Individual Differences in Fear and Social Reinstatement Behaviours in Laying Hens

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Abstract: Individual differences in behavioural responses are of growing interest in behavioural studies. The present study investigated the consistency of the individual differences over time and across social (social reinstatement responses) and non social test situations (tonic immobility response). Three breeds of commercial hybrid layers (ISA Brown, Lohmann Tradition and Lohmann Silver) were reared from hatch to 37 weeks of age. Individual birds were subjected to tonic immobility test at 3, 5, 7, 10, 11, 15, 16, 20, 24, 35 and 37 wk old and to runway test of sociality at 3, 5, 10, 16, 20 and 37 wk old. Fearfulness did not show breed differences either in the overall means or in a certain tested age. However, ISA Brown had a higher latency to emerge to a runway than LT (16 and 20 wk) and LS (at 37 wk). In addition, ISA Brown hens had a higher latency to reinstate with their companion than LS (10 and 37 wk). The individual ranks for behavioural traits of fear and sociality were consistent over time. These results indicate fear and sociality responses are behavioural strategies used by individuals in certain test situation when repeated. Moreover, the duration of TI response was positively correlated to both sociality traits (latency to emerge and reinstate with a companion) indicating that birds had overall behavioural traits that were consistent across different contexts. This suggests that hens can be categorized into behavioural types or styles based on their test responses. The highly fearful birds (longer TI duration) had a higher latency to emerge and reinstate with their companions (reactive style) and the less fearful birds (shorter TI duration) had a lower latency to emerge and socially reinstate with their companions (proactive style). In conclusion, these individual differences are consistent over time and the behaviour of hens in one test can predict their behaviour in other test situation. Thus it could be used to assess individual hens and potentially be used in a breeding program to select a hen with more desirable personality traits.

Key words: Laying hens, fearfulness, sociality, tonic immobility and social reinstatement

Introduction

Individual differences in behavioural responses are of growing interest in behavioural sciences. There could enormous benefits for poultry husbandry if a test could be developed that would identify categories or types of individuals unlikely to cope with subsequent challenges. Feather pecking as an undesirable behavioural activity remains the major welfare problem in commercial units of laying hens particularly in alternative housing systems. It can be genetically manipulated (Kaer et al., 2001) which may offer a solution to the problem (Savory, 1995; Jones and Hocking, 1999). However, successful genetic selection against feather pecking currently relies on collecting data on feather pecking behaviour in individual birds so that only low feather peckers are used for breeding. A less time consuming method would be the ability to identify high feather peckers by an easily measurable, phenotypically and genetically associated or, preferably, predictive behavioural trait could be useful in selective breeding programs (Cloutier et al., 2000). Blokhuis and Beuving (1993) reported that two lines of White Leghorns were differed in their propensity to feather peck differed in their level of fearfulness and the tonic immobility response was significantly longer in the higher feather pecking line than lower feather pecking line. In addition, a positive correlation between level of fearfulness as measured by tonic immobility duration and the rate of severe feather pecking was found (Blokhuis and Beutler 1992; Vestergaard et al., 1993). These lines differed also in their open field behaviour and social reinstatement behaviour (Jones et al., 1995). Consistent line difference in two lines of White Leghorn in the feather pecking behaviour was identified early after hatching (Riedstra and Groothuis, 2002) and in their physiological (corticosterone) and neurobiological characteristics (Van...
Hocking, 1999; Marin much attention not only due to its association to feather Hierden Fearfulness and sociality characteristics have received a companion, (Jones, 2003).

exhibited greater sociality, motivation to be with a breeds in addition to influences of age and habituation.

specific, apply across both certain genetically similar

whether results from behavioural tests are breed differences in behaviours over rearing and laying periods and over social and non social situations and whether results from behavioural tests are breed specific, apply across both certain genetically similar

Materials and Methods

One hundred non beak trimmed female chicks from each breed of ISA Brown, Lohmann Tradition (LT) and Lohmann Silver (LS) were obtained from a commercial hatchery 6 hours after the hatching period had initiated. The newly hatched chicks were reared in a partially slatted deep litter system. Chicks from each line were housed in single strain pen measuring 3.5 (width) x 6 (length) x 3 (height) m for 3 weeks. After this age the birds were grouped into 50 birds per pen in 6 pens of identical size (1.75 x 6 x 3 m), two pens for each strain with a stocking density of 4.6 birds/m². The separations between pens were made of wire and plastic. All of these pens of identical size were arranged along one side of a corridor. In each pen about 45% of the total floor was made of slats and the floor in the rest of the pen was covered with 15 cm layer of wood shavings and straw. Pens were provided with nipple and bell drinkers and two feeders. Water and commercial diet were available ad libitum. The light program and temperature followed commercial recommendations. The pens were provided with perches from one day old and wooden nests from 17 weeks of age. The disturbance of the birds was kept to the minimum required for routine feeding, watering and maintenance. At the third week of life 20 chicks from each line were subjected to individual identification by means of wing band (manually made from a sponge layer and strong carton paper and fixed by surgical tape).

Behavioural tests: The behavioural tests were carried out in a separate room adjacent to the bird pens and having the same climatic conditions as the bird pens. Birds were out of auditory and visual contact with the other birds. Tonic immobility and runway tests were done in separate days within the same week. Twenty birds from one pen for each strain were randomly selected and individually marked by wing bands with specific number for each bird (bands were made manually from a sponge layer and strong carton paper and fixed by surgical tape). These twenty marked birds from each breed were subjected individually to the behavioural tests at each age replicates. The same birds were tested each time for either tonic immobility or runway test. The testing controls was done to investigate the age effect on the group level and to test the effect of habituation of repeatedly tested individuals by comparing the results of both marked and non-marked individuals.

Tonic immobility test: The tonic immobility (TI) is induced by manual restraint. The bird was placed on its back in a U-shaped cradle covered with cloth. The bird
was then restrained with one hand on its sternum for 45 seconds while holding the head and neck by the other hand. Towards the end of the induction period, hand pressure was gradually lifted so that if the chick still moved, another induction period was started immediately, until the movement ceased. After removal of the hands, a stop watch was started. The experimenter then retreated one meter, moving out of sight of the bird and observed the behaviour of the bird through a monitor behind a wooden barrier. The recorded parameters were:

1. The number of induction trials (45 seconds period of restraint) to attain tonic immobility lasting at least 20 seconds.
2. The duration of tonic immobility reaction is the latency until self-righting. If the bird righted in less than 20 seconds, it was considered that tonic immobility had not been induced and the restraint procedure was repeated. Conversely, if a bird did not show a righting response over the 15 min test period, a maximum score of 900 seconds was given for duration. Twenty marked birds from each breed were tested at 3, 5, 7, 10, 11, 15, 16, 20, 24, 35 and 37 weeks old. The same twenty marked individuals of each breed were subjected to the test at each of age replicates. In addition 20 un-marked control birds from each breed were tested at 7, 11 and 16 weeks old (the test was repeated using another 20 un-marked birds each week). Tonic immobility test was carried out during one day between 09:00 h and 16:00 h.

Runway (sociality) test: The hen was placed in wooden box (start box) measuring about 40 x 30 x 40 cm (length x width x height), with a door at one side facing to the runway. The runway measured 2 x 1 m (length x width). A goal box made of wire measuring 0.4 x 0.6 x 0.3 meter (length x width x height) at the opposite end of the start. The goal box was used only for the stimulus bird (companion bird). Therefore the actual length of the runway was 160 cm. The tested bird has to traverse a runway and enter the goal zone after emergence from the start box. The floor of the runway was made of concrete. The goal zone was 20 cm near the goal box which was marked on the floor of corridor by chalk in a straight line. The individual bird was caught from the home pen and placed in the start box for 2 minutes to acclimatize the bird to the environment of the box before the test begun. The front door was opened via a rope by the experimenter sitting on a chair on the other side of the start box, out of the bird’s vision. The latency until full emergence was recorded by stop watch via screen in the front of the experimenter. After full emergence of the tested bird from the start box, the latency to enter the goal zone (reinstate with the stimulus bird) was also recorded. Twenty marked birds from each breed were tested at 3, 5, 10, 16, 20 and 37 weeks old. In addition 20 un-marked control birds from each breed for each tested age were tested at 10 and 20 weeks old (the test was repeated using another 20 un-marked birds each week).

Statistics: For statistical analysis of the data, the Statistical-Program SPSS for Windows, Version 15.0 was used. One way analysis of variance (ANOVA) and post hoc tests (Duncan Test) for the normally distributed data and Kruskai-Wallis Test and Mann-Whitney Test for the data which are not normally distributed were used to investigate the breed differences. Furthermore, age influences in each strain were tested either by Analysis of Variance of repeated measurements (ANOVA) for normally distributed data followed by Paired T test to compare each pair of age replicates and Friedman Test for not normally distributed data followed by Wilcoxon Signed Ranks Test for each of two age replicates. In control birds, age effect was tested within each strain by T test for Independent Samples for normally distributed data and Mann-Whitney Test for not normally distributed, being independent samples. Consistency of the individual behavioural characteristics of fear and sociality over time was tested in each breed of hens using Kendall’s Coefficient of Concordance and the consistency across the situations (correlation between measurements) was tested by Pearson Correlation Coefficient.

Results
The strain x age interaction variance estimates for TI duration, emergence time and social reinstatement time was equal to zero and non significant (P > 0.05) as indicated by Variance Component analysis. Therefore, both breed and age differences were statistically handled separately.

Breed differences:
Tonic immobility response: Although there were no significant strain differences in TI duration for individually tested birds, the control un-marked birds (group level) revealed a significant difference between the three strains. ISA Brown had a significantly longer TI duration than LS at week 16 (Table 1).

Social reinstatement response: There was a significant strain difference in the latency of the marked individuals to leave the start box at week 16, 20 and 37 of age (Table 2). ISA Brown hens had a higher latency to leave the start box compared with LT at week 16 and week 20 (P < 0.05; Table 2) and compared with LS at week 37. In addition LS had a higher latency compared with LT at week 16 and week 20 (P < 0.05; Table 2). ISA Brown
Table 1: Influence of breed on the fear behaviour of laying hens as measured by tonic immobility duration

<table>
<thead>
<tr>
<th>Strains</th>
<th>Age (weeks)</th>
<th>ISA Brown</th>
<th>Lohmann Tradition</th>
<th>Lohmann Silver</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marked birds</td>
<td>3</td>
<td>239.45±51.91</td>
<td>213.35±58.50</td>
<td>163.35±41.03</td>
<td>0.566</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>328.05±53.64</td>
<td>347.95±69.72</td>
<td>348.60±62.77</td>
<td>0.966</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>316.55±61.62</td>
<td>346.80±56.51</td>
<td>283.00±44.06</td>
<td>0.712</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>286.80±58.14</td>
<td>350.50±64.92</td>
<td>272.10±52.70</td>
<td>0.608</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>362.05±77.32</td>
<td>332.60±75.54</td>
<td>183.85±52.46</td>
<td>0.126</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>243.40±51.72</td>
<td>308.60±52.29</td>
<td>241.95±54.45</td>
<td>0.598</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>276.55±64.08</td>
<td>220.95±56.09</td>
<td>241.30±47.33</td>
<td>0.780</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>239.75±50.59</td>
<td>166.50±38.00</td>
<td>206.00±39.80</td>
<td>0.331</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>289.25±58.08</td>
<td>308.60±52.29</td>
<td>241.95±54.45</td>
<td>0.598</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>245.60±58.11</td>
<td>186.45±44.54</td>
<td>206.00±39.80</td>
<td>0.331</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>285.45±33.82</td>
<td>263.90±24.70</td>
<td>246.70±22.38</td>
<td>0.608</td>
</tr>
<tr>
<td>Un-marked birds</td>
<td>7</td>
<td>448.55±65.97</td>
<td>383.30±62.78</td>
<td>378.15±60.49</td>
<td>0.681</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>361.30±66.94</td>
<td>248.35±46.95</td>
<td>326.95±56.53</td>
<td>0.368</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>455.29±79.29</td>
<td>339.10±64.44</td>
<td>235.75±37.01</td>
<td>0.050</td>
</tr>
</tbody>
</table>

Within the same row, means with no common superscripts are significantly different (P < 0.05; one way ANOVA followed by Duncan’s test). Results are reported as means ± SEM.

Table 2: Influences of breed on emergence and social reinstatement behaviours of laying hens

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Age (weeks)</th>
<th>ISA Brown</th>
<th>Lohmann Tradition</th>
<th>Lohmann Silver</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marked birds</td>
<td>3</td>
<td>438.00</td>
<td>169.50</td>
<td>163.00</td>
<td>0.326</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>123.00</td>
<td>21.50</td>
<td>50.00</td>
<td>0.177</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>14.50</td>
<td>5.50</td>
<td>12.50</td>
<td>0.281</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>4.50</td>
<td>2.00</td>
<td>4.50</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7.00</td>
<td>3.00</td>
<td>3.00</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>14.50</td>
<td>9.00</td>
<td>6.00</td>
<td>0.048</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>134.00</td>
<td>43.50</td>
<td>37.00</td>
<td>0.180</td>
</tr>
<tr>
<td>Un-marked birds</td>
<td>10</td>
<td>82.00</td>
<td>36.50</td>
<td>43.50</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>253.50</td>
<td>518.00</td>
<td>242.50</td>
<td>0.368</td>
</tr>
<tr>
<td>Social reinstatement time:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marked birds</td>
<td>3</td>
<td>138.50</td>
<td>171.50</td>
<td>57.00</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>76.50</td>
<td>58.50</td>
<td>47.00</td>
<td>0.362</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>78.00</td>
<td>64.00</td>
<td>26.50</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>19.00</td>
<td>16.50</td>
<td>10.00</td>
<td>0.642</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>10.50</td>
<td>51.00</td>
<td>11.00</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>81.50</td>
<td>92.50</td>
<td>46.50</td>
<td>0.082</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>125.00</td>
<td>122.50</td>
<td>37.50</td>
<td>0.097</td>
</tr>
<tr>
<td>Un-marked birds</td>
<td>10</td>
<td>152.50</td>
<td>56.00</td>
<td>61.00</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>57.00</td>
<td>278.00</td>
<td>91.00</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Within the same row, medians with no common superscripts differ significantly (P ≤ 0.05; Kruskal-Wallis Test followed by Mann Whitney test).

had a higher latency to emerge from the start box compared with LT and LS birds. The latency of marked individuals to re-instate with their companion showed a significant strain difference at week 10 and 20 (Table 2) and there was a tendency towards significance at week 20 at 20 (P = 0.082) and in the overall mean (P = 0.097). LT had a higher latency to re-instate with a companion than LS at week 20 and 37 (Table 2). The latency of LT to re-instate with their companion had a tendency to be higher than ISA Brown at week 20 (Table 2). In addition, ISA Brown hens had a higher latency to re-instate with their companion compared to LS at week 10, 37 and in the overall mean (Table 2). The control un-marked birds showed also a significant breed differences in sociality. LT had a higher latency to re-instate with their companion compared with LS at week 20 of age. ISA Brown had a higher latency to re-instate with a companion at week 10 compared with LT and LS and at week 20 compared with LT.

Age changes: TI duration of ISA Brown marked individuals tends to increase from 239 se at week 3 to 362 se at week 11 (P < 0.1) and then tends to decrease to 239 se at week 24 and 245 se at week 37 (P < 0.1). Similarly, TI duration in LT increased with age up to week 10 and then decreased (P < 0.05). In LS, TI duration was significantly increased from 163 se at week 3 to 348 se at week 5 and then decreased with age up to week 11 (183 se) and then increased with age up to week 20 (359 se) and then decreased significantly up to week 35 (P < 0.05).

The latency of the marked individuals of LT to emerge from the box was decreased from week 3 to week 5, 10 and 16 and then it increased at week 20 and 37 compared to week 16. Similarly in LS it decreased with age from week 3 to week 5, 10, 16, 20 and increased at week 37 compared to week 16 and 20. In ISA Brown the latency to emerge from the start box also decreased with age from week 3 to week 5, 10, 16, 20 and 37 compared to week 20. In the un-marked controls the latency to leave the start box was increased week 10 week 20 in LT and in LS while the difference did not reach the significance in ISA Brown.

The latency of ISA Brown marked individuals to re-instate with their companion was decreased within age from
Table 3: Consistency of individual differences in fear behaviour and sociality over time in three breeds of laying hens (Kendall’s Coefficient of Concordance with 19 DF)

<table>
<thead>
<tr>
<th>Behavioural measurement</th>
<th>W</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. ISA Brown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tonic immobility (11 ages)</td>
<td>0.312</td>
<td>59.229</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of TI (s)</td>
<td>0.115</td>
<td>21.799</td>
<td>0.294</td>
</tr>
<tr>
<td>Number of TI induction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runway test (6 ages)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to leave start box (s)</td>
<td>0.497</td>
<td>56.652</td>
<td>0.001</td>
</tr>
<tr>
<td>Latency to inter goal zone (s)</td>
<td>0.429</td>
<td>48.925</td>
<td>0.001</td>
</tr>
<tr>
<td>B. Lohmann Tradition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tonic immobility (11 ages)</td>
<td>0.206</td>
<td>43.020</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of TI (s)</td>
<td>0.058</td>
<td>17.796</td>
<td>0.536</td>
</tr>
<tr>
<td>Number of TI induction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runway test (6 ages)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to leave start box (s)</td>
<td>0.432</td>
<td>49.233</td>
<td>0.001</td>
</tr>
<tr>
<td>Latency to inter goal zone (s)</td>
<td>0.470</td>
<td>53.536</td>
<td>0.001</td>
</tr>
<tr>
<td>C. ISA Brown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tonic immobility (11 ages)</td>
<td>0.254</td>
<td>52.990</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of TI (s)</td>
<td>0.139</td>
<td>29.060</td>
<td>0.065</td>
</tr>
<tr>
<td>Number of TI induction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runway test (6 ages)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to leave start box (s)</td>
<td>0.446</td>
<td>50.840</td>
<td>0.001</td>
</tr>
<tr>
<td>Latency to inter goal zone (s)</td>
<td>0.381</td>
<td>43.472</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 4: Correlation between the overall means of sociality parameters (emergence and social reinstatement time) and means of the corresponding ages of tonic immobility duration in three breeds of laying hens (Pearson Correlation Coefficient)

<table>
<thead>
<tr>
<th>Behavioural parameter</th>
<th>ISA Brown</th>
<th>Lohmann Tradition</th>
<th>Lohmann Silver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonic immobility duration</td>
<td>0.120</td>
<td>0.528**</td>
<td>0.191</td>
</tr>
<tr>
<td>Emergence time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISA Brown</td>
<td>0.254**</td>
<td>0.847**</td>
<td>0.191</td>
</tr>
<tr>
<td>Lohmann Tradition</td>
<td>0.473**</td>
<td>0.685**</td>
<td>0.191</td>
</tr>
<tr>
<td>Lohmann Silver</td>
<td>0.248**</td>
<td>0.916**</td>
<td>0.191</td>
</tr>
</tbody>
</table>

*Correlation is significant at the level 0.01(2-tailed).
**Correlation is significant at the level 0.05(2-tailed).

The emergence time was higher in the un-marked individuals compared with the marked ones in ISA Brown, LT and LS at week 10 and 20.

The social reinstatement time of the un-marked individuals was higher compared to marked ones at week 10 in ISA Brown (P < 0.05) and LS (P < 0.05) but not in LT. Furthermore, it was higher at week 20 in the un-marked individuals compared to the marked ones in all tested strains.

Consistency of individual behavioural traits over time:
Significant correlations were found. These implied that individual ranks for behavioural traits of fear and sociality were consistent over time. The correlations were large and high W values were not expected (Table 3). These results indicate the stability of individual behavioural characteristics of fear and sociality.

Consistency of individual behavioural traits over different situations: The correlation between the overall means of latency to emerge from the start box and latency of social reinstatement time was positive (Table 4) in all breeds (0.847, 0.685 and 0.916 in ISA Brown LT and LS respectively) and highly significant (P < 0.01) which means that individuals that have lower latency to leave the start box are quicker to be socially reinstated with their companion. Furthermore, the correlations between TI and both emergence and social reinstatement time were positive (0.528 and 0.473, respectively) and highly significant (P < 0.01) in Lohmann Tradition and were positive non significant in both ISA Brown and Lohmann Silver. This result suggested that LT individuals that have shorter tonic immobility duration are quicker to leave the start box and are quicker to be socially reinstated with their companions and vice versa.

Discussion
This study examined how breed, habituation and age at testing influenced the test responses in commercial layer type breeds. A clear breed differences were found in response to sociality test. However, breed differences were not clearly found in response to tonic immobility reaction. Age and habituation influenced both social reinstatement behaviour and fearfulness.

Fearfulness: Differences due to breed were considered important in behavioural studies when all the data from a test were aggregated and combined. Fearfulness as indicated by TI duration in the present study did not show breed differences on the individual level either in the overall means or in a certain tested age. However, ISA Brown was more fearful than LS at week 16 in the group level (un-marked control birds). Although significant differences were not found at other ages, these results could indicate that tonic immobility behaviour in laying hens could have a genetic background. This finding is in
the agreement with Albentosa et al. (2003) who found strain differences in tonic immobility behaviour. They found that White Leghorns had longer tonic immobility duration than ISA Brown, Columbian Blacktail and Ixworth. Similarly, Hocking et al. (2001) mentioned that ISA Brown had longer tonic immobility duration than Tetras at week 31 of age.

Fearfulness changed in all breeds in the repeatedly tested birds as the birds grew older. During the rearing period, ISA Brown TI duration was increased with age from week 3 up to 11 weeks. Fearfulness of LT breed increased as the birds get older up to 10 weeks and in LS breed it increased from wk 3 to wk 5. This result could be due to age changes and increase in bird weight.

The tonic immobility duration of LS increased from week 11-20. This significant increase in tonic immobility in this age period may reflect a direct effect of maturation. It was shown that the age related increase in TI may be associated with the approach of sexual maturity and the birds changing the endocrine state (Campo and Carnicer, 1993).

The decrease in TI duration with age after week 20 could be due to experience of the birds to the test (habituation) rather than age effects. This finding is in agreement with the finding of Hocking et al. (2001) who mentioned that TI duration was shorter at week 31 compared with week 2. After maturity, our data on the group level did not show any increase in TI duration. Contrary to that Hansen et al. (1993) reported an increase in TI duration from week 31 to week 70 in laying hens in cages and pens tested on the group level.

Social reinstatement behaviour: ISA Brown had a higher latency to leave the start box to an unfamiliar environment than LT in individual level (marked birds) and group level (un-marked birds). Furthermore, ISA Brown had higher latency to leave the start box than LS and LS had a higher latency to leave the start box than LT at week 16. This means that ISA Brown and LS hens are slower to emerge to an unfamiliar environment than LT. This result indicates that the emergence from the start box is strain specific and has a genetic background which is in line with the result of Jones (1992); Jones et al. (1995); Hocking et al. (2001).

The latency of birds to enter the goal zone showed significant strain differences. ISA Brown hens were slower to reinitate with their companion compared to LT and LS in both repeatedly tested individuals and birds tested once. Furthermore, LT hens are slower to reinitate with their companion compared to LS hens. This apparent strain difference in social reinstatement behaviour reveals the genetic background of this behavioural characteristic. These results confirm the findings reported by Hocking et al. (2001) and indicate that commercial laying hen breeds showed a relatively differences in the frequency of sociality traits although they are selected along the same criteria such as feed conversion efficiency and egg production.

The latency emergence time decreased as the bird grew older up to week 20 and afterwards it increased up to week 37 in repeatedly tested individuals (marked birds) of all strains and this could be due to maturation or habituation effect. In birds tested once (un-marked birds), there was no age effect on the latency to leave the start box in ISA Brown which indicates that the decrease in the latency to leave the start box up to week 20 is mainly due to experience of the bird to the test and habituation rather than to age changes.

It is conceivable that decreased latency to leave the start box in older birds either reflected their greater body weight or perhaps more likely, experience-dependent reductions in separation distress and the expression of social reinstatement behaviour that accompanied the repeated testing. The latter interpretation is consistent with our finding that the latency to enter the goal zone in runway test was decreased with age in the repeated testing individuals. Thus the bird may have been equally motivated to seek social contact initially but habituation induced reduction in fear and/or separation distress (Jones, 1996; Hocking et al., 2001). However, the increase in the emergence time after week 20 in repeated tested individuals in all strains could be due to age changes after maturation.

The social reinstatement time decreased with age up to week 20 in all strains tested for the repeatedly tested individuals. Afterwards, it increased again with age. Birds tested once of LT breed showed increase in the social reinstatement time. Furthermore, higher latency to enter the goal zone in the LT individuals tested once at week 20 compared to repeatedly tested individuals could indicate the habituation effect rather than age changes. Similarly, the higher social reinstatement time in group level testing of ISA Brown and LS compared with repeated testing individuals confirm the influence of the habituation. The increase in the social reinstatement time with age after week 20 could mainly be considered an age related change.

Consistency of individual behavioural traits over time: The organized pattern of behavioural characteristics in an individual can be referred to as a personality traits (Janczak et al., 2003) or behavioural strategies. A common prerequisite of this is that the same test carried out on the same individuals in consecutive times will give the same response (Erhard and Mendl, 1999). Furthermore, the individual differences in behaviour should show not only consistency over time but also consistency over the different situations to be considered as a personality traits because it should be independent of the situation (Erhard and Shouten, 2001). Behavioural strategy in contrast, is applied for
individual differences in behaviour which is consistent in specific situation (situation dependent) when repeated 
(Erhard et al. 1999; Erhard and Shouten, 2001). 
In the present study, the individual behavioural 
characteristics of fearfulness (tonic immobility duration) 
and sociality (latency to emerge to an open field arena 
and social reinstatement time) in ISA Brown, Lohmann 
Tradition and Lohmann Silver laying hens were 
consistent over time. This result confirms the 
consistency of these individual behavioural 
characteristics through the rearing and laying periods. A 
significant intra-situational consistency in some 
behavioural characteristics of laying hens as tonic 
immobility and reaction to a novel rod was reported for 
short time period of 3 days (Jones, 1987) and for capture 
ranks of quails for some weeks (Mills and Faure, 2000). 
Recently, Hocking et al. (2001) mentioned the stability of 
the individual behavioural characteristics of tonic 
immobility, open field and social reinstatement 
response. From our results, we can conclude that the 
 intra-situational consistency of individual behavioural 
characteristic of fearfulness and sociality in commercial 
laying hens tested and both fearfulness and sociality are 
behavioural strategies used by the individuals in 
challenge situations as predator attack, isolation and/or 
social stress.

**Consistency of individual behavioural traits over different situations:** The aggregation of the data from 
repeated behavioural measurements (the summing of 
sets of multiple observations) was thought to increase 
the correlation coefficient, presumably by averaging out 
errors of measurement (Ossenkopp and Mazmanian, 
1985; Tachibana, 1985; Jones, 1987). The aggregation 
of data from sets of tests and measurements used here 
may have acted similarly. The latency to explore an open 
field arena was positively correlated to the latency to 
reinstate socially with their companion in all lines. This 
means that individuals which are quicker to emerge 
from the start box and explore the open field arena, are 
also quicker to reinstate socially with their companion. 
These results indicated that the motivation of the birds 
to emerge from the start box was mainly to reinstate with 
their companions. The positive and significant 
correlations between fearfulness and both sociality 
measurements in Lohmann Tradion birds were 
surprising. This finding could support the coping style 
hypothesis in laying hens. The less fearful individuals 
(shorter tonic immobility birds) were quicker to emerge 
and reinstate with their companions in sociality test 
(proactive coping style) while highly fearful individuals 
(longer tonic immobility) were slower to emerge from the 
start box and to reinstate with their companions (reactive 
coping style).

The individual differences in behavioural responses 
were considered personality traits when they show not 
only consistency over time but also consistency across 
different situations of different contexts (social and non 
social situations). This consistent individual difference 
both over time and over situations could be explained by 
different coping styles (proactive/reactive). This is in 
agreement with Erhard et al. (1999); Erhard and Mendl 
(1999). In other wards, the reaction of an individual to the 
tonic immobility test predicts the behaviour in the other 
test situation. Thus tonic immobility is an indicator of 
individual personality characteristics, because the two 
test situations reveal consistency in the individual hen 
behaviour. Thus it could be used to assess individual 
hens and potentially be used in a breeding program to 
select a hen with more desirable personality traits. For 
example, Mills and Faure (2000) found that lines of 
Japanese quail selected for high sociality and short 
tonic immobility duration were easier to catch than lines 
selected for low sociality and long tonic immobility 
duration. Another example, pig farmers in Denmark 
used the back test (where a pig is turned on its back and 
restrained for a min) of Hessing et al. (1993) as a 
selection criterion for keeping the boars for breeding or 
not (Erhard et al., 1999).

In laying hens, Blokhuis and Beuving (1993) reported 
that two lines of White Leghorns were differed in their 
propensity to feather peck differed in their level of 
fearfulness and the tonic immobility response was 
significantly longer in the higher feather pecking line 
than lower feather pecking line. These lines differed also 
in their open field behaviour and social reinstatement 
behaviour (Jones et al., 1995). In addition, a positive 
correlation between level of fearfulness as measured by 
tonic immobility duration and the rate of severe feather 
pecking was found (Blokhuis and Beutler, 1992; 
Vestergaard et al., 1993). Consistent line difference in 
two lines of White Leghorn in the feather pecking 
behaviour was identified early after hatching (Riedstra 
and Groothuis, 2002) and in their physiological 
(corticosterone) and neurobiological characteristics (Van 
Hierden et al., 2002) and low feather pecking line 
exhibited greater sociality, motivation to be with a 
companion, (Jones, 2003).

Therefore, selection for short tonic immobility (low 
fearfulness) will produce birds characterized by higher 
sociality (low latency to emerge and reinstate with their 
companions) and these birds would have low feather 
pecking activity. However, feather pecking activity was not 
verified in the present study and further researches are 
needed to investigate the association between feather 
pecking activity and both tonic immobility (fear) and 
social reinstatement behaviours.

In conclusion, the individual differences in fear and 
social reinstatement behaviours of laying hens are 
consistent over time. Moreover, fear and social 
reinstatement responses are correlated and are 
personality traits in Lohmann Tradition hens and 
consequently these birds could be categorized to 
proactive and reactive types.
Ghareeb et al.: Individual Behavioural Differences in Laying Hens

References

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