egg production was slightly but significantly reduced in hens housed without access to straw, whereas weight gain did not differ between the housing conditions. The latter variable is possibly less influenced by stress in adult hens than in growing chicks. Freeman and Manning (1979) found a significantly decreased growth rate in chicks that were regularly handled (caught and moved to a new place for a short time) over a 3 week period. With adult laying hens, Hughes and Black (1976) observed a reduced egg production if unaccustomed birds were handled.

The housing conditions not only had significant effects on measures of stress but also on the rates of feather pecking. As expected, feather pecking was more pronounced in hens without access to straw as foraging material and fed on pellets. The effect of foraging material and food form on feather pecking was best characterised by the significant

interaction between these 2 factors (Aerni *et al.*, 2000), whereas stress measures were most consistently affected by the provision of foraging material. However, H/L ratios and duration of TI were also significantly affected by food form and with the latter there was a significant interaction between the 2 factors.

Hens housed without straw and fed on mash did not show high rates of feather pecking although they were characterised by increased stress. As a consequence, stress was not a reaction to feather pecking in the present study but should be regarded as a factor that may enhance the development of this abnormal behaviour. The stress measures were significantly influenced by the housing conditions in our study and indicated more stress in hens housed without straw and fed on pellets than in hens housed without straw and fed on mash. As only the former developed high rates of feather pecking, the hens' tendency to show pronounced feather pecking may be linked to a certain stress threshold.

In conclusion, the results of our study show that provision of foraging material and food form have significant effects on both feather pecking and indicators of stress, suggesting that feather pecking in laying hens is associated with stress. Given our results, it would be interesting to investigate systematically whether strain-specific differences in feather pecking (Cuthbertson, 1980; Quart and Adams, 1982; Craig and Lee, 1990; Kjaer and Sørensen, 1997; Walser, 1997) are paralleled by differences in the stress response (Blokhuis and Beuving, 1993; Jones *et al.*, 1995). This may help in finding a solution to reduce the problems caused by feather pecking in laying hens, especially in alternative housing systems.

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