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The phenotypic interrelationships between feather pecking, being feather pecked and fear criteria in White Leghorn lines selected for high and low severe feather pecking and their F2- crosses

Phänotypische Beziehungen zwischen Federpicken, bepickt werden und Furcht in Weißen Leghorn Linien, die auf hohes und niedriges Federpicken selektiert wurden und deren F2-Kreuzungen

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Introduction

It is generally assumed that high feather pecking (HFP) birds are more fearful than low feather pecking (LFP) birds. This assumption is mainly based on the co-existence of feather pecking and fear on the basis of group means. Housing in large groups or other environmental factors have been reported to produce fearful flocks and high levels of feather pecking or feather damages (Choudary et al., 1972; Hughes and Black, 1974; Sefton, 1976; Rott, 1978; Lee and Craig, 1991; Blokhuis and Beutler, 1992; Vestergaard et al., 1993). The relationship between feather pecking and fear has also been shown in genetic studies. Buitenhuis et al. (2005) found QTL's for fear criteria and feather pecking on the same chromosome (GGA1). Other studies, however, showed a rather complex relationship between feather pecking and fear, depending on the lines used, the age of observation and the type of fear test. Jones et al. (1995) reported that lines selected for high or low feather pecking differed in their fear reaction. Birds which showed lower fear in young age pecked more when they reached the adult age (Rodenburg et al., 2004). The authors considered fear reaction in the young birds as predictor of feather pecking behaviour in adults. Albentosa et al. (2003) found significantly higher fear in White Leghorn lines selected for high and low feather pecking than in three other layer strains (ISA Brown, Columbian Blacktail and Ixworth). Similar results have been reported by Uitdehaag et al. (2008, 2009, 2011). Rhode Island hens were less fearful than White Leghorn Hens. Fear usually declines with age. The latency to leave an emerge box (ET) decreased in two lines (Lohmann Tradition and ISA Brown) from 10 to 35 weeks of age (Ghareeb et al., 2008). Reduced fear with age was also found in OF and novel object tests in four different layer strains by Albentosa et al. (2003).

Rodenburg et al. (2010) found no significant difference in fear as measured by TI, human approach test and novel object test in White Leghorn hens of an earlier generation of the high and low feather pecking lines used in the present experiment. Hocking et al. (2001) observed a decrease in fear with age in Tetra but not in ISA Brown. The latency to vocalize and to walk in an open- field test increased from 1 to 30 weeks of age in two commercial lines (ISA Brown and Tetra). There was no consistent age effect with regard to the duration of tonic immobility.

The relationship between fear and damaging pecking resulting in cannibalism has also been studied in a line selected for low mortality by Rodenburg et al. (2009), Nordquist et al. (2011) and De Haas et al. (2012). This line is considered similar to the lines showing low feather pecking behaviour. The low mortality line showed higher activity in the open- field indicating a lower level of fear. However, Bolhuis et al. (2009) found no differences between the low mortality and the control line in a sudden human approach test. There was no difference in fear as measured by an approach test (pencil test) in two genetic lines and their crosses which differed in feather pecking and feather scores, and the correlation between feather conditions and fear was close to zero (Bessei et al., 1984a, b).

The aim of the present study was to investigate the phenotypic interrelationships between feather pecking, being pecked and fear criteria in White Leghorn hens selected for high and low severe feather pecking and their F2- crosses.

Materials and methods

The present study consists of two parts. In the first part feather pecking and fear- related criteria were studied in two lines of White Leghorns which have been divergently selected for high (HFP) and low severe feather pecking (LFP) for 9 generations. In the second part the same criteria were observed in F2-crosses of the above mentioned lines.

Selection lines

The selection started in the Danish Institute of Animal Sciences, Foulum, Denmark, for the first 5 generations as described by KJAER et al. (2001) and then for 4 more generations at the Institute of Animal Husbandry and Breeding, University of Hohenheim, Stuttgart-Hohenheim, Germany. The chicks of both lines, HFP and LFP, were vaccinated against Marek's disease at day-old and neck-banded. They were raised in separate deep litter pens under conventional lighting and feeding programmes. At 8 weeks of age the male chicks were separated from the female chicks and were raised separately. At 26 weeks of age a total of 224 hens, 114 HFP and 110 LFP, were randomly assigned to five floor pens of 16 m^2. The pens were separated by a wire mash grid. Group size varied between 44 and 45 birds with HFP and LFP at equal parts. All pens were equipped with hand filled round feeders (50 cm diameter), nipple drinkers (15–16 nipples), nests (1.20 m^2 nest space, lined with Astroturf mats), perches (23 cm/bird) and a litter area (1/3 of the total space). A conventional layer mash containing 16.6% crude protein and 11.4 ME/kg was provided ad libitum. The litter area was covered with wood-shavings (10 cm depth). The litter was dry and friable throughout the experiment. Access to the litter area was closed during the time of observation. Two third of the pen area were covered by a perforated plastic floor. The slats were raised at a height of 38 cm from the litter area. Artificial light (14 hrs) was provided through incandescent bulbs from 3 am to 5 pm. There was additional natural light through transparent plastic material at the upper part of the side wall of the pen. Light intensity provided by the electric bulbs was 20 lux. Depending on the fluctuation of the natural light, light intensity increased occasionally up to 2500 lux on sunny days. Climatic conditions were controlled by a thermostatically regulated ventilation system. All hens were individually identified by numbered plastic tags on their back. Each pen was visually observed for feather pecking behaviour by five experienced observers in sessions of 20 minutes. After each session the observers changed the pens in a rotational system so that all pens were observed by all observers at the same day from 1 pm to 3 pm. This procedure was repeated on two consecutive days. Bouts of vigorous pecks or pulls delivered and received were recorded. The breeding values for bouts of vigorous feather pecks were estimated using PEST (GROENEVELD, 1990) and VCE4 (GROENEVELD, 1998). Thirty birds each with the highest breeding values (breeding values between 4.54 and 20.85) for the number of feather pecking bouts delivered of the HFP and the lowest breeding values (breeding values between – 3.625 and – 3.715) for the number of feather pecking bouts delivered of the LFP were selected for the fear tests. At 33 weeks of age the selected hens were transferred to a windowless poultry house with individual cages measuring 46x46 cm. Fear tests started after an adaptation period of 5 days.

F2-crosses

The design of the F2-crosses has been described in detail by BENNEWITZ et al. (2014), in print). Briefly, an equal number of dams and sires of the HFP and LFP lines of 10th selection generation were reciprocally mated in order to produce 10 F1-families. One male of each family was mated to hens of the other families. A total of 967 female F2 birds were produced in 4 successive hatches (280, 255, 250 and 182 birds per hatch). Due to mortality and losses of identification tags the number of birds used for statistical analyses decreased slightly with age. The chicks were sexed, vaccinated against Marek's disease and neck-banded at day-old. The female chicks were randomly assigned to pens (1.20 m × 0.90 m) of 10 birds each. At 3 weeks of age all birds of the same hatch were transferred in larger deep litter pens (2.6 m × 2.6 m) where they were kept up to 18 weeks of age. The group size varied between 40 and 60 birds. At 18 weeks of age all birds of the same hatch were transferred to one large pen (5.90 m × 10.20 m) to measure their locomotor activity (data not shown in this study). The chicks were reared under commercial feeding and light programmes starting with 24 hours of light for 2 days. Day length was then reduced to 8 hours at 7 weeks of age and increased successively to 14 hours from 17 to 21 weeks of age. At 26 weeks of age the hens were transferred to the same deep litter pens as used in part one of the experiment for the observation of feather pecking behaviour. The same procedure was used as mentioned above. After the observations the birds were housed in furnished cages (1.20 m × 0.75 m) with 8 birds per cage.

Fear tests

Fear tests in the selection lines and in the F2- crosses were carried out using the same protocol. While the selection lines were tested as adults only, the F2- crosses were tested as chicks (7–9 days) and at 40 weeks of age. The tests were carried out in a separate room of the same building. The temperature of the test room was adapted to the temperature of the home pen.

Tonic immobility (TI). The TI responses of every bird were measured using a modified method described by JONES et al. (1982). Each bird was gently carried to a separate room next to their home pen. Tonic immobility was induced by restraining the bird on its back in specially constructed V-shaped wooden cradles. The cradles were adapted to the size of the birds. Duration of TI was recorded when the bird remained immobile for a maximum of 180 s. The numbers of inductions were recorded as well. A maximum of three attempts were made.

Open field test (OF). This test was modified from a method described in JONES et al. (1982).The open- field was a box measuring 1 m × 1 m × 1 m (length × width × height) with 4 wooden walls and a wooden floor. The chicks were gently taken of the home pen, carried to the test room and placed in the centre of the open field. The observer sat at the side of the box with a good view of the chick. The latencies to walk, to vocalize and the numbers of steps were recorded over a period of 180 s. The open field was cleaned after each test.

Emerge Box test (ET). The emerge box for the chicks was of plywood and measured 23 cm × 23 cm × 20 cm (length × width × height) as described in JONES et al. (1982). There was a lid on the top and a guillotine trapdoor covering a 10 cm^2 hole in one of the walls. The emerge box was adapted to the hens' size at 40 weeks of age measuring 32 cm × 32 cm × 35 cm (length × width × height) and the same procedure was applied. The boxes were situated in the larger box which was used for testing the open field behaviour. Each chick was gently taken from its home pen and placed in the emerge box. The box was closed and after resting in the dark for 60 s the trapdoor was opened and the latencies for the appearance of the head and the whole bird were recorded. The test was finished when the whole bird had left the box. If the bird had not put its head through the door or emerged fully within 3 minutes after raising the door, maximum latencies of 180 s were recorded for both characteristics.

Pencil test. A pencil with a length of 15 cm was moved towards the head of the bird when they were housed individually. Each hen was tested once at 34 weeks of age (selection lines) and twice at 29 and 30 weeks of age (F2-crosses). The reaction of the hens were recorded in scores from 1–5 (method described in BESSEI, 1980 as modified from HUGHES and DUNCAN, 1972).

The definition of the scores was as follows:

1- pecking at the pencil

2- no obvious response

3- moving to the left or right side

4- moving to the backside

5- trying to escape

The mean scores of both measurements in the F2- crosses were used for statistical analyses.

Statistical analysis

The effect of line (HFP, LFP) in part one of the study was tested by Wilcoxon Test. The correlations between the criteria were calculated within lines using Spearman's rank correlations (SAS, 2007: JMP 7 Statistics and Graphics Guide, SAS Institute Inc., Cary, NC, USA). In part two of the experiment the birds were split in high and low feather pecking subgroups (HFP, LFP) on the basis of the number of bouts of vigorous feather pecks delivered (≥ 2 HFP; < 2 LFP). Data were analyzed using Generalized Linear Mixed Model (SAS 9.3, 2013 TS Level 1 M 2, SAS Institute Inc.) in a 2-factorial design with line, age and the interaction line × age as fixed factors. Differences between means were tested for significance by linear contrasts. The correlations between the criteria were calculated within lines by Spearman's rank correlations.

The University of Hohenheim Committee on Animal Care and Use approved these experiments with the numbers S266/10, S335/12 and S338/12.

Results

Selection lines

The means, SD and p-values for feather pecking and fear-related criteria of the HFP and LFP groups of the selection lines are shown in Table 1. There were highly significant differences between HFP and LFP birds for feather pecks delivered (FPD) and received (FPR). HFP birds showed significantly higher means for feather pecks delivered and lower means for feather pecks received than LFP birds. There were also significant effects of the line in most fear-related criteria. The duration of TI was significantly shorter for HFP than for LFP birds. There was no significant difference in the number of inductions between the lines. The latency of the first step and to vocalize in the OF test was significantly shorter in HFP than in LFP. The HFP exhibited more than twice as much steps in the open- field than the LFP birds. The head and whole body emerge in the ET were also significant. HFP showed shorter latencies than LFP birds. There were no differences in the pencil test between the lines (HFP 3.38 ± 0.30 and LFP 3.19 ± 0.36) (data not shown in Table). Spearman's Rank correlation coefficients of the selection lines are shown in Table 3. The correlations between the fear criteria were generally very low and not significant. There was a tendency of a negative correlation between feather pecks delivered and the latency of the first step in the open field (-0.252) in the HFP and a positive correlation in the same criteria in the LFP (0.191). Feather pecks received showed the tendency of a positive correlation (0.129) with the duration of tonic immobility in the HFP and a negative correlation in the LFP (-0.267). Similar inverse relationships in both lines were found for feather pecks received and the pencil test scores. The correlation was negative in the HFP (-0.243) and positive in the LFP (0.265).

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Table 1. Means, SD and p-values of lines selected for high (HFP) and low (LFP) feather pecking for feather pecks delivered (FPD) and received (FPR) over a 20 min period and the criteria of different fear tests, Tonic immobility (TI), Open- field (OF), Emerge Box (ET) at 34 weeks of age. Means within the same column with different letters are significantly different ($p < 0.05$)

Mittelwerte, Standardabweichungen und p-Werte für Pickschläge ausgeteilt (FPD) und erhalten (FPR) über eine 20 min Periode von Linien, die auf hohes (HFP) und niedriges (LFP) Federpicken selektiert wurden und die Merkmale der verschiedenen Furchttests, Tonische Immobilität (TI), Open- field (OF), Emerge Box (ET) im Alter von 34 Wochen. Mittelwerte mit unterschiedlichen Buchstaben innerhalb derselben Spalte unterscheiden sich signifikant voneinander ($p < 0.05$)

	Feather pecking		TI		OF			ET	
Age Line	FPD	FPR	Duration of TI	No. of inductions	Latency 1st step	No. of steps	Latency vocalization	Latency head	Latency body
HFP	4.69[a] ± 4.44	0.81[a] ± 0.80	73.57[a] ± 72.24	2.03 ± 0.77	21.14[b] ± 35.76	29.62[a] ± 18.01	57.93[b] ± 74.63	34.87[b] ± 59.50	53.47[b] ± 70.04
LFP	0.27[b] ± 0.87	1.65[b] ± 0.89	116.01[b] ± 73.85	1.87 ± 0.73	65.35[a] ± 65.29	13.25[b] ± 11.79	85.07[a] ± 71.35	71.51[a] ± 71.68	120.32[a] ± 71.61
Line	<.001	<.001	0.032	0.391	<.001	<.001	0.023	0.024	0.002

Table 3. Spearman's rank correlation coefficients between feather pecks delivered (FPD) and received (FPR) and the main fear criteria of fear tests, Tonic immobility (TI), Open- field (OF), Emerge Box (ET) and pencil test of lines selected for high (HFP; above the diagonal) and low (LFP; below the diagonal) feather pecking at 34 weeks of age (selection lines).

Spearman's Rangkorrelationskoeffizienten zwischen ausgeteilten (FPD) und erhaltenen (FPR) Pickschlägen und den Hauptmerkmalen der Furchttests, Tonische Immobilität (TO), Open- field (OF), Emerge Box (ET) und dem Bleistifttests bei Linien, die auf hohes (HFP; über die Diagonalen) und niedriges (LFP; unter der Diagonalen) Federpicken selektiert wurden im Alter von 34 Wochen (Selektions Linien).

	FPD	FPR	Duration TI	Latency 1st step	Latency head	Pencil test score
FPD	–	0.020	−0.046	−0.252	0.037	0.027
FPR	−0.083	–	0.129	−0.015	−0.207	−0.243
Duration TI	−0.068	−0.267	–	0.002	−0.159	−0.129
Latency 1st step	0.191	−0.055	0.247	–	0.241	−0.001
Latency head	0.153	0.012	0.175	0.346	–	0.01
Pencil test score	−0.032	0.265	0.191	−0.275	0.125	–

F2-crosses

The means, SD and p- values of FPD, FPR and the fear responses of the F2- birds split in HFP and LFP groups are shown in Table 2. The line effect was significant for FPD, the duration of TI, latency of the first step and the number of steps in the OF, and latency of head emerge in the ET. HFP showed significantly higher means for FPD but not for FPR. LFP birds showed a longer duration of TI and a shorter latency of the first step in the OF than the HFP birds. There was however no significant difference between the lines for the latency to vocalize. The number of steps in the OF was significantly higher for LFP than for HFP birds. The latency of head emerge in the ET was significantly longer in the LFP as compared to the HFP birds, but the latency of body emergence was similar in both lines. There was no significant difference between lines in the pencil test scores (HFP 3.18 ± 0.75 and LFP 3.27 ± 0.67) (data not shown in table). There was a significant age effect for all fear criteria. The duration of TI was shorter at 40 weeks of age and the number of inductions higher than at 7 days of age. The latency of the first step in the OF was shorter and the number of steps lower at 40 weeks as compared to 7 days of age. In contrast to the latency of the first step the latency to vocalize was shorter at 7 days of age. Similarly to the latencies in the OF the latencies of head and body emerge in the ET were shorter at 40 weeks of age. Significant line by age interactions were found for the number of inductions in the TI test and the latency of head emerge in the ET. The number of inductions was significantly higher at 40 weeks than at 7 days of age in both lines. There was a significant difference between the lines at 40 weeks only, where LFP showed a higher mean than HFP (Figure 1). The latency of head emerge was significantly higher at 7 days than at 40 weeks of age. But the difference was significant between the lines at 40 weeks only. The LFP showed higher means than the HFP (Figure 2). Similarly to the results of the selection lines the correlations between feather pecking and fear criteria were generally low in the F2 birds (Table 4). Because of the higher number of birds even very small correlation coefficients reached the level of significance. There was a significant positive correlation of FPD and FPR in LFP and a significantly negative correlation in HFP birds. There were no significant correlations between FPD and FPR with fear criteria within both lines, with the exception of FPD and the pencil test scores for the LFP birds. Among the fear criteria (at 7 days and 40 weeks of age) all significant correlations showed a positive direction in both lines.

Table 2. Means, SD, p- values and interactions of F2- cross divided in high (HFP) and low (LFP) feather pecking birds for pecks delivered (FPD) and received (FPR) over a 20 min period and the criteria of different fear tests, Tonic immobility (TI), Open field (OF), Emerge Test (ET) at 7 days and 40 weeks of age. Means within the same column with different letters are significantly different (p < 0.05)

Mittelwerte, Standardabweichungen, p-Werte und Interaktionen der F2- Kreuzungen unterteilt in Tiere mit einer hohen (HFP) und einer niedrigen (LFP) Pickaktivität für Pickschläge ausgeteilt (FPD) und erhalten (FPR) über eine 20 min Periode und die Merkmale der verschiedenen Furchttests, Tonische Immobilität (TI), Open- field (OF), Emerge Box (ET) im Alter von 7 Tagen und 40 Wochen. Mittelwerte mit unterschiedlichen Buchstaben innerhalb derselben Spalte unterscheiden sich signifikant voneinander (p < 0.05)

	Feather pecking		TI		OF			ET	
Age Line	FPD	FPR	Duration of TI	No. of inductions	Latency 1st step	No. of steps	Latency of vocalization	Latency head	Latency body
7 days									
HFP	–	–	157.91 ± 42.04	1.12 ± 0.39	128.54[a] ± 64.16	20.72[b] ± 38.36	73.38 ± 70.92	156.99[c] ± 47.46	160.18 ± 43.53
LFP	–	–	161.18 ± 40.51	1.07 ± 0.28	115.32[b] ± 68.02	29.18[a] ± 45.23	71.31 ± 70.58	157.38[c] ± 45.28	162.09 ± 40.88
40 weeks									
HFP	3.66[a] ± 5.47	2.70 ± 1.87	104.13 ± 62.62	1.52[b] ± 0.76	84.25[a] ± 61.74	5.60 ± 6.30	102.45 ± 74.82	54.87[a] ± 68.30	96.82 ± 76.69
LFP	0.08[b] ± 0.08	2.61 ± 1.96	110.88 ± 66.19	1.57[c] ± 0.80	72.57[b] ± 61.31	6.21 ± 6.80	101.86 ± 75.50	69.76[b] ± 74.99	104.62 ± 76.05
HFP	–	–	131.35[b] ± 59.84	1.28 ± 0.55	106.47[a] ± 67.10	12.97[b] ± 27.73	88.22 ± 74.30	106.08[b] ± 77.93	128.72 ± 69.83
LFP	–	–	137.22[a] ± 58.95	1.32 ± 0.63	93.26[b] ± 66.95	18.63[a] ± 36.55	85.37 ± 74.55	113.14[a] ± 75.68	132.70 ± 67.77
7 days	–	–	158.89[a] ± 41.35	1.10[b] ± 0.33	125.30[a] ± 65.36	22.80[a] ± 40.29	72.88[b] ± 70.80	157.09[a] ± 46.91	160.65[a] ± 42.88
40 weeks	–	–	105.79[b] ± 63.55	1.48[a] ± 0.69	81.39[b] ± 61.80	5.75[b] ± 6.43	102.30[a] ± 74.94	58.45[b] ± 70.22	98.70[b] ± 76.57
Line	<.0001	0.53	0.045	0.215	0.0003	0.027	0.490	0.012	0.279
Age	–	–	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Line* Age	–	–	0.168	0.023	0.813	0.096	0.980	0.011	0.398

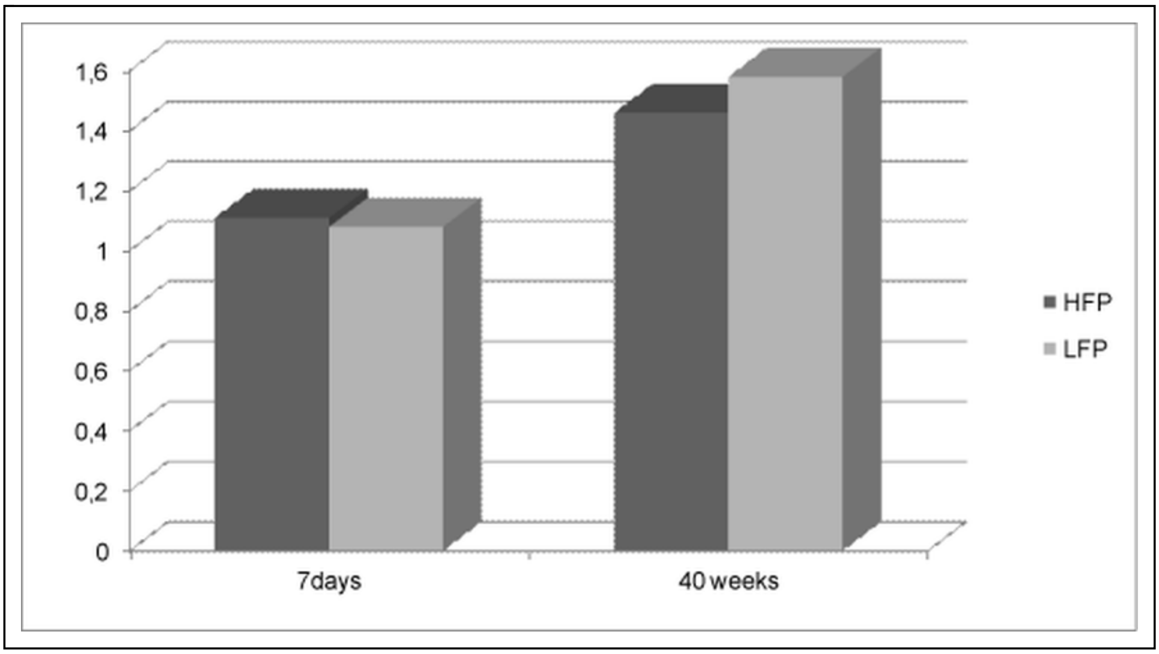

Figure 1. Mean number of inductions in the TI test of F2-crosses split in high feather pecking (HFP) and low feather pecking birds (LFP) at 7 days and 40 weeks of age

Mittelwerte der Induktionsversuche im TI Test der F2- Kreuzungen, eingeteilt in hoch (HFP) und niedrig (LFP) pickende Tiere im Alter von 7 Tagen und 40 Wochen

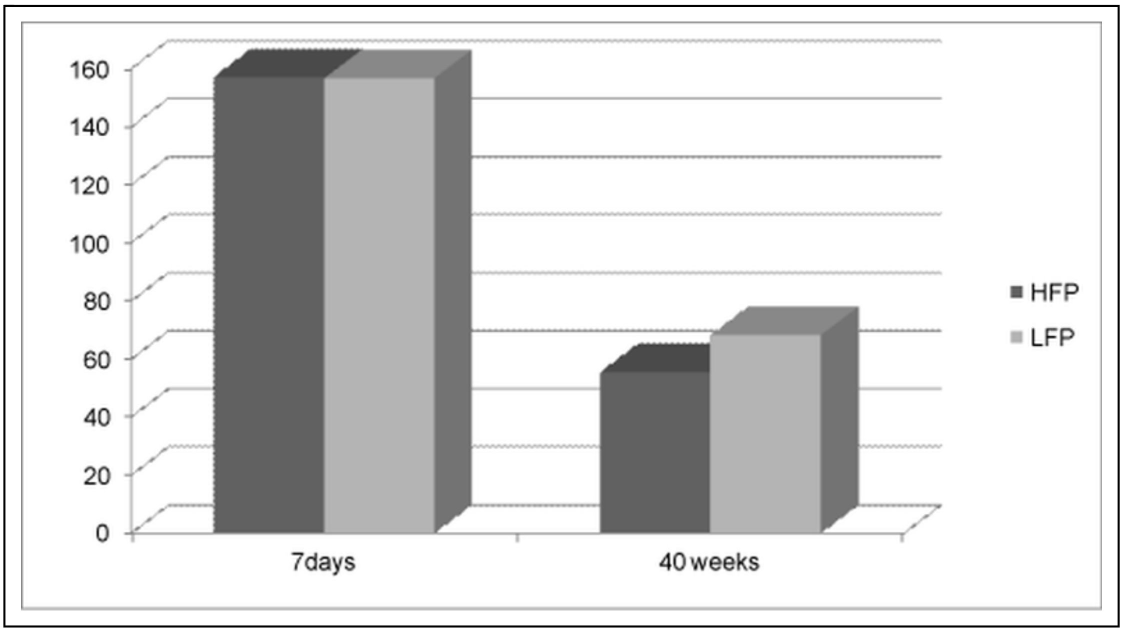

Figure 2. Mean latencies of head emerge in the Emerge test of F2-crosses split in high feather pecking (HFP) and low feather pecking birds (LFP) at 7 days and 40 weeks of age

Mittelwerte der Latenzen bis zum Erscheinen des Kopfes im Emerge Test der F2- Kreuzungen eingeteilt in hoch (HFP) und niedrig (LFP) pickende Tiere im Alter von 7 Tagen und 40 Wochen

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Table 4. Spearman's rank correlation coefficients between feather pecks delivered (FPD) and received (FPR) and the main fear criteria of fear tests, Tonic immobility (TI), Open field (OF), Emerge Box (ET) and pencil test of F2- crosses divided in high (HFP; above the diagonal) and low (LFP; below the diagonal) feather pecking birds at 7 days and 40 weeks of age. Significant coefficients ($p < 0.05$) are marked in bold letters (F2- crosses).

Spearman's Rangkorrelationskoeffizienten zwischen ausgeteilten (FPD) und erhaltenen (FPR) Pickschlägen und den Hauptmerkmalen der Furchttests, Tonische Immobilität (TO), Open- field (OF), Emerge Box (ET) und dem Bleistifttests bei F2- Kreuzungen, die in hoch (HFP; über die Diagonalen) und niedrig (LFP; unter der Diagonalen) Picker selektiert wurden im Alter von 7 Tagen und 40 Wochen. Signifikante Koeffizienten ($p < 0.05$) sind durch dick gedruckte Buchstaben markiert (F2 Kreuzungen).

	FPD	FPR	Duration TI 7 days	Latency 1st step 7 days	Latency Head 7 days	Duration TI 40 weeks	Latency 1st step 40 weeks	Latency head 40 weeks	Pencil test score
FPD	–	**-0.080**	0.009	0.052	0.036	−0.037	−0.031	−0.067	0.002
FPR	**0.211**	–	0.083	0.013	−0.041	0.047	−0.043	−0.057	0.058
Duration TI 7 days	0.025	−0.006	–	0.047	0.007	0.055	0.026	0.047	0.033
Latency 1st step 7 days	−0.077	−0.032	0.104	–	**0.335**	0.058	**0.148**	0.047	−0.030
Latency head 7 days	−0.099	0.049	0.028	**0.318**	–	−0.009	0.052	**0.080**	−0.051
Duration TI 40 weeks	−0.028	−0.044	0.042	0.032	0.073	–	0.050	−0.002	0.040
Latency 1st step 40 weeks	−0.067	−0.020	0.047	**0.146**	**0.198**	0.097	–	**0.283**	0.071
Latency head 40 weeks	−0.079	0.047	0.082	0.026	0.071	0.070	**0.258**	–	**0.140**
Pencil test score	**0.180**	0.045	0.084	−0.035	−0.096	0.063	0.052	0.015	–

Discussion

The selection for severe high (HFP) and low (LFP) feather pecking resulted in a significant difference between the HFP and LFP sublines already after 2 generations (KJAER et al., 2001). The difference increased in the following generations, and the means of feather pecking of the HFP were about ten times higher than of the LFP. Previous studies have shown that the sublines not only differed in the selection criterion but also in gentle feather pecking (KJAER et al., 2001), feather eating (HARLANDER-MATAUSCHEK and BESSEI, 2005) and aggressive pecking (BESSEI et al., 2013; BENNEWITZ et al., 2014 in print). The F2-crosses represent a large number of birds of a wide range of phenotypic variation in the selection criterion. Splitting the F2- crosses into HFP and LFP subgroups using a threshold of ≥ 2 and < 2 bouts of FPD resulted in similar means and differences as shown in the selection lines. It has been shown in a study on social behaviour that the F2-subgroups represent the selection lines on a larger scale (BESSEI et al., 2013). Most studies on feather pecking and fear in chickens considered active feather pecking (FPD) only. Since fear may influence both, active feather pecking (FPD) and being feather pecked (FPR) both criteria have been observed in the present study. The interrelationships between these criteria will be discussed on the basis of results of the selection lines and the F2-crosses and their relation to the fear criteria are being dealt with thereafter.

Feather pecks delivered and feather pecks received

The interrelationships between feather pecks delivered and feather pecks received are highly variable among the studies reported in the literature and obviously are not consistent throughout the experiments using the same lines. CUTHBERTSON (1978) found that high feather pecking hens were less frequently the target of being pecked. KJAER and SØRENSEN (1997) reported contrasting correlations between feather pecking performed and feather pecks received depending on the age of observation. Using the same lines as in the present study of an earlier generation KJAER et al. (2001) did not find a difference in the number of pecking bouts received. These results are in agreement with our results in the HFP and LFP of the F2-crosses (Table 2). In the selection lines, however, the HFP received a significantly lower number of feather pecks than the LFP (Table 1). The controversial results may be explained by the fact, that only 30 birds of each selection line, representing the highest and lowest breeding values for FPD, have been used. There was no significant difference for FPR when all birds of the selection lines were considered (BESSEI et al., 2013). The lower number of FPR of the extreme HFP in the selection line could be the result of social rank: HFP showed to be higher in aggression and in social rank than LFP (BESSEI et al., 2013). The low correlations between aggression and FPR within both lines, however, do not support this hypothesis. They rather support the results of the F2 subgroups and underline the independence of feather pecking and being pecked. This is also demonstrated when the percentage of birds identified as feather peckers (≥ 2 bouts of pecks delivered) and as being feather pecked (< 2 bouts of pecks received) are considered. Nearly all birds of both F2 sub-groups were found to be the target for feather pecking (99.4% and 98.9% for the HFP and LFP respectively). The corresponding values for the selection lines were 80.0% and 100% for the HFP and LFP respectively. The lacking difference between active feather pecking and being feather pecked has also been confirmed in physiological criteria related to feather pecking. KOPS et al. (2013) showed that feather peckers and their victims were very similar in their serontonin level (5-HT) and serotonin turnover in the brain.

Feather pecking and fear criteria

Fear in chickens is usually measured in special tests, such as TI, OF and ET. These tests have been found to express general fear in chickens (FAURE, 1981; JONES, 1986) and quail (JONES et al., 1982). The initial reaction in all tests is freezing or immobility and is measured as latency to express other behaviours, such as walking or vocalizing. The latency to express other behaviours is usually considered a primary reaction to fear eliciting stimuli. The activity after freezing is being related to exploration, social reinstatement (JONES, 1983) or escape from an unpleasant environment (GALLUP et al., 1976). Since fear inhibits other activities, a long duration of freezing or of latency to move is considered to indicate higher fear while other activities, mainly locomotion, indicate lower fear (FAURE, 1981, GALLUP et al., 1976). Long TI and long latency to emerge in the ET corresponded to freezing in the OF and are usually positively related. Our results of the OF test in the F2- crosses (Table 2) are in agreement with the general assumption that feather pecking and fear are positively correlated (JONES et al., 1995 RODENBURG et al., 2009; and DE HAAS et al., 2012). However the opposite is shown in the duration of TI and the latency of head emergence in the ET of the F2 crosses as well as in all fear criteria of the selection lines (Table 1). In line with our findings JENSEN et al. (2005) reported that high feather peckers from a F2-cross between Red Jungle Fowl and White Leghorn hens showed shorter duration of TI. However the differences were not significant. Similarly, ALBENTOSA et al. (2003) and RODENBURG et al. (2010) found no significant difference in the duration of TI between HFP and LFP birds from earlier generations of the same lines used in our experiment. The higher fear of LFP of the selection lines in our study may be related to the higher level of feather pecks received as suggested by CUTHBERTSON (1978) and LEE and CRAIG (1991). But this could not be confirmed by the data of the F2- crosses (Table 2) where HFP and LFP received the same amount of feather pecks. The relationships between feather pecking delivered and received and fear criteria as shown in the group means are not being reflected in the phenotypic correlations. The correlation coefficients were neither significant in the selection lines nor in the F2- crosses (Table 3, 4).

We found no significant difference between HFP and LFP in the pencil test. Similar results have been reported by BESSEI (1984b). Rhode Island Reds with a high feather pecking activity showed a similar fear score in the pencil test as Sussex with a low feather pecking activity. Using the human approach test, BOLHUIS et al. (2009) found no significant differences between a low mortality and a control line. In contrast to these results recorded in adult birds, KOPS (2014) reported a significantly shorter latency to approach a human in young HFP birds. The approach test in adult birds obviously represents fear of humans rather than fear in general. This may explain the lack of difference between the high and low feather pecking lines in this criterion. The controversial results may be caused by several factors. A major problem is the development with age of both, feather pecking and fear. It has been shown that the responses of birds in the different fear tests change with age (FAURE, 1981; BESSEI, 1982; HOCKING et al., 2001; ALBENTOSA et al., 2003). Although there was a high consistency of the latencies to move in the open field, latency to leave the emerge box and the duration of tonic immobility in young chicks (GALLUP et al., 1970, 1976; JONES et al., 1982, 1995), different results can be expected when birds are being tested at different ages. It has been shown that younger birds are more fearful than adults in the open-field (ALBENTOSA et al., 2003) and in the TI test (GHAREEB et al., 2008). RODENBURG et al. (2004) found even a negative genetic correlation in the open- field test of young and adult birds. Our results are in line with these findings in so far as young birds of both, HFP and LFP from the F2-crosses showed higher fear levels in the tonic immobility test, the open- field test and the emerge test.

The age effect of fear may not be consistent among all breeds and lines. HOCKING et al. (2001) found that the latency to vocalize and to walk in an open- field test increased from 1 to 30 weeks of age in two lines (ISA Brown and Tetra). But the duration of TI in Tetra decreased and ISA Brown showed no change with age. In contrast to the latencies to move in the OF and to emerge in the ET the latency to vocalize increased with age in the present study. This is in agreement with the results of HOCKING et al. (2001). The relatively high number of inductions required to induce TI in adult birds may be due to the higher body weight, which impairs handling while turning the birds on their back. The number of inductions and the duration of TI have been found to coincide in so far as lines showing longer duration of TI need less attempts of induction in Japanese quail (JONES et al., 1982; BENOFF and SIEGEL, 1976). GALLUP (1974) and GALLUP et al. (1976), however, did not find a relationship between the ease of induction and the duration of TI. The locomotor activity in the OF test is usually inversely related to the latency to move. In our study, however, there was a low locomotor activity in the OF after a relatively short latency to the first step in adult birds. This may be due to a change of motivation with age. While young chicks usually show a high motivation to reinstate social contact with their group mates through frantic pacing, adults spent more time exploring their environment while standing or ground pecking as reported by ALBENTOSA et al. (2003). The longer latency to vocalize in adult hens can also be explained by the lower social motivation as compared to chicks.

The phenotypic correlations between feather pecking and fear criteria were generally very low and despite the high number of birds in the F2 crosses they did not reach the level of significance (with the exception of FPD and the pencil test in the LFP, Table 4). This can be attributed to the inconsistent response of the birds in the different fear tests. Both, feather pecking and fear responses are highly susceptible to environmental influences, and individual birds obviously react in different ways to this stimuli. In addition, feather pecking and fear develop differently with age in response to breeds or lines and individuals within lines. Therefore the low correlation between feather pecking and fear in the present study may not be generalized, and differentresults may be found in other lines.

The hypothesis of a general relationship between fear and feather pecking needs to be reconsidered.

Conclusions

1) The hypothesis that feather pecking is generally related to fear could not be confirmed in the present study.
2) On the basis of group means the high feather pecking selection lines and subgroups built in the F2- crosses there was a certain co-existence of feather pecking and fear criteria assessed in the OF test. This is in agreement with various experiments reported in the literature.
3) Other tests (TI, ET) revealed either no differences in fear between HFP and LFP or the opposite tendency.
4) Both feather pecking and fear are subjected to age-related developments.
5) Fear develops differently with age, depending on the method of fear assessment and genetic line. Therefore results of the present study can not be extended to other breeds or commercial lines.
6) The within line phenotypic correlations between feather pecking, being feather pecked and fear were low and not significant.
7) These findings exclude a causal relationship between feather pecking and fear

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Summary

On the basis of observations that flocks of chickens with high incidence of feather pecking and feather damages show a high fear level, it is generally assumed that feather pecking and fear are positively correlated. This hypothesis was tested in two experiments using adult laying hens of lines selected for high (HFP) and low feather pecking behaviour (LFP) and their reciprocal crosses. A total of 60 adult birds, 30 HFP and 30 LFP, of the selection lines were used in part one of the experiment. The birds were first observed for the number of bouts of severe feather pecks delivered (FPD) and received (FPR) when kept in groups of equal numbers of both lines. Thereafter all birds were subjected to several fear tests: Tonic immobility test (TI), open- field test (OF), emerge box test (ET) and pencil test. In part two of the experiment a total of 967 birds of the F2- crosses of both lines were used. All birds were tested using the same fear tests as above at 7 days and 40 weeks of age. FPD and FPR were observed in adults only. The whole population was split for FPD in HFP and LFP using the threshold of ≥ 2 (HFP) and < 2 (LFP). HFP and LFP of the selection lines and the F2- crosses clearly differed in FPD. LFP of the selection lines received more feather pecks than HFP. There was no significant difference for FPR in HFP and LFP in the F2- crosses. In contrast to our expectation HFP from the selection lines showed a significant shorter duration of TI, shorter latency to move and to vocalize in the OF and a shorter latency to leave the emerge box, indicating lower fear. Similar results were found in the HFP and LFP of the F2- crosses for the duration of TI and latency of head appearance in the ET. Latency of the first step and to vocalize in the OF, however showed the opposite tendency. Line by age interactions appeared for the number of inductions in the TI and the latency of head emerge in the ET. There were no differences between HFP and LFP in the pencil test in both experiments. The phenotypic correlations between FPD and FPR with all fear criteria were low and not significant in both experiments. There is obviously no consistent relationship between feather pecking and fear in this population. Depending on type of fear test and age the HFP may show higher, lower or no difference in fear. Genotypes by age interactions further contribute to the variability of the results. The low phenotypic correlations among the criteria confirm this conclusion.

Key words

Feather pecking, fear, laying hens, age

Zusammenfassung

Phänotypische Beziehungen zwischen Federpicken, bepickt werden und Furcht in Weißen Leghorn Linien, die auf hohes und niedriges Federpicken selektiert wurden und deren F2- Kreuzungen

Aufgrund von Beobachtungen, dass in Hühnerherden mit einem hohen Federpicken und starken Gefiederschäden auch ein hohes Furchtniveau herrscht, wird allgemein angenommen, dass Federpicken und Furcht in positiver Beziehung stehen. Diese Hypothese wurde in zwei Experimenten mit Legehennen, die auf eine hohe (HFP) und eine niedere (LFP) Federpickaktivität selektiert wurden und deren F2- Kreuzungen getestet. In Teil 1 des Experiments wurden 60 Legehennen, 30 HFP und 30 LFP, der Selektionslinien getestet. Die Tiere wurden hinsichtlich ihres Zuchtwertes für ausgeteilte (FPD) Federpickschläge aus einer Herde von 224 Legehennen ausgewählt. Hierfür wurden die Tiere in Gruppen mit gleichen Teilen beider Linien beobachtet. Anschließend wurden alle Tiere folgenden Furchttests unterzogen. Tonische Immobilität (TI), Open- field (OF), Emerge Box Test (ET) und Bleistifttest.

In Teil 2 des Experiments wurden 967 Tiere aus den F2- Kreuzungen der Selektionslinien in denselben Furchttests im Alter von 7 Tagen und 40 Wochen getestet. Federpickverhalten und Bleistifttest wurden nur im erwachsenen Alter beobachtet. Die Population wurde mit Hilfe einer Schwelle von ≥ 2 und < 2 Serien von starkem Federpicken (FPD) in HFP und LFP- Tiere unterteilt. HFP und LFP der Selektionslinien und der F2- Kreuzungen unterschieden sich signifikant in der Höhe der FPD. LFP der Selektionslinien erhielten mehr Pickschläge als die HFP Tiere. Bei den F2-Kreuzungen gab es für dieses Merkmal keine Unterschiede zwischen den HFP und den LFP. Die HFP der Selektionslinie zeigten im Gegensatz zu unserer Annahme eine kürzere Dauer der TI, eine kürzere Latenz bis zum ersten Schritt und zum ersten Laut im OF und eine kürzere Latenz bis zum Verlassen der Emerge Box. Dies deutet auf eine niedrigere Furcht hin.

Ähnliche Ergebnisse wurden bei den F2- Kreuzungen für die Dauer der TI und die Latenz bis zum Erscheinen des Kopfes im ET gefunden. Die Latenz bis zum ersten Schritt und bis zum ersten Laut im OF zeigte allerdings in die entgegengesetzte Richtung. Für die Anzahl der Induktionsversuche im TI und für die Latenz bis zum Erscheinen des

Kopfes im ET zeigte sich eine Interaktion zwischen Linie und Alter. In beiden Experimenten wurden im Bleistifttest keine Unterschiede zwischen den Linien gefunden. Die phänotypischen Korrelationen zwischen FPD und FPR und allen Furchtmerkmalen waren in beiden Experimenten sehr niedrig und nicht signifikant. Es gibt offensichtlich keine feste Beziehung zwischen Federpicken und Furcht. Vielmehr tragen die stark ausgeprägten Effekte von Alter und der Interaktion von Linie × Alter zu einer hohen Variablität der Ergebnisse bei. Die niedrigen phänotypischen Korrelationen bestätigen diese Annahme.

Stichworte

Federpicken, Furcht, Legehennen, Alter

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