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## 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

Hierden *et al.*, 2002) and low feather pecking line exhibited greater sociality, motivation to be with a companion, (Jones, 2003).

Fearfulness and sociality characteristics have received much attention not only due to its association to feather pecking behavioural vice but also due its correlation to human animal interactions. 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.

Fearfulness leads to behavioural and physiological changes that assist an animal to cope with that stimulus (Moberg, 1985) and can be measured by the duration of tonic immobility (TI) reaction (Gallup, 1979; Jones, 1986; Hocking *et al.*, 2001; Albentosa *et al.*, 2003). The bird's underlying level of sociality (the motivation to be with conspecifics) was measured by runway test (Jones and Hocking, 1999; Marin *et al.*, 2001, Hocking *et al.*, 2001; Albentosa *et al.*, 2003). Individual differences in animal behaviour are the rule rather than an exception and are of increasing interest in behavioural sciences (Bekoff, 1977; Manteca and Deag, 1993; Schrader, 2000). Moreover, variation in behaviour and physiology are considered biological individual characteristics to cope with relevant environmental changes that threaten homeostasis (Hessing *et al.*, 1994) and the organized pattern of behavioural characteristics of an individual can be referred as personality (Janczak *et al.*, 2003).

The possibility to identify stable behavioural stable behavioural characteristics would aid understanding the origins of individual differences in behaviour giving insight into behavioural mechanisms (Spooler *et al.*, 1996).

In laying hens studies of personality traits and individual behavioural characteristics become necessary nowadays due to the presently trend towards diversification of husbandry systems to deep litter and free range systems where welfare related problems like feather pecking are more difficult to control compared to battery cages (Zeltner *et al.*, 2000). So that, studies of behavioural patterns of birds and welfare related behaviours are moving from group level towards the study of individual birds (Keeling, 1994) because knowledge of the individual behavioural characteristics of birds may give insight in the process of welfare related behavioural problems (Rodenburg *et al.*, 2004). Such data on the individual differences in fear and social reinstatement behaviours could help for understanding their stability over time and how fear and sociality are correlated. Therefore, the present study was conducted to investigate the consistency of the individual 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

breeds or across a range of genetically dissimilar breeds in addition to influences of age and habituation.

## 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/m<sup>2</sup>. 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 programme 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 for each tested age 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 (reinstatement 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 Kruskal-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 ( $P < 0.05$ ; Table 4). Moreover, the un-marked controls showed a significant differences between the three strains at week 10 ( $P < 0.05$ ; Table 2). ISA Brown

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Table 1: Influence of breed on the fear behaviour of laying hens as measured by tonic immobility duration

Age (weeks)	Strains			P
	ISA Brown	Lohmann Tradition	Lohmann Silver	
<b>Marked birds</b>				
3	239.45±51.91	213.35±58.50	163.35±41.03	0.566
5	328.05±53.64	347.95±69.72	348.60±62.77	0.966
7	316.55±61.62	346.80±56.51	283.00±44.06	0.712
10	286.80±58.14	350.50±64.92	272.10±52.70	0.608
11	362.05±77.32	332.60±75.54	183.85±32.46	0.126
15	243.40±51.72	308.60±52.29	241.95±54.45	0.598
16	276.55±64.08	220.95±56.09	241.30±47.33	0.780
20	304.57±61.11	220.15±38.00	359.00±61.44	0.196
24	239.75±50.59	166.50±31.59	160.70±19.28	0.236
35	289.25±58.08	209.20±43.74	252.80±56.70	0.385
37	245.60±58.11	186.45±44.54	206.00±39.80	0.331
Mean	285.45±33.82	263.90±24.70	246.70±22.38	0.608
<b>Un-marked birds</b>				
7	448.55±65.97	383.30±62.78	378.15±60.49	0.681
11	361.30±66.94	248.35±46.95	326.95±56.53	0.368
16	455.29±79.29	339.10 <sup>a</sup> ±64.44	235.75 <sup>b</sup> ±37.01	0.050

Within the same row, means with no common superscripts are significantly different ( $P \leq 0.05$ ; one way ANOVA followed by Duncan's test). Results are reported as means  $\pm$  SEM

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

Age (weeks)	Breeds			P
	ISA Brown	Lohmann Tradition	Lohmann Silver	
<b>Emergence time:</b>				
<b>Marked birds</b>				
3	438.00	169.50	163.00	0.326
5	123.00	21.50	50.00	0.177
10	14.50	5.50	12.50	0.281
16	4.50 <sup>a</sup>	2.00 <sup>b</sup>	4.50 <sup>a</sup>	0.034
20	7.00 <sup>a</sup>	3.00 <sup>b</sup>	3.00 <sup>b</sup>	0.014
37	14.50 <sup>a</sup>	9.00 <sup>ab</sup>	6.00 <sup>b</sup>	0.045
Mean	134.00	43.50	37.00	0.180
<b>Un-marked birds</b>				
10	82.00 <sup>a</sup>	36.50 <sup>b</sup>	43.50 <sup>b</sup>	0.020
20	253.50	518.00	242.50	0.368
<b>Social reinstatement time:</b>				
<b>Marked birds</b>				
				3
5	138.50	171.50	57.00	0.116
10	76.50	58.50	47.00	0.362
16	78.00 <sup>a</sup>	64.00 <sup>b</sup>	26.50 <sup>b</sup>	0.050
20	19.00	16.50	10.00	0.642
37	10.50 <sup>b</sup>	51.00 <sup>a</sup>	11.00 <sup>b</sup>	0.031
Mean	81.50 <sup>a</sup>	92.50 <sup>a</sup>	46.50 <sup>b</sup>	0.082
Mean	125.00 <sup>a</sup>	122.50 <sup>a</sup>	37.50 <sup>b</sup>	0.097
<b>Un-marked birds</b>				
10	152.50 <sup>a</sup>	56.00 <sup>b</sup>	61.00 <sup>b</sup>	0.010
20	57.00 <sup>b</sup>	278.00 <sup>a</sup>	91.00 <sup>b</sup>	0.005

Within the same row, medians with no common superscripts differ significantly ( $P \leq 0.05$ ; 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 reinstate with their companion showed a significant strain difference at week 10 and 20 (Table 2) and there was a tendency towards significance at week 37 ( $P = 0.082$ ) and in the overall mean ( $P = 0.097$ ). LT had a higher latency to reinstate with a companion than LS at week 20 and 37 (Table 2). The latency of LT to reinstate 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 reinstate 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 reinstate with their companion compared with LS at week 20 of age. ISA Brown had a higher latency to reinstate 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 increased at week 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 reinstate 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)

Behavioural measurement	W	$\chi^2$	P
<b>A. ISA Brown</b>			
Tonic immobility (11 ages)			
Duration of TI (s)	0.312	59.229	0.001
Number of TI induction	0.115	21.799	0.294
Runway test (6 ages)			
Latency to leave start box (s)	0.497	56.652	0.001
Latency to inter goal zone (s)	0.429	48.925	0.001
<b>B. Lohmann Tradition</b>			
Tonic immobility (11 ages)			
Duration of TI (s)	0.206	43.020	0.001
Number of TI induction	0.058	17.796	0.536
Runway test (6 ages)			
Latency to leave start box (s)	0.432	49.233	0.001
Latency to inter goal zone (s)	0.470	53.536	0.001
<b>C. Lohmann Silver</b>			
Tonic immobility (11 ages)			
Duration of TI (s)	0.254	52.990	0.001
Number of TI induction	0.139	29.060	0.065
Runway test (6 ages)			
Latency to leave start box (s)	0.446	50.840	0.001
Latency to inter goal zone (s)	0.381	43.472	0.001

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)

	Tonic immobility duration	Emergence time
<b>Emergence time</b>		
ISA Brown	0.120	
Lohmann Tradition	0.528**	
Lohmann Silver	0.191	
<b>Social reinstatement time</b>		
ISA Brown	0.254	0.847**
Lohmann Tradition	0.473*	0.685**
Lohmann Silver	0.248	0.916**

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

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

week 3 to week 20 and then it increased at week 37 compared with week 20 ( $P < 0.05$ ). Similarly, the latency of LS marked individuals decreased within age from week 3 up week 20 and then it increased significantly at week 37 compared with week 20. While in LT, it decreased within age from week 3 up to week 16 and then it increased within age up to week 37. The latency of the unmarked LT birds increased from week 10 to week 20.

**Difference between repeated and single testing:** The mean TI duration of ISA Brown laying hens tended to be higher in the single testing (un-marked birds) individuals at week 16 ( $P < 0.1$ ) compared with repeatedly tested individuals (marked birds). The mean TI duration of LT did not show any significant difference between marked and un-marked individuals at all tested weeks ( $P > 0.05$ ). The mean tonic immobility duration of Lohmann Silver was significantly higher in the single testing individuals at week 11 compared to the repeated testing individuals ( $P = 0.034$ ), but there were no significant differences at week 7 and 16 ( $P > 0.05$ ).

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 reinstate with their companion compared to LT and LS in both repeatedly tested individuals and birds tested once. Furthermore, LT hens are slower to reinstate 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 Tradition 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 words, 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 programme 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.



## References

- Albentosa, M.J., J.B. Kjaer and C.J. Nicol, 2003. Strain and age differences in behaviour, fear response and pecking tendency in laying hens. *Br. Poult. Sci.*, 44: 333-344.
- Bekoff, M., 1977. Mammalian dispersal and the ontogeny of individual phenotypes. *Am. Nat.*, 111: 715-732.
- Blokhuis, H.J. and A. Beutler, 1992. Feather pecking damage and tonic immobility response in two lines of White Leghorn hens. *J. Anim. Sci.*, 70: 170.
- Blokhuis, H.J. and G. Beuving, 1993. Feather pecking and other characteristics in two lines of laying hen. In: Savory, C.J., B.O. Hughes, (Eds.) *Proceedings of the 4<sup>th</sup> European Symposium on Poultry Welfare*. Universities Federation for Animal Welfare, Potters Bar, UK., P: 266.
- Campo, J.L. and C. Carnicer, 1993. Realised heritability of tonic immobility in White Leghorn Hens: A Replicated Single Generation Test. *Poult. Sci.*, 72: 2193-2199.
- Cloutier, S., R.C. Newberry, C.T. Foster and K.M. Girsberger, 2000. Does pecking at inanimate stimuli predict cannibalistic behaviour in domestic fowl. *Appl. Anim. Behav. Sci.*, 66: 119-133.
- Erhard, A.H., M. Mendl and S.B. Christiansen, 1999. Individual differences in tonic immobility may reflect behavioural strategies. *Appl. Anim. Behav. Sci.*, 64: 31-46.
- Erhard, A.W. and M. Mendl, 1999. Tonic immobility and emergence time in pigs-more evidence for behavioural strategies. *Appl. Anim. Behav. Sci.*, 61: 227-237.
- Erhard, H.W. and W.G.P. Shouten, 2001. Individual differences and personality. In: Keeling, L.J. and H.W. Gonyou, (eds): *Social behaviour in farm animals*. CAB International Wallingford, pp: 333-352.
- Gallup, G.G.J., 1979. Tonic immobility as a measure of fear in domestic fowl. *Anim. Behav.*, 27: 316-317.
- Hansen, I., B.O. Braastad, J. Storbraten and M. Tofastrud, 1993. Differences in fearfulness indicated by tonic immobility between laying hens in aviaries and cages. *Anim. Welf.*, 2: 105-112.
- Hessing, M.G.C., A.M. Hagelso, W.G.P. Shouten, P.R. Wiepkema and J.A.M. Van Beek, 1994. Individual behavioural and physiological strategies on pigs. *Physio. Behav.*, 55: 39-46.
- Hessing, M.G.C., A.M. Hagelso, J.A.M. Van Beek, P.R. Wiepkema, W.G.P. Schouten and R. Krukow, 1993. Individual behavioural characteristics in pigs. *Appl. Anim. Behav. Sci.*, 37: 285-295.
- Hocking, P.M., C.E. Channing, D. Waddington and R.B. Jones, 2001. Age-related changes in fear, sociality and pecking behaviours in two strains of laying hen. *Br. Poult. Sci.*, 42: 414-423.
- Janczak, A.M., L.J. Pedersen and M. Bakken, 2003. Aggression, fearfulness and coping styles in female pigs. *Appl. Anim. Behav. Sci.*, 81: 13-28.
- Jones, R.B., 1986. The tonic immobility reaction of the domestic fowl: A review. *World's Poult. Sci. J.*, 42: 82-96.
- Jones, R.B., 1987. The assessment of fear in the domestic fowl. In: Zayan, R. and I.J.H. Duncan. (Eds.): *Congestive Aspects of Social Behaviour in the domestic Fowl*. Elsevier, Amsterdam, P: 40-81.
- Jones, R.B., 1992. The nature of handling immediately prior to test affects tonic immobility fear reactions in laying hens and broilers. *Appl. Anim. Behav. Sci.*, 34: 247-254.
- Jones, R.B., 1996. Fear and adaptability in poultry: insights, implications and imperatives. *World's Poult. Sci. J.*, 52: 131-174.
- Jones, R.B., 2003. Feather pecking in poultry: The application of science in a research for practical solutions. In: *Science in the service of animal welfare*, University Federation for Animal Welfare (UFAW) Symposium, Edinburgh, UK. 2-4 April. 13 supplement.
- Jones, R.B. and P.M. Hocking, 1999. Genetic selection for poultry behaviour: Big bad or Friend in need? *Anim. Welf.*, 8: 343-359.
- Jones, R.B., H.J. Blokhuis and H.J. Beuving, 1995. Open field and tonic immobility responses in domestic chicks of two genetic lines differing in their propensity to feather peck. *Br. Poult. Sci.*, 36: 525-530.
- Kaer, J.B., P. Sorensen, D. Gianola and G. Su, 2001. Divergent selection on feather pecking behaviour in laying hens (*Gallus gallus domesticus*). *Appl. Anim. Behav. Sci.*, 71: 229-239.
- Keeling, L.J., 1994. Feather pecking-who in the group does it, how often and under what circumstances? In: *Proceeding of the 9<sup>th</sup> Poultry Conference*, Glasgow, UK., p: 288-289.
- Manteca, Y. and J.M. Deag, 1993. Individual differences in temperament of domestic animals: A review of methodology. *Anim. Welf.*, 2: 247-268.
- Marin, R.H., P. Freytes, D. Guzman and R.B. Jones, 2001. Effects of an acute stressor on fear and on the social reinstatement responses of domestic chicks to cagemates and strangers. *Appl. Anim. Behav. Sci.*, 71: 57-66.
- Mills, A.D. and J.M. Faure, 2000. Ease of capture in lines of Japanese quail (*Coturnix japonica*) subjected to contrasting selection for fear or sociality. *Appl. Anim. Behav. Sci.*, 69: 125-134.
- Moberg, G.P., 1985. Biological response to stress: key to assessment of animal well being?, in: Moberg, G.P. (ed) *Animal stress* (Bethesda, MD, Am. Physiological Soc.).

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- Ossenkopp, K.P. and D.S. Mazmanian, 1985. The principle of aggregation in psychological correlation research: an example from the open field test. *Anim. Learn. Behav.*, 13: 339-344.
- Riedstra, B. and T.G.G. Groothuis, 2002. Early feather pecking as a form of social exploration: the effect of group stability on feather pecking and tonic immobility in domestic chicks. *Appl. Anim. Behav. Sci.*, 77: 127-138.
- Rodenburg, T.B., Y.M. Van Hierden, A.J. Buitenhuis, B. Riedstra, P. Koene, S.M. Korte, J.J. Van Der Poel, T.G.G. Groothuis and H.J. Blokhuis, 2004. Feather pecking in laying hens: new insights and directions for research? *Appl. Anim. Behav. Sci.*, 86: 291-298.
- Savory, C.J., 1995. Feather pecking and cannibalism. *Worlds Poult. Sci.*, 51: 215-219.
- Schrader, L., 2000. Consistency of individual behavioural characteristics of dairy cows in their home pen. *Appl. Anim. Behav. Sci.*, 77: 55-266.
- Spoolder, A.M.H., A.J. Burbidge, B.A. Lawrence, H.P. Simmins and A.S. Edwards, 1996. Individual behavioural differences in pigs: intra-and iter-test consistency. *Appl. Anim. Behav. Sci.*, 49: 185-198.
- Tachibana, T., 1985. Higher reliability and closer relationship between open field test measures on data aggregation. *Anim. Learn. Behav.*, 13: 345-348.
- Van Hierden, Y.M., S.M. Korte, W. Ruesink, C.G. Van Reenen, B. Engel, A.H.G. Korte-Bouws, J.M. Koolhaas and H.J. Blokhuis, 2002. Adrenocortical reactivity and central serotonin and dopamine turnover in young chicks from a high and low feather pecking line of laying hens. *Physiol. Behav.*, 75: 1-7.
- Vestergaard, K.S., J.P. Kruijtt and J.A. Hogan, 1993. Feather pecking and chronic fear in groups of red jungle fowl: their relations to dustbathing, rearing environment and social stress. *Anim. Behav.*, 62: 413-419.
- Zeltner, E., T. Klein, B. Huber and B. Eicher, 2000. Is there social transmission of feather pecking in groups of laying hens chicks? *Anim. Behav.*, 60: 211-216.