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Presence of Males Within Laying Hens Affects Tonic Immobility Response and Sociality

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Abstract: Fear can be a damaging factor, resulting in poor production and impaired animal welfare. Often fear reactions are elicited in situations that are in some way related to predator defense. As males have a guarding role, mixing the sexes might be a way of reducing fear in flocks of laying hens. Tonic Immobility (TI) is anti-predator behaviours shown by poultry and has been used in behavioural studies to measure the level of fear or stress. A runway test was used in many studies to measure level of sociality in poultry. The effect of males on duration of TI and sociality in female laying hens was studied. ISA Brown and Lohmann Tradition lines were used in each treatment. The treatments were: 1- Female hens and males mixed in a ratio of 1 male to 10 females; 2- All female hens. The presence of males had an effect ($p<0.05$) on TI-duration and Emergence Time (ET) and Social Reinstatement Time (SRT); females in the mixed-sex groups had shorter TI-duration and showed shorter latency to emerge from the start box and quicker to reinstate with a companion bird than females in the all-female groups. These results indicate that female laying hens show less signs of fear and higher level of sociality if the flock contains males.

Key words: Fear, hens, males, rearing, sociality

INTRODUCTION

Research on the welfare of domesticated birds reared for meat and egg production has identified a number of traits related in one way or another to the well-being of birds. Fearfulness and sociality of laying hens are two of them and they have been examined in different strains and ages across both rearing and laying periods (Ghareeb *et al*., 2008; Hocking *et al*., 2001). Fear, an important component of stress and duration of Tonic Immobility (TI) are considered to be a useful index of general fearfulness in fowl (Jones, 1989). TI is an unlearned state of reduced responsiveness to external stimulation also known as death feigning and is induced by physical restraint. Several factors affect the duration of TI, such as previous handling, management, social factors, housing system and genetic background (Jones, 1996; Nash and Gallup, 1976; Jones, 1986). The duration of TI has been shown to correlate positively with fear level measured by the plasma corticosterone levels and other physiological measurements such as heterophil/lymphocyte ratio (Jones *et al*., 1988). Tonic immobility is an anti-predator behaviour shown in situations where the animal has been caught by a predator (Gallup, 1977; Thompson and Liebreich, 1987). By pretending to be dead, there is a better chance to escape in an unguarded moment.

The level of fearfulness as measured by tonic immobility duration was shown to be correlated with the level of sociality (Ghareeb *et al*., 2008). Furthermore, previous studies provided values for fearfulness and sociality that could be applicable in practice for assessing laying hen welfare. Because a mismatch between underlying sociality and the bird's social environment elicits either acute stress responses or chronic distress, which damage welfare and productivity. A technique that has been used in many studies to measure the level of sociality in laying hens is to investigate the latency of birds to reinstate with a companion bird from the same group after they had emerged to a runway, also is known as social reinstatement test (Hocking *et al*., 2001; Ghareeb *et al*., 2008).

The red jungle fowl, ancestor of modern laying hens, are typically gregarious animals. The male gathers a harem of females, which he fertilizes and protects (McBride *et al*., 1969). There are many different animals predating on poultry and chickens have various ways of protecting themselves. Depending on the type of predator, they can either freeze motionless, run for cover or escape by jumping up to higher levels. Especially when the group is engaged in activities like searching for food and eating or dust-bathing, it is the males that scan the environment for possible predators and give the appropriate alarm call (McBride *et al*., 1969; Johnson, 1963; Sullivan, 1991). It is likely that males through this behaviour may also have a fear reducing effect on the flock.

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Fear reactions in poultry are elicited in situations that can in some way be related to predator-prey situations (Suarez and Gallup, 1983) such as unfamiliar environments, novel objects as well as humans, sounds and odours (Duncan, 1985; Jones, 1986). Fear responses enhance survival if animals find themselves in an adequate environment. In many modern husbandry systems, however, fear can be detrimental both to the welfare and the production of the animals. Environmental enrichment is proposed to reduce fear in poultry (Jones, 1996). One form of enrichment of both the physical and the social environment for laying hens, based on Newberry's (1995) definition "improvement of biological functioning" would be having males in the flock. Previous study was carried out to investigate the effect of males on female fearfulness at age of 32-43 weeks and indicated that female hens were less fearful when reared with males in a mixed sex groups (the ratio was ratio 1 male to 100 female) than females reared in all female groups (Ode´n *et al*., 2005). Furthermore, mating ratio of 1:1, 1:3, 1:5 and 1:11 did not affect the fearfulness of female hens at 30 wk when they are reared in 12 birds per pen or 60 birds per pen (Campo and Davila, 2002). However, the effect of males on female fearfulness and sociality were not studied at younger ages. During the rearing period including adolescence and age of maturity, fearfulness of female was higher compared with laying period (Ghareeb *et al*., 2008). But there was no data regarding the presence of males within female laying hens in the ratio of 1 male to 10 females on fearfulness and sociality of laying hens during rearing, adolescence period and age of maturity. Therefore, the present experiment was conducted to investigate the effects of males on the fearfulness and sociality of laying hens.

MATERIALS AND METHODS

Birds and housing: Two different lines of laying hens chicks (ISA Brown and Lohman Tradition) were used in the current study. The newly hatched chicks (100 females and 10 males from each line) were reared in a partially slatted deep litter system. Birds of each line were divided into two groups; 1- mixed sex group (5 males and 50 females), 2- all female group (50 females). Birds of each group were housed in pen measuring 1.75 x 6 m (width x length). Pen divisions were of wire and plastic. In each pen about 45% of the total floor was slatted and the remaining floor was covered with a 15 cm layer of wood shavings and straw. Perches were provided from one day old and wooden nests from 17 weeks of age. Pens were provided with nipple and bell drinkers and two feeders. Water and a commercial diet were available *ad libitum*. Photoperiod and heating followed standard recommendations.

Behavioural observations: The tonic immobility and runway test were done on separate days within the same tested age. Twenty birds from each group of both lines were selected at random and individually marked by wing bands with a specific number for each bird. The same birds were tested each time for either tonic immobility or runway test. The behavioural observations were carried out at 7, 10, 20 and 24 wk in a separate room adjacent to the unit and had the same conditions as the home pens. Birds were isolated from auditory and visual contact with the other birds in the test situations.

Tonic immobility reaction: To measure the level of fear in both groups (mixed sex group and all female group) of each strain (ISA Brown and LT), tonic immobility was induced as described earlier (Ghareeb *et al*., 2008). As soon as a bird was caught, by placing the bird on its back with the head hanging in a U-shaped wooden cradle. The bird was restrained for 45 s. The observer sat in full view of the bird, about 1 m away and fixed his eyes on the bird because of the fear-inducing properties of eye contact. If the bird remained immobile for 20 s after the experimenter removed his hands, a stopwatch was started to record latencies until the bird righted itself. If the bird righted itself in less than 20 s, then it was considered that tonic immobility had not been induced and the restraint procedure was repeated. If the bird did not show a righting response over the 15-min test period, a maximum score of 900 s was given for righting time.

Runway test: To measure the social reinstatement behaviour and thereby the underlying sociality of birds of each treatment (mixed sex group and all female group) in each strain (ISA Brown and LT), a runway test was used as described earlier (Ghareeb *et al*., 2008). The hen was placed in a start box measuring about 40 x 30 x 40 cm (length x width x height) with a door at one side facing the runway. The runway measured 2 x 1 m (length x width). A goal box made of wire measuring 0.4 m x 0. 6 x 0.3 m (length x width x height) was placed at the opposite end to the start box. Thus the actual length of runway was 160 cm. The goal box was used only for the stimulus bird that was selected from the same pen of the tested bird. The stimulus bird was female and changed after each block of 4 tests. The tested bird had to traverse the 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 the corridor by chalk in a straight line. The individual bird was caught from the home pen and placed in the start box for 2 min to acclimatise the bird to the environment of the box before the test began. The front door was opened using 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 (ET) was manually recorded via a stop watch. After full emergence of the tested bird from the start box, the latency until the whole bird entered the goal zone (SRT) was also recorded. If the bird did not emerge from the start box or did not enter the goal zone within 10 min, the test was stopped and the tested bird was given the maximum score of 600 s.

Statistics: Statistical analyses were conducted with the Statistical Package for Social Science (SPSS for Windows Version 12; SPSS GmbH®, Munich, Germany) to determine if variables differed between groups. The Kolmogorov Smirnov test was used to test the normal distribution of the data before statistical analysis was performed. For tonic immobility duration, the data were normally distributed therefore t-test for independent samples was used between mixed sex group and all female group in each strain. For parameters of the runway test, the data were not normally distributed therefore Mann-Whitney test was performed to find the difference between mixed sex and all female groups in each strain. Probability values of less than 0.05 ($p \leq 0.05$) were considered significant.

RESULTS

Fear levels: Female birds of both Lohmann Tradition and ISA Browns had shorter duration of tonic immobility in mixed sex group compared with all female group (Table 1). Indeed, at 10 wk female birds of mixed sex group of ISA Browns had significantly ($p = 0.001$) shorter tonic immobility duration (287 s) compared with all female group (620 s). Similarly females of mixed sex group of Lohmann Tradition had significantly ($p = 0.026$) shorter tonic immobility duration (351 s) compared with all female group (583 s). Moreover, female birds of mixed sex group of ISA Browns at 7, 20 and 24 wk had tended to have shorter tonic immobility ($p<0.1$) compared with all female group (Table 1). Furthermore, females of mixed sex group of Lohmann Tradition had a numerically shorter duration of tonic immobility compared with all female group (Table 1).

Sociality: The female birds of the mixed sex group of both Lohmann Tradition and ISA Browns were quicker to explore an unfamiliar runway than females reared in all female group (Table 2). At wk 10 females of mixed sex group of ISA Browns were significantly ($p = 0.001$) quicker (21 s) to emerge from the start box than all female group (334 s). Similarly females of mixed sex group of Lohmann Tradition were significantly ($p = 0.001$) quicker (18 s) to emerge from the start box than all female group (50 s). Moreover, females of the mixed sex group in both lines were numerically quicker to emerge from the start box compared with all female group at wk 20 (Table 2).

Table 1: The level of fearfulness measured as Tonic Immobility duration (TI) for females in mixed and all-female groups of laying hens

	Sex composition		
Age (wk)	All female group	Mixed group	P
ISA brown			
7	476	317	0.090
10	620*	287	0.001
20	497	305	0.060
24	410	240	0.082
Lohmann tradition			
7	444	347	0.302
10	583*	351	0.026
20	274	220	0.373
24	239	167	0.255

t-test for independent samples, $p \leq 0.05$ is significant

Table 2: The latency to explore unfamiliar runway for females in mixed and all female groups of laying hens

	Sex composition		
Age (wk)	All female group	Mixed sex group	P
ISA brown			
10	334*	21	0.001
20	41	28	0.495
Lohmann tradition			
10	50*	18	0.001
20	21	19	0.355

Mann-Whitney test, $p \leq 0.05$ is significant

Table 3: The latency to reinstate with a companion bird in a runway test for females in mixed and all female groups

	Sex composition		
Age (wk)	All female group	Mixed sex group	P
ISA brown			
10	256	93	0.096
20	102*	50	0.023
Lohman tradition			
10	140*	93	0.050
20	64	99	0.547

Mann-Whitney test, $p \leq$ is significant

Females in mixed sex group were quicker to reinstate a companion bird in the goal zone than all female group in both ISA Browns and Lohmann Tradition (Table 3). Females of ISA Brown mixed sex group at wk 20 were significantly quicker ($p = 0.023$) to reinstate a companion bird (50 s) compared with all female group (102 s). Females of Lohmann Tradition mixed sex group at wk 10 were significantly quicker ($p = 0.050$) to reinstate a companion bird (93 s) compared with all female group (140 s). Moreover, Females of ISA Brown mixed sex group at wk 10 tended to be quicker ($p<0.1$) to reinstate with a companion bird (93 s) compared with all female group (256 s).

DISCUSSION

In this study significant effects of males on TI duration, Emergence Time (ET) and Social Reinstatement Time (SRT) of ISA Brown and Lohmann Tradition laying hens were found. Females in mixed sex groups had shorter TI

durations and quicker to emerge and reinstate a companion bird in a runway test. This indicates that there were a reduction of fear and higher sociality in the groups that contained males in a ratio of 1:10 females. These results are in agreement with Ode´n *et al*. (2005) who found that the level of fear was reduced when laying hens were reared with males in the ratio of 1:100 females compared with those females reared alone. This could indicate that presence of males itself decrease the fear levels of females rather than the male to female ratio. It was shown that the mating ratios of 1:1, 1:3, 1:5 and 1:11 females did not affect the fear levels of laying hens, however presence of males decrease the stress of hens as indicated by Heterophil to lymphocyte ratio (Campo and Davila, 2002).

Previously TI-duration has been found to be useful when measuring the effect of different and presumingly stressful situations like transportation and handling procedures (Mills and Nicol, 1990; Scott *et al*., 1998) and new environments, but also fear in social situations (Jones, 1996; Bilcik *et al*., 1998). Riedstraa and Groothuis (2002) found that TI righting time was longer in chicks confronted with unfamiliar birds than when among familiar ones. This supports the view that TI may also be useful for measuring fear in the social environment among conspecifics, where a multifold of motivations exist. Another aspect of the social impact is that there may be great individual variation in fearfulness of hens as indicated by tonic immobility duration (Ode´n, 1996). This could possibly be related to rank order (Cunningham *et al*., 1988). However, fearfulness was shown to be correlated to sociality in laying hens; more fearful birds were quicker to emerge to unfamiliar runway and were also quicker to reinstate a companion bird in runway (Ghareeb *et al*., 2008). In the present study rearing of males within laying hens resulted in high sociality of hens reared within males compared with hens reared alone. These results could be valuable in practice to increase the sociality characteristics of laying hens and decrease their fearfulness during adolescence and age of maturity before they began to lay their eggs. Because a mismatch between underlying sociality and the bird's social environment elicits either acute stress responses or chronic distress, which damage welfare and productivity.

Environmental enrichment has been suggested as a means to reduce fear in poultry (Jones, 1996). Newberry (1995) critically reviewed the concept of ''enrichment'', which she meant has been too anthropomorphically used. She defined the term as ''improvement in the biological functioning of captive animals'' and proposed this to be measured by for example increased reproductive success and improved health. One important improvement being the reduction of harmful aggression. It has been suggested that an environment with opportunities for chickens to escape possible predators, as well as avoiding threatening conspecifics reduces aggression and makes the birds use more of their available space (Cornetto *et al*., 2002; Bizeray *et al*., 2002). The reason for this is possibly that they become less fearful. In this light, the inclusion of males in a flock may well act as such enrichment as it enhances the biological functioning by improving the early warning of possible predators.

As females in the mixed-sex groups showed significantly shorter TI-duration as well as lower latency to emerge to unfamiliar environment and to reinstate their companion birds in a runway, it can be concluded that laying hens are less fearful and more social if the flocks also contain males.

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