Effects of genetic background and social environment on feather pecking and related behavioural characteristics in laying hens

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Abstract

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Woldwide, but especially in Europe, poultry husbandry will undergo significant changes due to the prohibition of both battery cage systems and beak-trimming. In laying hens, these changes will increase the risk of feather pecking. Feather pecking is defined as the non-aggressive pecking towards the plumage of other birds. It may result in feather damage and mortality due to cannibalism, which can be considered the ultimate phase of severe feather pecking. Feather pecking may therefore have negative consequences for bird welfare and the economic situation in poultry industry. To gain further insight in risk factors related to feather pecking, this thesis investigated the effects of genetic background and social environment on feather pecking and related behavioural characteristics in laying hens. In several experiments, behaviour, performance and physiology of cage-housed birds from pure-bred genetic lines was studied in different social environments at different ages. Results indicated that birds from different pure-bred lines show differences in feather damage due to severe feather pecking (an indicator for feather pecking) and in their response towards a novel object. This indicates that it is possible to select against high levels of both feaher pecking and fear related behaviour. The tendency to develop feather pecking was also related to the response towards a novel object, although this relation differed between birds from different backgrounds and from different ages. Other results showed that the response in the novel object test was also related to performance, which should be taken into account if such a test would to be used in a breeding program. Feather pecking and fear related behaviour were also affected by group mates (social environment): non-fearful birds became more fearful in presence of fearful birds. This effect could only be established at 18, but not at 5-6 weeks of age. At adult age, fearful birds showed more feather damage in presence of non-fearful birds, whereas the social environment during rearing had no effect on the occurrence of feather pecking. This indicates that fearful behaviour predisposes adult birds both to more easily develop and to be targeted by feather pecking. The changes in social environment were, however, not accompanied by physiological changes in brain serotonine or dopamine activity. These neuro-transmission systems have been related to feather pecking. Results did indicate that the role of serotonin uptake does require further attention. According to the results from this thesis, laying hens should be kept in behavioural uniform groups to minimize the damage due to feather pecking. Additionally, reducing the expression of feather pecking could be achieved by breeding against expression of fearful behaviour, but possible correlated changes in performance should be accounted for. It remains to be investigated how the results with respect to social environment can be translated towards more extensive systems, such as floor-housing.

Voorwoord

In april 2004 ben ik in Wageningen gestart met het onderzoek naar de robuuste leghen bij de vakgroepen Fokkerij en Genetica en Adaptatiefysiologie. De exacte richting van mijn onderzoek moest toen nog bepaald worden en de nadruk kwam gaandeweg te liggen op de sociale- en gedragsmatige aspecten van verenpikken, met dit proefschrift als resultaat. In de totstandkoming van dit proefschrift, hebben de afgelopen 4 jaren, uiteraard, een verscheidenheid aan personen een bijdrage gehad.

Ik wil in eerste instantie mijn directe collega's op Zodiac bij Fokkerij en Genetica en bij Adaptatiefysiologie bedanken voor de positieve formele en informele sfeer waarin ik heb kunnen werken.

Bas, Henk (het 1^{e} jaar) en Hans zijn de afgelopen periode, ieder op hun eigen wijze, dagelijks betrokken geweest bij mijn wetenschappelijke vorming. Mijn waardering voor de energie die jullie hebben gestoken in mijn begeleiding en de vele besprekingen die we de afgelopen vier jaar hebben gehad. Johan en Bas Kemp wil ik, als leden van mijn begeleidingscommissie, bedanken voor de continue heldere kijk op de inhoud en structuur van mijn project.

Het contact met de andere twee 'Robuustheids'-AIO's was intensief, alhoewel ik denk dat het delen van een kamer met Laura en Esther zijn vruchten heeft afgeworpen. Beide bedankt voor de vele gezamenlijke uren kantoor, stal, cursus, congres en, natuurlijk, koffiepauze (waarbij ik Kimm niet mag vergeten te noemen) en kroeg!

Ook het uitvoeren en analyseren van de proeven heb ik niet alleen gedaan. In chronologische volgorde wil ik daarvoor bedanken: alle studenten (voor de veerscores), Yvonne en Liesbeth (voor alle manual restraint testjes en de hulp bij het voorbereiden van de experimenten), Albert en Sander (voor het bloedtappen) en Bart (voor hulp met de statistiek). De experimenten zijn uitgevoerd op bedrijven van I.S.A. en waren niet mogelijk geweest zonder de nadrukkelijke medewerking van Theu, Harry en Theo (Stevensbeek) en Bennie en Peter (Landhorst).

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A part of my project was spent at the Division of Farm Animal Science of the University of Bristol and I thank Christine Nicol for giving me this opportunity. The stay in Langford accelerated my own research and it was a great pleasure to collect chicken-faeces with Mike and to guide Luca's pigs through his maze ;-)!

In afgelopen vier jaar zijn, niet te vergeten, ook familie en vrienden een luisterend oor geweest. Naast vervelen, hoop ik dat ik jullie ook heb kunnen enthousiasmeren voor hetgeen waarmee ik bezig was?! Ik wil jou, Lotte, in het bijzonder bedanken als steun en toeverlaat, ook in de mindere periodes. Veel succes met jouw promoveren, het valt mee.

Gezien mijn nieuwe baan lijken de eerste voorzichtige schreden in de kippenwereld ook niet de laatste te zijn geweest.

Koen

Contents

Chapter 1:
Framework

The research presented in this thesis was carried out as part of the project 'Genetics of Robustness in Laying Hens'. The main objective of this project was to identify heritable characteristics that could be incorporated in breeding programs to improve the robustness of laying hens. A robust laying hen should, under normal physical conditions, have the potential to keep functioning and take short periods to recover under varying environmental circumstances. Worldwide, but especially in Europe, poultry husbandry in the near future will undergo significant changes due to the prohibition of both battery cage systems and beak-trimming. In the future, the poultry industry needs animals that:

1. Do not peck with intact beak,
2. Keep functioning/producing at high temperatures,
3. Keep functioning/producing at high disease challenge,
4. Keep functioning/producing with changing feed quality.

A robust laying hen will be able to cope with these environmental changes, which were, except for the change in feed quality, addressed in three PhD projects. Robustness was assumed to be related to partly genetic differences between laying hens in (possibly correlated) behavioural, immunological and physiological responses towards environmental stressors. The three projects therefore took either a behavioural, genetic or immunological approach to investigate robustness. Besides technical- also ethical considerations related to robustness need to be considered in designing commercial breeding programs.

Societal concern exists on the extent to which animals should be adapted to their environment. Indeed, managerial actions like vaccinating will remain important in order to improve health and welfare of laying hens. Breeding for improved disease resistance or for reduced occurrence of damaging behaviours can also enhance the animal's ability to cope with environmental challenges, which could, however, be achieved at the cost of the integrity of the animals. Irrespective of a possible loss of integrity, breeding for robustness can have positive effects on health and welfare. These ethical issues have been addressed in a study (Star et al., 2008) related to this project, in which it was argued ethically justifiable to incorporate robustness traits into a commercial breeding program.

Behavioural approach

The research presented in this thesis focused on genetic and behavioural risk factors for the development of feather pecking, a behavioural disorder occurring in laying hens. A robust laying hen is considered to show no feather pecking. Results indicated that birds from different pure-bred lines show differences in feather damage due to severe feather pecking, indicating that it is possible to select against feather pecking (chapter 3). In addition, feather damage due to severe feather pecking and fear related behaviour were found to be affected by group mates: non-fearful birds became more fearful in presence of fearful birds (chapter 5). Fearful birds, on the other hand, showed more feather damage in presence of non-fearful birds (chapter 6). This indicates that fearful behaviour predisposes birds both to more easily develop and to be targeted by feather pecking. Reducing the expression of feather pecking could be achieved by breeding against expression of fearful behaviour.

Genetic approach

Expression of feather pecking is affected by group mates and group- rather than individual selection might therefore be more effective in improving robustness. This was investigated in a second project, which was aimed at reducing mortality due to cannibalism by incorporating social effects into a breeding program. Selection on group rather than on individual level has been shown to be effective in reducing mortality due to cannibalism. A theoretical framework based on genetic effects of individuals on group performance was therefore developed (Bijma et al., 2007a, Bijma et al., 2007b) and subsequently tested using commercial, pure-bred lines. On individual level, so-called associative effects were indeed found heritable (Ellen et al., 2008). It is currently tested if incorporation in a breeding program will reduce mortality due to cannibalism.

Immunological approach

In the third project, effects of genetic background, environmental conditions and early-life experiences on immunological parameters related to robustness were investigated. It was found that birds from different genetic background clearly differ in their immunological and physiological response to high temperatures and disease challenge (Star et al., 2007a). Results further indicated a predictive value for the level of natural antibodies (indicative for the innate immune system) binding to KLH (Keyhole Limpet Hemocyanine; a protein which laying hens will normally never encounter) for survival of the laying period of laying hens (Star et al., 2007b). Since natural antibodies have a moderate heritability, it might be possible to select for this trait. Selection on innate immune parameters will probably not be on the expense of hen-day egg production, as these traits are most likely not correlated. The main conclusion was therefore that implementation of selection for natural antibodies into a breeding goal might improve robustness of laying hens.

Chapter 2:
General Introduction

Introduction

The keeping of wild and domesticated animals in captive environments reduces the possibility of these animals to fully express their behavioural repertoire. The keeping of migratory birds in zoo enclosures or the unavailability of substrate to perform dust bathing by laying hens in battery cages are examples of the restrictions an environment can put on the expression of specific behaviours. Such animals have an increased risk to develop abnormal behaviour. Pacing in zoo-populations of polar bears (Ross, 2006), feather picking in captive-populations of parrots (Van Hoek and King, 1997) or tail-biting in domesticated pigs (Schrøder-Petersen et al., 2004) are examples of such abnormal behaviours which are possibly expressed in an animals' attempt to cope with the sub-optimal conditions it is exposed to (Broom, 1991a, 1991b). That not all animals develop abnormal behaviour in captivity indicates that individual differences exist in the susceptibility to develop these behaviours.

Feather pecking

Feather pecking in domestic laying hens is another example of such an abnormal behaviour. Feather pecking is defined as non-aggressive pecking towards the plumage of other birds and two major forms of feather pecking can be distinguished: gentle and severe feather pecking. Gentle feather pecking does not cause feather damage and may have a social, exploratory function, especially early in life (Riedstra and Groothuis, 2002). Severe feather pecking, on the other hand, is more forceful and causes feather- and even tissue damage (Savory, 1995). Gentle and severe feather pecking are uncorrelated behaviours (Rodenburg et al., 2004a; Newberry et al., 2007). Feather damage and mortality due to cannibalism, which can be considered the ultimate phase of severe feather pecking, constitute welfare and economic problems in the current poultry industry.

Risk factors

The risk to develop feather pecking depends on the interaction between the genetic and environmental factors a bird is exposed to during the incubation-, the rearing- and the laying period (Figure 1). Difference between lines, differences within lines and epigenetic effects constitute the genetic factors which contribute to the risk to develop feather pecking. The environmental factors not only include the physical aspects of the housing system (e.g. nutrition, lighting etc.), but also the social aspects, i.e. the group mates an individual bird has to interact with. Effects of conditions during incubation on feather pecking have not been studied yet, although it is assumed that these may well exist. Recent studies indicate that behaviour, for instance feather pecking, may even depend on the environment in which grandparent animals were kept (Figure 1). In the following, some of the risk factors will be discussed, after which the aims and outline of this thesis will be described.

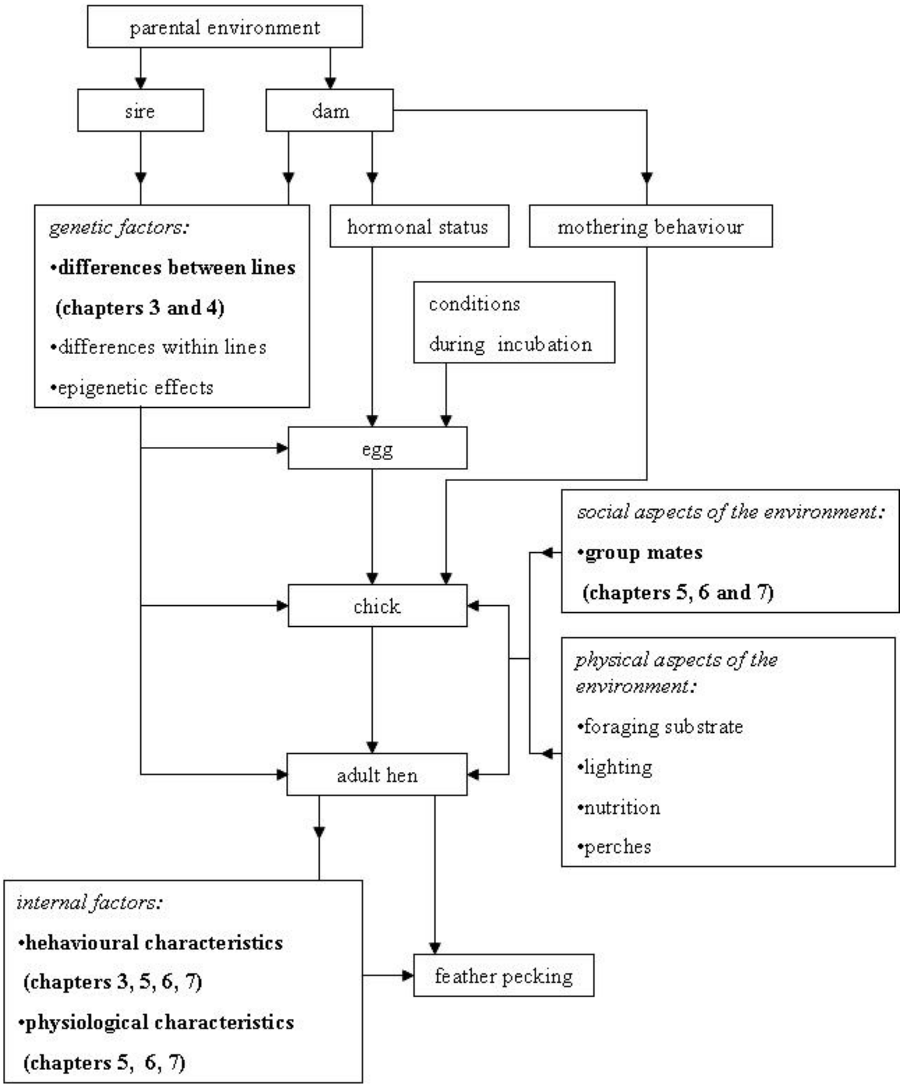

Figure 1: Schematic representation of the factors which, according to current knowledge, may contribute to the risk to develop feather pecking under commercial conditions. The factors indicated in bold will be studied in this thesis.

Genetic factors

It is generally agreed upon that genetic factors contribute to the development of feather pecking (Rodenburg et al., 2004b; Sedlačková et al., 2004). Birds originating from different lines (Hocking et al., 2004) as well as birds originating from the same line (Kjaer and Sørensen, 1997) may differ in the tendency to develop feather pecking. Recently, heritable differences in 'sociality' (Bijma et al., 2007a) and in stress-induced gene-expression (Lindqvist, 2007) provided new insights on the behavioural transmission between parents and their offspring.

Differences between lines

Differences between genetic lines indicate that there is genetic variation in feather pecking (Hocking et al., 2004) and that specific genetic lines might preferably be used in order to reduce feather pecking. The ranking of specific genetic lines in terms of feather pecking is, however, not fixed but influenced by environmental conditions. Kjaer and Sørensen (2002) found, for instance, that early access to a range area reduced feather pecking in two specific genetic lines, but enhanced feather pecking in another genetic line.

Rhode Island Red and White Leghorn lines

The genetic lines that are used in this thesis are either from Rhode Island Red (producing brown eggs; RIR) or from White Leghorn origin (producing white eggs; WL). In a floor housing system, a WL and an ISA brown line (comparable with RIR) were recently found to differ in feather damage at 37 weeks of age (Kjaer and Sørensen, 2002). The difference in feather damage was accompanied by a difference in feather pecking behaviour between these lines, which was, however, not significant (Kjaer and Sørensen, 2002). In another study (Kjaer, 2000), also in a floor housing system, similar differences in feather pecking at 38 weeks of age were found between two WL lines and two medium heavy-strain lines (comparable with RIR). The ranking of RIR and WL lines in terms of feather pecking might, however, be different in another housing system, for instance, in battery cage systems.

Differences within lines

Genetic variation in feather pecking also exists within genetic lines, allowing selection against feather pecking on individual level. Reports on heritability estimates for feather pecking range from 0.00 (Rodenburg et al., 2003) to 0.38 (Kjaer and Sørensen, 1997). Genetic selection is therefore expected to result in reduction of feather pecking in subsequent generations. The effects of divergent selection on feather pecking were shown by (Kjaer et al., 2001). They found less feather pecking after two (1.37 vs. 3.10 feather pecking bouts per bird per hour) and three generations of selection (0.63 vs. 4.56 feather pecking bouts per bird per hour) in birds from the low compared with the high feather pecking line respectively. Under commercial conditions, direct selection against feather pecking requires collection of sufficient observations, which is time-consuming.

Group selection

Selection on group- rather than on individual performance was found to be effective in reducing mortality due to cannibalism (Craig and Muir, 1996; Muir, 1996). A theoretical framework based on genetic effects of individuals on group performance (Muir, 2005; Bijma et al., 2007b; Bijma et al., 2007a) could possibly explain the results found by Muir (1996) and by Craig and Muir (1996). In laying hens, these 'social' models resulted in a larger genetic variation for mortality due to cannibalism than traditional models which use individual observations only (Ellen et al., 2008). Hence, greater progress in reducing mortality due to cannibalism could be made with these selection techniques rather than with the traditional ones. The results are less surprising if one would consider that expression of feather pecking as a 'social' behaviour is affected by victim characteristics as plumage pigmentation and activity level (Keeling et al., 2004; Riber and Forkman, 2007). In this sense, group selection against feather pecking or mortality due to cannibalism is expected to alter the phenotype of both performers and victims of feather pecking, although its effects on behaviour are largely unknown (Rodenburg et al., 2008).

Epigenetic factors

The difference in learning ability between laying-hen offspring from stressed and non-stressed parents has recently been suggested to be caused by different inheritance of gene expression patterns between these parental groups (Lindqvist, 2007). These different patterns were suggested to result from direct parent-offspring transmission or from indirect effects of maternal hormones in the egg on gene expression of the embryo. Learning ability as such has not been linked to feather pecking, but these results do suggest that additive genetic variance is not the only source of heritable genetic variance which should be explored when studying feather pecking.

Maternal factors

Presence of a mother hen was found to reduce fear related behaviour in young chicks (Roden and Wechsler, 1998; Perre et al., 2002). High fear related behaviour in young chicks has been related to increased feather pecking at adult age (Rodenburg et al., 2004a). The presence of a mother hen did, however, not have direct effects on occurrence of feather pecking at adult age (Roden and Wechsler, 1998; Riber et al., 2007). In rodents, fear related behaviour was affected in offspring as well as in grand offspring of dams showing impaired maternal care (Curley et al., 2008). These results suggest that behavioural problems related to fear and feather pecking partly originate from the absence of maternal care under commercial conditions. Deprivation of maternal care may negatively affect these behaviours in offspring, even across several generations. Actual presence of a mother hen in commercial farming systems does not seem feasible, but rearing laying hens under more natural conditions could potentially reduce behavioural problems related to feather pecking and fear (Rodenburg et al., 2008).

Hormonal status of the mother hen

In birds, offspring behaviour is affected by the deposition of maternal hormones in the egg (Eising et al., 2006). Egg corticosterone concentrations, for instance, are a direct reflection of these concentrations in the mother hen and have an influence on embryonic development (Hayward and Wingfield, 2004). Alterations in egg corticosterone concentrations have been found to affect fear related behaviour in offspring from these eggs (Janczak et al., 2006). Stress-induced elevations of corticosterone concentration in the mother could thus affect offspring behaviour, i.e. feather pecking, by the mediating effects of egg corticosterone on embryonic development.

Internal factors

Apart from their genetic background, other internal factors such as their behavioural, and physiological characteristics also differentially predispose birds to develop of feather pecking.

Behavioural characteristics of feather pecking

At young age, birds characterized as high feather peckers were less active in the open field (Jones et al., 1995) and more active in the manual restraint test (Van Hierden et al., 2004b) than birds characterized as low feather peckers. No difference in tonic immobility response was found (Jones et al., 1995). It was hypothesized that fearful birds in the open field at young age were at a higher risk to develop feather pecking at later age than less fearful birds (Rodenburg et al., 2004a). Because tonic immobility response did not differ between birds from a high and low feather pecking line, social motivation was suggested to be another risk factor in the development of feather pecking (Jones et al., 1995).

At adult age, however, birds showing most open field activity showed most feather pecking behaviours (Rodenburg et al., 2004a; Newberry et al., 2007). The different correlations between feather pecking and open field activity at either young or adult age could be explained by an age-dependent change in the expression of fearfulness. Low open field activity could reflect high fearfulness at young age, but low fearfulness at adult age (Candland and Nagy, 1969). Another study found that adult birds that were more active in their home-cage also showed more feather pecking (Newberry et al., 2007). The higher activity could be necessary to approach the birds at which feather pecking is targeted.

Physiological characteristics of feather pecking

Birds from a high feather pecking line were found to have lower baseline as well as stress induced corticosterone levels (consistent at young and adult age; (Korte et al., 1997;

Hierden et al., 2002). Both baseline adrenaline and nor-adrenaline concentrations as well as stress-induced adrenaline concentration did not differ between these same lines, whereas stress-induced nor-adrenaline was found higher in birds from the high feather pecking line (Korte et al., 1997). Birds from the high feather pecking line were subsequently characterized as having an increased sympathetic, but reduced hypothalamic-pituitary-adrenal (HPA) stress response, whereas birds from the low feather pecking line as having a reduced sympathetic, but increased HPA stress response. These distinct stress responses show similarities with the two coping styles as assumed to exist in rodents and pigs (Benus et al., 1989; Benus et al., 1990; Hessing et al., 1994). Based on these similarities, it was hypothesized that high feather pecking birds would represent the proactive coping style, whereas low feather pecking birds would represent the reactive coping style. This hypothesis seemed, however, not in agreement with the higher open field activity at young age found for birds from a low- as compared with birds from a high feather pecking line. It was argued that inability of high feather peckers to successfully interact with their (social) environment, predisposed them to more easily develop routine like-behaviours, i.e. feather pecking (Korte et al., 1997).

The brain serotonergic as well as the dopaminergic neurotransmission systems have both been related to the occurrence of abnormal behaviour in animals (Dantzer, 1986; Chaouloff, 2000). Serotonin and dopamine turnover levels at brain-level were, for instance, higher in birds from a low than in birds from a higher feather pecking line (Hierden et al., 2002). Stimulation of the serotonergic neurotransmission system by dietary supplementation of the serotonin precursor L-tryptophan (Van Hierden et al., 2004a) and inhibition of this system by administration of an serotonin auto-receptor agonist (Van Hierden et al., 2004b) resulted in an expected decrease or increase of feather pecking. Administration of haloperidol, a dopamine-antagonist, reduced feather pecking in adult laying hens, probably by stimulating dopaminergic neurotransmission (Kjaer et al., 2004). Recently, it was reported that administration of either serotonin 1A or 1B receptor-antagonists differently increased feather pecking in birds selected for either low or high group productivity and survivability respectively (Curley et al., 2008; Dennis et al., 2008). The function of these serotonergic receptors in the development of feather pecking might therefore be different for birds from different genetic backgrounds. It was, however, reported by Van Hierden (2003) that increased expression of feather pecking was similarly related to decreased serotonin-turnover in birds from two genetic lines (based on equal regression coefficients of serotonin turnover on feather pecking in both lines). This indicates a causative effect of serotonin-turnover on feather pecking, which is independent from genetic background.

Environmental factors

Genetic differences between birds will differentially predispose birds to develop feather pecking. Modification of environmental factors such as light and nutrition, or of substrates which are required to express behaviours like perching, however, enables farmers to effectively reduce the risk of feather pecking to develop.

Physical aspects of the environment

Under natural conditions, laying hens spent more than 90% of the daytime foraging (Dawkins, 1989). For laying hens in captivity, expression of foraging behaviour will thus have a high priority and inability to express this behaviour could result in pecking directed at plumage of conspecifics rather than at food particles (Blokhuis, 1986). In order to reduce feather pecking, it therefore seems sensible to at least provide laying hens with the opportunity to perform foraging behaviour. Keeping birds in battery cages rather than in pens, for instance, indeed leads to more feather pecking as birds will be less able to express foraging without an appropriate substrate available (Blokhuis, 1986). Feather pecking will also develop if other behaviours, i.e. dustbathing (Vestergaard and Lisborg, 1993) can not be expressed. In a recent study, however, foraging rather than dust bathing behaviour was found to have the strongest association with feather pecking (Huber-Eicher and Wechsler, 1997).

Perching is another component of laying hen behaviour. Possibility to perch during the rearing period improved plumage quality and reduced mortality due to cloacal cannibalism in the laying period (Gunnarsson et al., 1999). The importance of the rearing period was also emphasized in another study, in which a low light intensity (3 vs. 30 lux) until 15 weeks of age led to less severe pecking at 45 weeks (Kjaer and Vestergaard, 1999). Low light intensities generally result in less feather pecking, although it could be a disadvantage for the development of perceptual mechanisms involved in other behaviours (Kjaer and Vestergaard, 1999).

Nutritional factors such as feed form and dietary content may have their effects on feather pecking. Providing a mash rather than a pelleted diet reduces the occurrence of feather pecking (Van Krimpen et al., 2005). Such effects are probably explained by the increased time hens spend foraging when provided a mash diet. Protein deficient diets generally increase the risk of feather pecking and additional dietary protein will, conversely, reduce this risk (Van Krimpen et al., 2005). In a study by (Van Hierden et al., 2004a), for instance, gentle feather pecking was reduced by adding tryptophan to the diet. Increased levels of tryptophan, a precursor of serotonin, may have stimulated serotonergic neurotransmission. Increased activity of the serotonergic system has been related to the decreased expression of feather pecking (Van Hierden et al., 2004b). Alternatively, feathers contain more than 90% protein and protein deficient diets may consequently result in impaired feather development (Van Krimpen et al., 2005), which increases the risk of feather pecking (McAdie and Keeling, 2000).

Social aspects of the environment

Expression of behaviour can be affected by interaction with group mates (Nicol, 1995). Occurrence of such effects have been studied, amongst others, by exposing naive birds to birds which are conditioned in performing a specific task or by intermingling birds from different genetic lines with distinct behavioural characteristics (Noble et al., 1993; Nicol and Pope, 1999). Application of these methods has shown that expression of foraging (Noble et al., 1993), 'key-pecking' (Nicol and Pope, 1999; Zeltner et al., 2000), tonic immobility response (Campo et al., 2005) and of feather pecking (McAdie and Keeling, 2002) could possibly be affected by group mates. The underlying motivations by which either foraging and 'key-pecking' or tonic immobility response and feather pecking are affected, could, however, be quite different. Visual perception of a bird engaged in foraging or 'key-pecking' (with a subsequent reward) may decide the 'observer' bird to perform similar behaviour. According to Zeltner et al. (2000), introduction of high feather pecking birds also leads to spread of feather pecking by social transmission. Intermingling high and low feather pecking birds may, however, provide a relatively stressful environment for birds from a low feather pecking line. The stressful environment rather than the observation of another bird performing feather pecking behaviour, may cause birds from a low feather line to actually engage in feather pecking (McAdie and Keeling, 2002).

That behaviour of group mates can indeed cause stress was suggested by Cheng et al. (2002). In their study, presence of a standard competitor bird differentially affected plasma corticosterone and dopamine concentrations in birds selected for either high or low group productivity and survivability. Plasma dopamine concentrations in absence of a standard competitor bird, differed similarly between birds from these genetic lines (Cheng et al., 2001). As the study by Cheng et al., (2002 did not include the required control groups without the standard competitor bird, it can therefore not be generally assumed that the difference in corticosterone or dopamine concentrations are simply due to presence of the competitor bird.

Aim and outline of this thesis

The experiments as conducted in this thesis had three main objectives: i) to investigate the genetic variation in feather pecking and in response towards a novel object among 12 purebred lines from either Rhode Island Red (RIR) or White Leghorn origin (WL; chapter 3), ii) to investigate the use of the novel object test to predict performance in birds from RIR origin (chapter 4) and iii) to investigate the effects of mixed housing of birds from RIR and WL origin on feather pecking and related behavioural and physiological characteristics (chapters 5, 6 and 7). Chapter 8 contains the summarizing discussion.

Next to the genetic differences in feather pecking and response towards the novel object, it was particularly investigated in chapter 3 if the response towards the novel object in battery-caged laying hens from RIR or WL origin was able to predict feather damage due to severe feather pecking. In chapter 4, it was subsequently investigated if the response towards a novel object was also possibly able to predict performance parameters, like egg production and mortality, in birds form RIR origin.

The remaining chapters 5, 6 and 7 focused on effects of social environment, which were defined as either pure- (groups with birds from one genetic origin) or mixed groups (groups with birds from both genetic origins). It was first investigated if these social environments at young age affected behavioural and physiological responses in two fear eliciting test situations. And, secondarily, if possible occurrence of these effects was age-dependent (chapter 5). In chapter 6, it was investigated if possible effects of social environment on these fear-elicited responses at young age, would be accompanied by changes in feather pecking at adult age. Possible carry-over effects of social environment during rearing period on feather pecking in the laying period were also investigated. Finally, in chapter 7, RIR and WL birds from pure and mixed groups were characterized in terms of their physiological responses, which could be causally related to the tendency to develop feather pecking.

Chapter 3:

The novel object test as predictor of feather damage in cage-housed Rhode Island Red- and White Leghorn laying hens.

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Abstract

The propensity to develop feather pecking (FP) has a genetic component and has been related to fear responses in laying hens. A convenient test measuring the fear response might therefore be used to identify hens with a stronger propensity to develop FP. However, genetic origin and age can influence the relation between fear response and FP. The present study investigated the use of a novel object test in the home cage as predictor of FP in 6 lines of laying hens from Rhode Island Red origin, and 6 lines from White Leghorn origin at adult age. Incidence of FP was assessed by scoring feather damage at back, rump and belly at 35, 43, 51, 63 and 69 weeks of age. A novel object test, measuring fear response to a rod, was performed at 23, 46 and 69 weeks of age. For RIR lines, a stronger fear response at 23 weeks was related to decreased feather damage at 51 weeks and for both RIR and WL lines stronger fear responses at 46 weeks were related to increased feather damage at 51 weeks. These results suggest that the relation between FP and fear response differs between ages and between Rhode Island Red and White Leghorn lines.

Introduction

The European poultry industry is facing significant changes in legislation concerning welfare of laying hens. In the near future, the battery cage system (under EU Council directive 99/74/EC) and beak trimming in some EU member countries (depending on national legislation) will be banned, which is assumed to increase damage due to feather pecking (FP) and cannibalism (Jendral and Robinson, 2004). FP behaviour in laying hens is the non-aggressive pecking towards the plumage of other birds. FP without removal of feathers is also known as gentle FP and is usually ignored by the recipient. Severe FP is more forceful than gentle FP and leads to damage and loss of feathers (Savory, 1995). Without beak trimming, FP is more likely to result in haemorrhages, which may eventually lead to mortality due to cannibalism.

It is known that genetic background and environmental factors such as housing system, feed composition and light (colour, intensity and frequency) play a role in the development of FP (Sedlačková et al., 2004). Several studies have investigated differences in feather pecking between commercial and/or traditional lines of laying hens (i.e. Hughes and Duncan, 1972; Hocking et al., 2001; Hocking et al, 2004; Kjaer, 2000; Kjaer and Sørensen, 2002). Based on these studies it can be concluded that differences in line or origin, White Leghorn (WL) or Rhode Island Red (RIR), influence the occurrence of FP at different ages. Methods to assess the incidence of FP in these studies involved direct observations of FP or indirect measurements on plumage condition; it has been shown that higher levels of (severe) FP using direct observations are correlated to poorer plumage condition scores (Bilčik and Keeling, 1999; Kjaer, 2000; Kjaer and Sørensen, 2002; Hocking et al., 2004).

Breeding companies are currently trying to develop lines with a reduced propensity to perform FP. In order to efficiently select against FP, a test is needed that accurately predicts

FP behaviour while damage to the selection candidate is avoided. One such test could be the response to a novel object test. Novel object tests are used to measure the degree of fearfulness (Jones, 1996). Fearfulness can be defined as the negative emotional state that results from perception of potential danger (Boissy, 1998) and it was found to have a genetic component in the domestic laying hen (Faure, 1981). Excessive fearfulness in itself may result in impaired welfare and it has also been found to have a negative impact on performance (Jones, 1996; Jones et al., 1997; Schutz et al., 2004). Elevated levels of fearfulness have been related to increased expression of FP (Jones et al., 1995; Rodenburg et al., 2004; Buitenhuis et al., 2005). Although opposite relations have been reported (Rodenburg et al., 2004; Jensen et al., 2005). Other studies were unable to detect a relation whatsoever (Hocking et al., 2001; Albentosa et al., 2003a; Albentosa et al., 2003b; Hocking et al., 2004). Possible explanations for these contradicting results include the method of measuring fear (i.e. novel object, open field test or tonic immobility), test environment (home cage/pen or experimental setting), type of line used (RIR or WL) and age of testing.

The aim of the present study was to evaluate response to a novel object test in the home cage as a predictor of FP. It has been suggested that the way fearfulness is expressed in chickens is age dependent (Candland and Nagy, 1969; Rodenburg et al., 2004). Therefore, different relations between FP and fear responses may be found at different ages. In this paper we report observations on the development of fear and FP during the entire laying period in 12 commercial, purebred lines of laying hens of White Leghorn and Rhode Island Red origin.

Material and Methods

The 12 purebred, commercial lines of laying hens used in this experiment had intact beaks and were housed in cages, both during the rearing and experimental period. These lines were either of Rhode Island Red type (RIR; laying brown eggs) or of White Leghorn type (WL; laying white eggs). Number of hens and cages for each line and for each origin are presented in table 1. Routine vaccinations against Marek's disease, New Castle disease, infectious bronchitis, infectious bursal disease, fowl pox and avian encephalomyelitis were applied during rearing.

Table 1: Distribution of hens and cages in the experiment over both origins and 12 lines

Rhode Island Red			White Leghorn		
line	number of hens	number of cages	line	number of hens	number of cages
B1	200	50	W1	198	49
B2	200	50	WA	210	53
B3	180	46	WB	204	51
BA	200	50	WC	233	61
BB	245	65	WD	206	56
BE	230	60	WF	200	50
total	*1255*	*321*	*total*	*1251*	*320*

During the experiment, all hens were housed in cages on the middle tier of a three level battery system for a period of one year (June 2004 until June 2005). Cages measured 44 cm (height) x 46 cm (depth) x 39 cm (width). Each cage housed four hens which equals 448,5 cm^2 per hen. Feed was available ad libitum from a trough measuring 39 cm (=total width of cage). From the beginning of the experiment (at 19 weeks of age) until 42 weeks of age hens were fed a standard commercial phase 1 diet (159 g/kg crude protein, 43 g/kg crude fibre and 11.17 MJ ME/kg). From 42 weeks onwards, until the end of the experiment, a standard commercial phase 2 diet (152 g/kg crude protein, 47.0 g/kg crude fibre and 11.01 MJ ME/kg) was given. Water was available ad libitum by two drinking nipples, placed in between two adjacent cages, and accessible from both cages. Wing bands allowed individual identification of the hens. Except for the experimental observations, contact between hens and humans occurred frequently: stockpersons collected and registered dead hens daily and manually collected eggs three times a week.

Hens arrived at the laying facility at 17 weeks of age and were kept at 9L: 15D light scheme (light from 7.00 until 16.00 hrs). After one week, the light period was increased with half an hour, starting at 6.30 hrs. Hereafter, the light period was increased with approximately 10 min / day. From 30 weeks onwards hens received light from 00.00 until 16.00hrs (16L : 8D).

Feather damage

Feather damage was assessed at 35, 43, 51, 63 and 69 weeks of age by evaluating the plumage condition on back and rump following the classification of Bilčik and Keeling (1999; range 0-5, higher scores indicating more damage). Damage to rump and back area were combined into one score: *backrump* = individual back score + individual rump score (ranging from 0 to 10). The mean *backrump* score was calculated for each cage at each timepoint.

Novel Object test

A novel object test (NOT) was performed at 23, 46 and 69 weeks of age. A wooden rod with a white tip was used as a novel object. Before an experimental cage was exposed to the wooden rod for a period of 30 seconds, a test person stood still for 10 seconds in front of the cage, hereafter he placed the rod on the middle of the feed trough with the white tip pointing through the bars into the experimental cage. Response to presence of rod in front of the cage was scored as follows:

1: hens within cage approaching the rod
2: hens within cage holding still
3: hens within cage moving away from the rod

Individual hens within a cage responded alike. Two test persons tested all cages in one day between 7.30hrs and 16.00hrs (at all three ages) and test sequence was also recorded. Prior to the experiment scoring methods of both test persons were brought into conformity. After testing a cage, four cages were skipped before testing the next cage. At the end of a row, the test person moved back to the beginning of the row to test the next sequence of cages. If the rod was accidentally touched and/or removed out of position and sight, then no score was assigned.

Statistical analyses

All analyses for plumage condition and novel object test were done using SAS® 9.1.3 (SAS Institute Inc., 2002-2003).

Feather damage. For 35, 43, 51, 63 and 69 weeks, separate analyses were performed to test effects of origin and line within origin on backrump cage means using the GLM procedure.

Novel Object test. Spearman correlations were calculated between NOT scores at 23, 46 and 69 weeks for each line separately. Out of 36 within line correlations, only 5 were found significantly positive ($P<0.05$). Therefore analyses of NOT was performed separately for 23, 46 and 69 weeks of age using the GENMOD procedure for a multinomial distribution with a cumulative logit link function. At each age, models tested effects of origin, line within origin on NOT score while adjusting for order of testing. Estimated probability levels equalled $e^x/(1+ e^x)$, with x = solution obtained from estimate statement in GENMOD analysis including effects tested significant according to table 3. Because mean line probabilities of moving away (score 3) and approaching (score 1) the rod were highly correlated at 23 (-0.90), 46 (-0.84) and 69 (-0.81) weeks, only probabilities of moving away from the rod (score 3) were analyzed.

Relation between novel object test and FP. It was decided to use backrump scores at 51, 63 and 69 weeks, because from 51 weeks onwards >50% of the lines (8 out of 12) had a mean backrump score >2, which is defined as ‘more damaged feathers’ (Bilčik and Keeling, 1999).

Main interest was to evaluate the relation between NOT and *backrump* score within lines. The only relation between lines was evaluated by regression of mean line *backrump* score at 51 weeks on mean line NOT score at 23 weeks.

For within line relations, separate analyses for 23 and 46 weeks and for each age, separate analyses for estimation of regression coefficients and least square means were performed. Composition of the models to estimate regression coefficients and least square means are presented in table 2.

Table 2: Model composition for estimation of regression coefficients and least square means for dependent *backrump* scores at 51 and 69 weeks and independent NOT scores at 23 and 46 weeks of age.

data source	model	age of NOT	number of analyses	included effects					
				origin	line (origin)	1)NOT score			
						2)b_{23}	b_{46}	class$_{23}$	class$_{46}$
all	I	23	1	X	X	X			
		46	1	X	X		X		
	II	23	1	X	X			X	
		46	1	X	X				X
within origin	III	23	2		X	X			
		46	2		X		X		
	IV	23	2		X			X	
		46	2		X				X

1) b_{23}=regression coefficient of backrump at 51 on NOT score at 23 weeks; b_{46}= regression coefficient of backrump at 51 on NOT score at 46 weeks; class$_{23}$= (class) effect of NOT score at 23 weeks on backrump at 51 weeks; class$_{46}$= (class) effect of NOT score at 46 weeks on backrump at 51 weeks.

2) in model I at 23 weeks, residual NOT scores were adjusted for origin, line(origin) and order of testing effects and at 46 weeks for origin and line(origin) effects. In model III, residual NOT scores at 23 weeks were adjusted for line and order of testing effects and at 46 weeks for line effects.

The relation between NOT at 23 or 46 and *backrump* at 51 weeks was investigated on two levels: with all data (table 2; models I and II) and within each origin (models III and IV). In model I at 23 weeks, residual NOT scores were adjusted for origin, line(origin) and order of testing effects and at 46 weeks for origin and line(origin) effects. In model III, residual NOT scores at 23 weeks were adjusted for line and order of testing effects and at 46 weeks for line effects. The GLM procedure was used for estimation of regression coefficients, least square means and obtaining residuals of NOT scores.

Because distributions of NOT responses within lines were heavily skewed, interaction effects of lines with NOT responses were not included in the models.

Results

Feather damage

Origin and line within origin were found to affect *backrump* cage means at every age (table 3).

Table 3: Significance of origin- and line-effects on *backrump* score at 35, 43, 51, 63 and 59 weeks and on NOT score at 23, 46 and 69 weeks of age, indicated by F- and Chi-square values respectively.

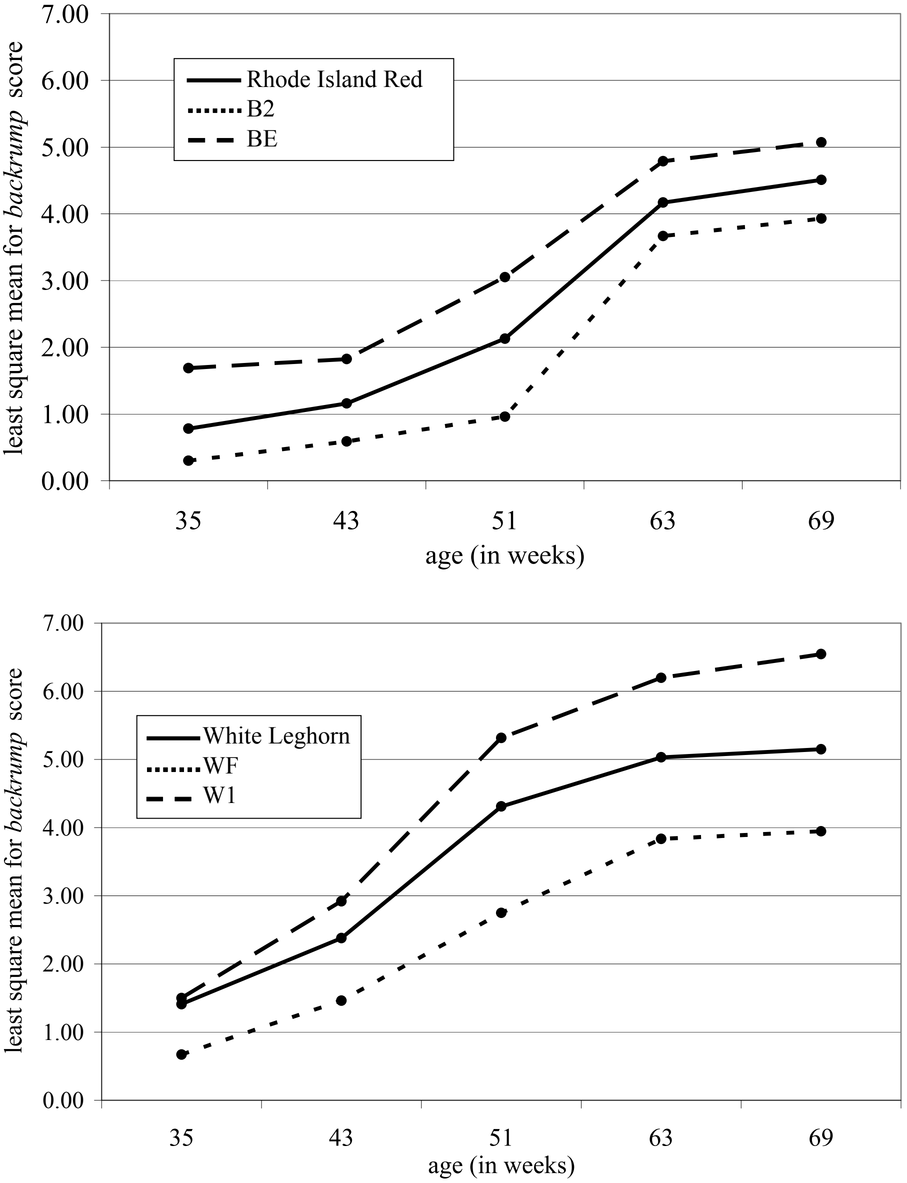
source[1]	d.f.[2]	*backrump* age (weeks)					NOT age (weeks)		
		35	43	51	63	69	23	46	69
origin	1	30.6	87.3	300.8	62.8	36.8	121.4	420.9	423.4
line (origin)	10	6.2	5.1	14.4	12.6	13.2	79.1	115.0	114.7

1) all F- and Chi-square values were significant at P<0.001.

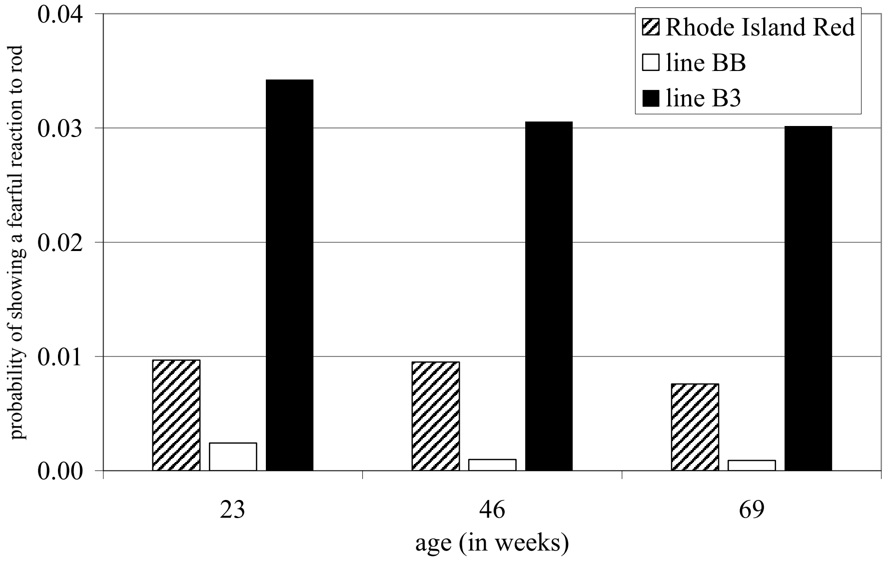
2) NOT score at 23 weeks was found to be affected by order of testing $(\chi_1^2 = 5.25; P < 0.05)$. Cages with higher order of testing showed less moving away from the rod. 2) d.f.= degrees of freedom

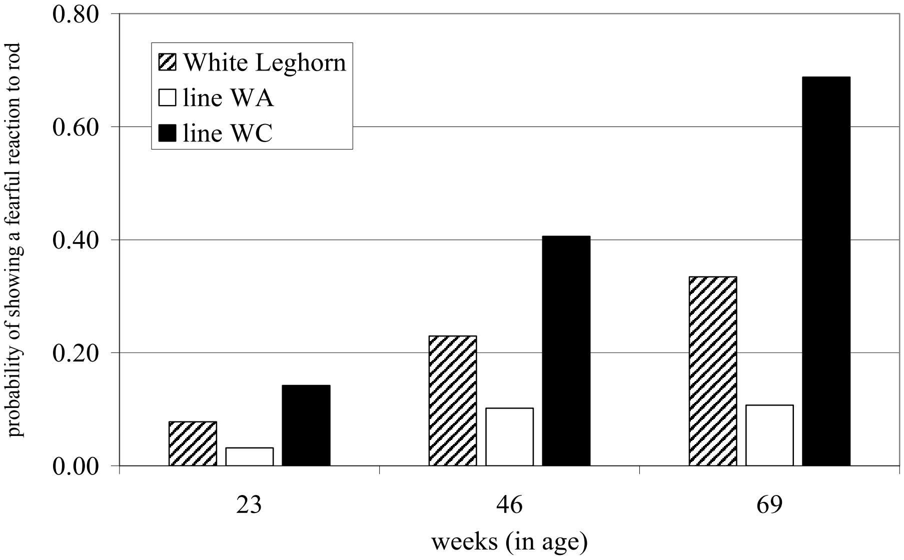
Backrump cage means increased with age and were higher for White Leghorn lines than for Rhode Island Red lines at all ages. Rhode Island Red lines had significantly lower *backrump* least square means than White Leghorn lines (P<0.05, after bonferroni adjustment; see figures 1a and 1b) at 35 (0.78 ± 0.05 vs. 1.41 ± 0.09), 43 (1.16 ± 0.07 vs. 2.38 ± 0.11), 51 (2.13 ± 0.08 vs. 4.31 ± 0.09), 63 (4.17 ± 0.06 vs. 5.03 ± 0.08) and 69 (4.51 ± 0.06 vs. 5.15 ± 0.08) weeks of age.

The range of *backrump* scores between Rhode Island Red lines at different ages is shown in figure 1a. At all ages, line B2 had lowest and line BE had highest *backrump* least square means. The range of *backrump* scores between White Leghorn lines at different ages is shown in figure 1b. At all ages, line WF had lowest and line W1 had highest *backrump* least square means (except for 35 weeks when line WA had the highest *backrump* least square mean).



Figures 1a and 1b: *Backrump* least square means at 35, 43, 51, 63 and 69 weeks of age for Rhode Island Red (1a; top panel) and White Leghorn (1b; bottom panel) lines. Development of *backrump* score with age for the most extreme WL lines is also indicated.





Figures 2a and 2b: Probabilities of showing score 3 to *rod* for Rhode Island Red (2a; top panel) and White Leghorn (2b; bottom panel). Development of fear related behaviour for two individual RIR and WL lines with lowest and highest probability of showing score 3 at 69 weeks is also indicated. Estimated probability levels equalled $e^x/(1+ e^x)$, with x = solution from estimate statement in GENMOD analysis including effects tested significant according to table 3. At 23 weeks of age, mean testing order for each origin or individual line at 23 weeks was included in their corresponding estimations.

Novel object test

Effects of origin and line within origin on NOT score were significant at 23, 46 and 69 weeks (table 3). At 23 (0.01 vs. 0.08), 46 (0.01 vs. 0.23) and (0.01 vs. 0.33) Rhode Island Red lines (figure 2a) had significantly lower probabilities (P<0.001) to move away from the rod (score 3) than White Leghorn lines (figure 2b).

The range of NOT scores between the Rhode Island Red lines at different ages is shown in figure 2a. At every age, line BB had lowest and line B3 had highest probabilities to move away from the rod. The range of NOT scores between the White Leghorn lines at different ages is shown in figure 2b. At 23, 46 and 69 weeks of age, line WA had lowest and at 69 weeks line WC had highest probability to move away from the rod. At 23 weeks line WF (0.20) and at 46 weeks line W1 (0.51) had highest probability to move away from the rod.

Relation between feather damage and novel object test

The relation of NOT score at 23 weeks with *backrump* score at 51 weeks between all lines was positive (b = 2.32 ± 1.13), but non-significant (NS; P=0.07; figure 3). This figure clearly illustrates the grouping of RIR and WL lines. On line level, this regression coefficient of *backrump* score at 51 weeks on NOT response at 23 weeks varied from -0.02 ± 0.35 (NS; line B1) to -0.91 ± 0.34 (P<0.01; line B3) for RIR lines and between 0.37 ± 0.41 (NS; line WC) to 0.44 ± 0.41 (NS; line WB) for WL lines. In general, line regression coefficients were consistent with origin regression coefficients.

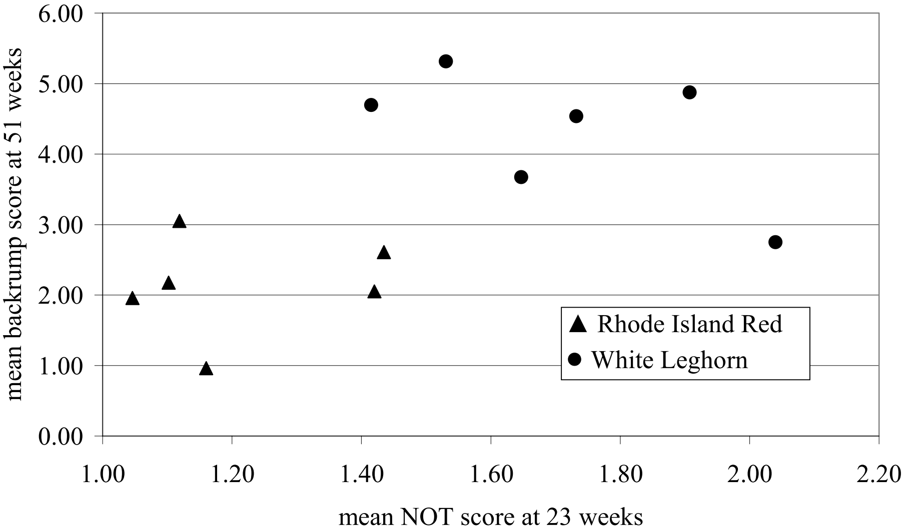


Figure 3: Line mean NOT score at 23 weeks on x-axis and line mean *backrump* score at 51 weeks on y-axis. The corresponding regression coefficient of *backrump* on NOT score was positive (2.32±1.13), but non significant (P=0.07).

For the relation within lines, *backrump* least square means at 51 weeks for NOT scores at 23 and 46 weeks obtained from models II and IV are shown in table 4.

For RIR lines, hens in cages approaching the rod (2.25 ± 0.10; score 1) at 23 weeks had more damage at *backrump* at 51 weeks ($P<0.05$) than cages holding still (1.68 ± 0.20; score 2). For all data (3.83 ± 0.19 vs. 3.19 ± 0.11 and 3.07 ± 0.12), RIR (3.25 ± 0.48 vs. 2.06 ± 0.09) and WL (4.75 ± 0.20 vs. 4.12 ± 0.12) lines, hens in cages moving away from the rod (score 3) at 46 weeks, had more damage to *backrump* at 51 weeks ($P<0.05$) than cages holding still (score 2) or approaching the rod (score 1).

Table 4: *Backrump* least square means at 51 weeks for NOT scores at 23 and 46 weeks for all data and for RIR and WL lines separately. Between brackets is the number of observations used to estimate each least square mean.

age of NOT	model[1]	data	NOT score		
			1 (approach)[2)]	2 (passive)	3 (fearful)
23	II	all	3.32±0.09 (376)	3.03±0.12 (217)	3.19±0.28 (35)
	IV	RIR	2.25±0.10^{b} (254)	1.68±0.20^{a} (58)	1.72±0.85^{ab} (3)
		WL	4.33±0.16 (122)	4.24±0.13 (159)	4.35±0.30 (32)
46	II	all	3.07±0.12^{a} (297)	3.19±0.11^{a} (250)	3.83±0.19^{b} (87)
	IV	RIR	2.06±0.09^{a} (251)	2.25±0.20^{ab} (55)	3.25±0.48^{b} (10)
		WL	4.18±0.25^{ab} (46)	4.16±0.12^{a} (195)	4.75±0.20^{b} (77)

1) when included, origin and line(origin) effects were tested significant at $P<0.001$.

2) *backrump* least square means within one row lacking a common letter superscript differ at $P<0.05$ after Bonferroni adjustment.

In model I, moving away from the rod (score 3) at 46 weeks was related to increased damage at *backrump* at 51 weeks (b = 0.34 ± 0.12; $P<0.01$; table 5).

In model I, no significant relation between NOT at 23 weeks and damage at *backrump* at 51 and 69 weeks was found. In model III, for RIR lines, moving away from the rod at 23 weeks related to decreased damage at *backrump* at 51 (b = -0.52 ± 0.21; $P<0.05$) and 69 (b = -0.48 ± 0.16; $P<0.01$) weeks, but moving away from the rod at 46 weeks related to increased damage at *backrump* at 51 weeks (b = 0.38 ± 0.18; $P<0.05$). For WL lines, moving away from the rod at 46 weeks related to increased damage at *backrump* at 51 weeks (b = 0.32 ± 0.16; $P<0.05$). For WL lines, no significant relation between NOT at 23 weeks and damage at *backrump* at 51 and 69 weeks was found.

Table 5: Regression coefficients (± standard error) of *backrump* score at 51 and 69 weeks on NOT scores at 23 and 46 for all data and for RIR and WL lines separately.

age of NOT	model[1)]	data	age (weeks) 51	age (weeks) 69
23	I	all	-0.16 ± 0.12	-0.09 ± 0.10
	III	RIR	-0.52 ± 0.21*	-0.48 ± 0.16**
		WL	-0.01 ± 0.16	0.07 ± 0.14
46	I	all	0.34 ± 0.12**	0.16 ± 0.10
	III	RIR	0.38 ± 0.18*	0.14 ± 0.13
		WL	0.32 ± 0.16*	0.17 ± 0.15

*P<0.05, **P<0.01.

1) when included, origin and line(origin) effects were tested significant at P<0.001.

In each model I, II, III and IV effects of origin (when included) and line within origin on damage at *backrump* at 51 weeks were all found to be highly significant (P<0.001). Results in relation to *backrump* score at 63 weeks were comparable with those from *backrump* scores at 51 and 69 weeks.

Discussion

The aim of the present study was to evaluate the novel object test in the home cage as a predictor of FP in adult laying hens. It was found that higher fear related responses at 46 weeks were associated with increased damage at back and rump area at 51 and 69 weeks. Corresponding relations were found within RIR and WL lines. Surprisingly, in RIR lines, an opposite relation was found for fear related behaviour at 23 weeks with damage at back and rump at 51 and 69 weeks, with less fearful animals having more damage at back and rump at these ages. In contrast, for WL lines, no relation between NOT at 23 and back and rump score at 51 or 69 weeks was found.

Relation between feather damage and novel object test

The relation between fear related behaviour and FP or feather damage has been shown in several studies (Craig et al., 1983; Bessei, 1986; Jones et al., 1995; Rodenburg et al., 2004; Buitenhuis et al., 2005). Associations between FP and fear arise because they are genetically related traits or because specific environmental conditions affect the expression of both traits. Phenotypic expression of a genetic correlation between FP and fear may be obscured by the environmental conditions under which experiments are conducted.

Independent of such a correlation, FP and fear may not always be affected by the same environmental conditions (Hughes and Black, 1974), i.e. FP may be fully expressed whereas fear may be not expressed under specific environmental conditions. If the relation between FP and fear is age dependent, as found in this study, than an increasing level of FP is a specific environmental condition affecting fear behaviour, probably by increasing the fear level of the FP recipient.

Using home-cage observations, Craig et al. (1983), found increasing feather damage at 40 weeks to be related to higher fearfulness using several 'fear' tests (i.e. Hansen's nervousness score) at approximately 40 weeks for WL hens housed in 10- or 14-bird cages. Bessei (1984) found increased FP during the rearing period to be predictive for higher fear levels during the laying period in RIR hens. Rodenburg et al. (2004) found higher fear levels in the rearing period to be predictive for increased FP during the laying period in WL hens. Results from the latter study suggest that fear could be predictive for FP in both WL and RIR lines.

White Leghorn

We did not find higher fearfulness at 23 weeks to be predictive for increased damage at later age, but ad hoc analysis showed that for WL lines, cages with a fearful response (score 3) had more feather damage (lsmean = 1.83 ± 0.21; $P=0.04$) at back and rump at 35 weeks than cages with more passive response (lsmean = 1.19 ± 0.13; score 2). This suggests that within WL lines, FP develops independent of the fear levels at 23 weeks, but that increased damage at 35 weeks leads to higher fearfulness at 46 weeks, which corresponds with findings of Bessei (1984) in RIR hens.

Early identification of WL hens with higher propensity to develop FP may require individual testing of hens at younger age, as done by Jones et al. (1995) and Rodenburg et al. (2004). A purebred low FP line showed less fearful behaviour in an open field test than its high FP counterpart (Jones et al., 1995) and in the crossbreed high fearfulness at 5 weeks was genetically correlated to expression of FP at 30 weeks (Rodenburg et al., 2004).

Rhode Island Red lines

For RIR lines, back and rump score at 51 weeks were negatively related with NOT at 23, but positively related with NOT at 46 weeks. This indicates that hens in cages changing from a lower fear response at 23 weeks to a higher fear response at 46 weeks had more damage to back and rump at 51 weeks than hens in cages changing in the opposite direction. Like WL lines, RIR cages with more damage at back and rump due to FP at 43 weeks tended to be more fearful at 46 weeks than cages with less damage ($b = 0.28 \pm 0.16$; $P=0.07$; not presented). Ad hoc analysis showed that RIR cages scoring 2 at 23 and 1 at 46 weeks (lsmean = 1.71 ± 0.24; $n=42$) had less damage ($P<0.01$) to back and rump at 51 weeks than cages scoring 1 at 23 and 2 at 46 weeks (lsmean = 2.69 ± 0.24; $n=41$). Rodenburg et al. (2004) found positive correlations between general pecking activity (including FP and ground pecking) at 29 weeks and activity in an open field test at 30 weeks. In general, activity in an open field test reflects exploration (Candland and Nagy, 1969), although specific activity patterns, such as pacing, are suggested to be indicative for higher fear levels (Hocking et al. 2001). For RIR lines at 23 weeks, explorative behaviour indicated by approaching the rod (score=1) may therefore be accompanied by higher occurrence of explorative pecking, shifting to actual FP at later age. Low FP hens have been found to be more environment directed (Rodenburg and Koene, 2003). In a cage environment, lack of an appropriate substrate to peck at may have redirected explorative pecking towards cage mates in RIR birds, as been suggested by Blokhuis and Arkes (1984). For hens in cages with a passive response at 23 weeks, a lower tendency to show explorative pecking did not lead to increased FP, feather damage and higher fear related behaviour (reinforcing each other), resulting in approaching the rod at 46 weeks.

Fear related behaviour indicated by response to a novel object

The novel object test as carried out in the present study measures the response to a rod at cage level (Jones, 1996). Fearfulness is an individual's propensity to respond fearfully under a wide range of potentially alarming stimuli (Jones et al., 1994). One way to measure this propensity is to expose hens to novelty in their home cage environment (Jones, 1987a), as was done in our study. Avoidance of the rod, scored '3' in our study is associated with high fear levels (Jones, 1985) corresponding to values of '3' and '4' from Sefton's (1976) scoring system. We therefore used probabilities of showing a fearful (=3) response to rod as indicators for fear related behaviour in our study. Approach of the rod (score 1) is associated with low fear levels as it indicates exploration of novelty, but interpretation of score 2 (=holding still/passivity) is ambiguous. It may reflect both indifference and arousal as reaction to the test situation, without engaging in either avoidance (score 3) or approach (score 1) of the rod.

Correlations between fear responses in different or repeated tests are important when single tests are to be used to select against fearfulness. Significant intra individual correlations within novel object/rod, novel food, emergence, predator surprise and tonic immobility tests have been used to argue that fear related behaviour in laying hens and Japanese quail is a stable trait over time (Jones, 1988; Hocking et al., 2001; Miller et al., 2005). Based on correlations between novel object/rod, tonic immobility, open field and restraint tests, studies have also emphasized the generality of the fear response under different situations (Jones, 1987b; Jones et al., 1991; Hocking et al., 2001; Schutz et al., 2004; Beaumont et al., 2005). Other authors found a lack of correspondence between similar tests (Albentosa et al., 2003b; Mignon-Grasteau et al., 2003; Miller et al., 2006) and argued that fear responses are merely context dependent (Murphy, 1978). With lack of correspondence, assessing fearfulness should be done by using multiple tests.

Origin (at 23, 46 and 69 weeks) and line within origin (at 23, 46 and 69 weeks) were found to influence response to NOT. For all WL lines, probability of showing a fearful response increased with age, whereas it remained low and stable for all RIR lines. WL lines showed more fear related behaviour than RIR lines at every age. Craig (1983) also found differences in fear related behaviour between two cage housed WL lines, whereas Fraisse and Cockrem (2006) did not find a difference in response to a NOT between a RIR and WL line. In the study by Fraisse and Cockrem (2006) the test person stood 3 m away from the tested cage, whereas in our study the test person remained in front of the tested cage. Higher fear of humans in WL lines in our study compared to the study by Fraisse and Cockrem (2006) might therefore be a possible explanation for the difference between both studies. Anderson et al. (2004) used two cage housed commercial WL hybrids and found an increase in fear related behaviour with age, but no effect of genotype. Ranking lines according to fear response showed that at 23 weeks, only one WL line (WA) was less fearful than two RIR lines (B1 and B3), whereas at both 46 and 69 weeks each WL line was more fearful than each RIR line. Based on the clear difference between RIR and WL, it can therefore be concluded that independent of the specific test and environment, lines from WL origin will show stronger fear responses than lines from RIR origin. Ranking of lines according to fear response within these origins, however, will change dependent of the specific test and environment.

Feather damage

For back and rump score, effects of origin and line within origin were found at every age. Damage to back and rump increased with age as expected, and plumage deterioration with age is generally observed (Johnsen et al., 1998; Kjaer, 2000). WL lines had more damage than RIR lines at every age. At specific ages, two WL lines were found to have less damage than at least one of the RIR lines: WF at 35, 43, 51, 63 and 69 weeks and WB at 63 and 69 weeks. Difference between origins with respect to damage at back and rump therefore do not seem as clear-cut as difference in fear response. This is in accordance with Kjaer (2000), who also found a specific commercial WL line to have the best and a specific commercial RIR line to have worst plumage condition at 51 and 69 weeks (kept in an aviary system). So dependent on specific lines and experimental environment different outcomes may be expected.

Receiving severe feather pecking is related to feather damage at the back and rump area (Bilčik and Keeling, 1999), although other studies did not find such a relation (Tind and Ambrosen, 1988; Kjaer, 2000). Kjaer (2000) argued that increased FP may not have lead to increased damage of already denuded areas. In our study, most damage to the back and rump area was scored for line W1 at 69 weeks (6.54), indicating that even at older age maximum damage (score 10; assigned to completely denuded back and rump area) had not yet occurred. It can therefore be assumed that in our study mean (cumulative) damage to back and rump within a single cage reflects occurrence of severe FP directed towards back and rump within that cage.

Assuming that FP can be spread by social learning (Zeltner et al. 2000) the use of mean feather damage scores as indicator for occurrence of FP within a group can overestimate the number of hens that actually perform FP. Increased variation in feather damage within group housed sibs (i.e. one hen with no and three hens with severe feather damage) may indicate that few hens actually perform FP, although such assumptions should be confirmed by direct observations on expression of FP. As feather damage reflects FP inflicted on the recipient, its use in breeding programmes will be restricted to group housed sibs of selection candidates as the interest would lie in identifying the candidates with higher tendency to develop FP using information from their group housed relatives (Ellen et al. 2006). If groups of non-relatives were used, the performer and receiver of FP would not have a common ancestral selection candidate.

Conclusion

For RIR lines, a NOT at 23 weeks can predict damage to back and rump due to FP, with a low fear response related to increased damage at back and rump due to FP. For WL lines, cages developing more FP after 23 weeks will show higher fearfulness at 46 weeks, which is independent of the fear response at 23 weeks. In both RIR and WL lines, fear response at 46 weeks is affected by preceding occurrence of FP. These results indicate that by using a NOT, early identification of cages with high feather damage at later age is possible in pure-bred RIR, but not in pure-bred WL lines. Future experiments should focus on crosses between the pure bred lines to evaluate the consistency of the relation between FP and fear in hybrids. Our results suggest that a NOT could be used in a breeding program to test cage housed relatives of individually kept selection candidates from RIR origin on their propensity for FP. Increasing societal concern on animal welfare can be an argument for breeding companies to adjust their breeding programs, although evidence for direct economic benefit is lacking.

Furthermore, this study provides good information on the amount of variation in feather damage due to FP and fear response in pure-bred laying hens from RIR or WL origin that are used in present breeding programs. The results clearly show that cage housed RIR and WL lines differ in fear response and FP during the laying period.

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Chapter 4:

The association of response to a novel object with subsequent performance and feather damage in adult, cage-housed, pure-bred Rhode Island Red laying hens

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Abstract

In laying hens, behavioural responses measured late in the laying period are associated with decreased performance. If measured early in the laying period, these behavioural responses could be used to predict performance later in life. The main objective of the present study was to investigate the association of the behavioural response towards a novel object at 23 weeks of age, with subsequent performance during the laying period. A total of 1251 hens from six different pure-bred Rhode Island Red lines (on average 208 hens per line) were housed in 318 cages (on average 56 cages per line). Performance variables included egg production (both per hen-present and hen-housed), BW at 19, 51 and 69 weeks of age, mortality and feather damage at 63 weeks. Hens from cages with an approach response realized a higher total egg production (hen-day and hen-housed) and a higher egg production in the middle (hen-housed) and end of the laying period (per hen-present and hen housed), as compared with hens from cages with a passive response towards the novel object. Approach responses were also associated with more feather damage, but not with BW or mortality. Low correlations were found between higher egg production and more feather damage. These results suggest that an approach response towards a novel object measured early in the laying period is associated with better performance later in the laying period. The novel object test early in the laying period can be used to predict subsequent performance, although possible causal mechanisms for this association remain to be investigated.

Introduction

Behavioural responses measured after 35 wk of age (Kujiyat et al., 1984), have been associated with lower productivity and higher mortality in laying hens (Sefton, 1976; Craig et al., 1983; Kujiyat et al., 1984; Barnett and Hemsworth, 1989; Hemsworth and Barnett, 1989; Barnett et al., 1992; Jones, 1996). When measured later in the laying period, the association between behaviour and low performance is most likely to occur as a consequence of the conditions to which susceptible hens have been exposed (Craig et al., 1983). Behavioural responses measured in an earlier stage of production could, however, be used to predict performance later in life.

The association of performance with behavioural response measured either early or late in the laying period may differ, as fear responses measured at different ages are not always correlated (Hocking et al., 2001; Miller et al., 2005, 2006). The association of behavioural response with both egg production (Sefton, 1976; Kujiyat et al. 1984) and feather damage (Uitdehaag et al. 2008) is also affected by genetic background, which should therefore be taken into account when studying these associations. The importance of research on the predictability of later performance by behavioural responses has been emphasized (Jones, 1996; Boissy, 1998), although scientific reports on such relations in laying hens are, to our knowledge, not available. Broilers and Japanese quail chicks rapidly escaping from a maze at young age had higher subsequent BW (Marin et al., 1999) and high egg production (Marin et al., 2002) as compared with their slow escaping counterparts. It was suggested that increased underlying fearfulness for slow escaping chicks could explain their lower growth rates during the growth period (Marin et al., 1999).

The present study investigated if the behavioural response to a novel object test in the home cage at 23 wk was associated with egg production, mortality, BW and feather damage. It was hypothesized that hens that approached the rod showed improved performance variables. Effects of genetic background were taken into account by using 6 pure-bred lines of Rhode Island Red origin.

Material and Methods

In this experiment, we used 1251 non-beak trimmed laying hens from six pure-bred lines (lines B1, B2, B3, BA, BB and BE; number of hens per line varying from 180 to 245) of Rhode Island Red origin (RIR). These laying hens were housed in 318 four-bird battery cages (number of cages per line varying from 45 to 60). Groups of laying hens within one cage were categorized based on their behavioural response towards a novel object at 23 wks. A novel object test (**N**OT) in the home cage is assumed to measure an aspect of fear and is especially suitable for application under semi-commercial conditions (Jones, 1996). The categorization resulting from the novel object test was used to predict subsequent egg production, BW and feather damage of the laying hens within these same cages. Data on the association of NOT responses with feather damage due to severe feather pecking have been presented elsewhere (Uitdehaag et al. 2008).

Housing and Management

After arrival at the laying facility at 17 wks of age, hens were housed in four-bird cages (44 cm (height) x 46 cm (depth) x 39 cm (width); 448.5 cm^2 per hen) on the middle tier of a three level battery system for a period of 1 yr (June 2004 until June 2005). Wing bands allowed identification of each individual hen. Except for the experimental observations, contact between hens and humans occurred frequently: stockpersons collected and registered dead hens daily and manually collected eggs three times / wk.

From 19 wks of age until 42 wks of age, a standard commercial phase 1 diet (159 g/kg crude protein, 43 g/kg crude fibre and 11.17 MJ ME/kg) was provided ad libitum from a trough measuring 39 cm (= total width of cage). From 42 wks until the end of the experiment, a standard commercial phase 2 diet (152 g/kg crude protein, 47.0 g/kg crude fiber and 11.01 MJ ME/kg) was provided. Water was available by two drinking nipples, placed in between two adjacent cages, and accessible from both cages.

At 17 wks of age, hens were kept at 9L: 15D light scheme (light from 7.00 until 16.00 hrs) and for the following week, the light period was increased by half an hour, starting at 6.30 hrs. Hereafter, the light period was gradually changed (turning on the lights 30 minutes earlier in each consecutive week) into a final 16L : 8D scheme, which was applied from 30 wks of age onwards.

Novel Object Test

A novel object test (NOT) was performed at 23 wks of age to obtain a behavioural response from a group of hens within the same cage. The NOT procedure (Uitdehaag et al., 2008) can be considered a modification of procedures that have been described elsewhere (Craig et al., 1983; Sefton, 1976). We used a wooden rod with a white tip as a novel object, which was placed in front of a cage in the feed trough for maximum period of 30 s, after the test person had been standing still for 10 s in front of that cage. Responses of hens within one cage were categorized as either ‘fearful’ (hens moving away from the rod), ‘passive’ (hens cage holding still) or ‘approach’ (hens approaching the rod). Our personal observations indicated that there was strong tendency for individual hens within a cage to respond similarly. All tests were carried out by the same test person.

Egg Production

Eggs were collected three times each wk, from 25 until 68 wks of age. From these data, hen-day egg production (corrected for mortality) and hen-housed egg production (based on initial number of hens within a cage) were calculated for three periods, namely: the start of the laying period (25 to 40 wks), the middle of the laying period (41 to 54 wks) and the end of the laying period (55 to 68 wks). Total egg production was also calculated for the hens within each cage (25 to 68 wks).

Mortality

Dead birds were collected daily and cages in which mortality occurred were assigned a score of one, whereas cages in which no mortality occurred were assigned a score of zero. Mortality rates were too low to distinguish between cages with one or more cases of mortality.

Body Weight

Individual BWs were collected at 19, 51 and 69 wks of age. Individual growth rates were calculated between 19 and 51 wks and between 51 and 69 wks of age. From these individual observations, mean BW and growth rates were calculated for each cage at each time point.

Feather Damage

Feather damage due to severe feather pecking was assessed at 63 wks of age by evaluating the plumage condition on back and rump (Bilcik and Keeling, 1999; range 0-5, higher scores indicating more damage). Damage to rump and back area were combined into 1 score (ranging from 0 to 10) and from these individual observations, the mean feather damage score at 63 wks of age was calculated for each cage.

Statistical Methods

Statistical analyses were performed to investigate the NOT effect on the performance variables while adjusting for possible line effects on these performance variables. In order to do so, egg production, BW, growth rates and feather damage were analyzed as dependent variables in a GLM procedure with NOT score and line included as independent class effects, according to the following model:

$$y_{ijk} = \mu + NOT23_i + LINE_j + e_{ijk} \qquad \text{(model 1)}$$

with y_{ijkl} = cage observation on one of the performance variables, μ = general mean of variable to be analyzed, $NOT23_i$ = cage response to NOT (i = 2), $LINE_k$ = line from which hens originate (k = 6 lines) and e_{ijkl} = residual term not explained by the model. Cages with hens showing the ‘fearful’ response were excluded from the analysis, because this response was very rarely observed (n = 3 cages). Least square means were estimated for each NOT response. Occurrence of mortality on cage level was analyzed as dependent variable using the GENMOD procedure for binomial distributions with a logit link function and NOT score at 23 wks and line included as independent class effects (similar to model 1). Additional analyses were performed to obtain more detailed information about lines B1 and B3, the lines with the most uniform distribution of cages over the passive and approach response (Table 1). In order to do so, model 1 was extended with the interaction of line with NOT response, which was run with data from lines B1 and B3 only.

Correlations of feather damage with hen-day egg production, bodyweight and growth rates were calculated using their residuals which were obtained from model 1 (nine correlations in total). These correlations were also calculated within each individual line (6 x 9 = 54 correlations in total).

Results

Distribution of NOT responses over cages differed between lines (Table 1). Lines B1 and B3 showed more passive responses, while lines B2, BA, BB and BE had a high tendency to show only approach responses. There were only three cages that showed a fearful response. It was decided to exclude these cages in the GLM analysis, leaving the passive and approach response as the two levels for the NOT score.

Table 1: Distribution of cages over novel object responses for each line.

line	NOT at 23 weeks			total
	fearful	passive	approach	
B1	1	19	30	50
B2	0	8	42	50
B3	1	18	27	46
BA	1	3	45	49
BB	0	3	62	65
BE	0	7	52	59

Descriptive statistics for egg production, mortality, BW, growth rates and feather damage for each NOT score are presented in Table 2.

Line Effects

Line affected ($P < 0.01$) each of the performance variables (except for hen-day egg production at the start and in the middle of the laying period; Table 3). Line BE had lowest total production, highest probability of at least 1 case of mortality, highest BW at 19 wks of age and most feather damage at 51 wks of age (Table 4). Other lines did not stand out in having extreme values for more than three of the performance variables.

Effects of Novel Object Test

Hens from cages with an approach response realized a higher hen-day egg production at the end of laying period, than hens from cages with a passive response ($F_{1,309} = 5.56$; $P = 0.02$, Table 3). Next to a higher realized hen-housed egg production in the middle ($F_{1,309} = 5.01$; $P = 0.03$) and at the end of the laying period ($F_{1,309} = 5.67$; $P = 0.02$), hens from cages with an approach response also realized a higher hen-housed total egg production, than hens from cages with a passive response ($F_{1,309} = 5.68$; $P = 0.02$). Realized hen-housed egg production during the entire laying period for hens from cages with either an approach or passive response is shown in Figure 1. Hens from cages with an approach response had more feather damage ($F_{1,305} = 5.92$; $P = 0.01$; Table 3) than hens from cages with a passive response.

In the additional analyses of lines B1 and B3, line and NOT were also found to affect the performance variables, whereas no effect of their interaction term was found. Differences between hens from cages with an approach and passive response were similar to results from model 1; these were found significant for hen-day egg production in the middle of the laying period (91 ± 0.9 vs. 88 ± 1.1; $F_{1,90} = 5.04$; $P = 0.03$), total hen-day egg production

(283 ± 2.5 vs. 273 ± 3.0; $F_{1,90} = 5.73$; $P = 0.02$) and hen-housed egg production at the start the laying period (106 ± 1.0 vs. 102 ± 1.1; $F_{1,90} = 6.71$; $P = 0.01$).

Table 2: Descriptive statistics (raw means and SD between brackets) on total egg production, mortality and BW for each novel object test (NOT) response.

NOT response	#cages	mortality[1]		total egg production (25-68 wks)		BW (kg)		feather damage (63 wks)
		#hens	#cages	hen-day	hen-housed	19 wk	51 wk	
fearful	3	2	2	288.77 (12.70)	263.28 (33.67)	1.57 (0.08)	2.11 (0.24)	1.89 (0.19)
passive	58	26	20	271.91 (26.65)	255.92 (38.01)	1.52 (0.09)	2.00 (0.15)	1.95 (0.57)
approach	258	107	83	273.42 (23.29)	261.32 (33.72)	1.55 (0.10)	2.03 (0.16)	2.12 (0.62)

[1] Mortality is presented on hen level (#hens) and on cage level (#cages; number of cages in which mortality occurred).

Table 3: Results from model 1, investigating the effect of line- and novel object test (NOT) on performance variables. Least square means of these performance variables (±SE) for the passive and approach response towards the novel object are presented

performance variable		F / Chi square-values[1]		NOT least square means	
		line	NOT	approach	passive
hen-day egg production	start	1.93†	0.87	104 ± 0.5	103 ± 1.0
	middle	2.20†	2.45	89 ± 0.5	87 ± 1.2
	end	6.05***	5.56*	82 ± 0.8	78 ± 1.7
	total	4.02**	2.89†	275 ± 1.5	269 ± 3.2
hen-housed egg production	start	3.66**	2.53	103 ± 0.6	100 ± 1.3
	middle	4.23**	5.01*	85 ± 0.8	81 ± 1.7
	end	6.82***	5.67*	76 ± 1.0	70 ± 2.2
	total	5.79***	5.68*	264 ± 2.2	251 ± 4.6
BW	19 wk	11.44***	3.77†	1.55 ± 0.01	1.52 ± 0.01
	51 wk	36.34***	0.07	2.03 ± 0.01	2.02 ± 0.02
	69 wk	35.13***	0.18	2.00 ± 0.01	2.01 ± 0.02
	growth1[2]	30.10***	0.98	0.49 ± 0.01	0.50 ± 0.02
	growth2	33.26***	2.19	-0.03 ± 0.00	-0.01 ± 0.01
mortality (18-68 wk)		16.88**	0.49	0.30 (0.24-0.36)[3]	0.35 (0.26-0.50)
feather damage (63 wk)		7.14***	5.46*	2.13 ± 0.04	1.91 ± 0.08

†P<0.10, *P<0.05 **P<0.01 ***P<0.001

[1] Chi-square values are presented for line and NOT effects on mortality. The number of error df was 309 in analysis of egg production and bodyweights, 305 in the analysis of feather damage and 306 in the analysis of mortality.

[2] Growth1 and growth2 are the increases in BW between 19 and 51 and between 19 and 69 wk of age respectively.

[3] Lower and upper 95%-confidence limits for the probability of at least one case of mortality per cage are indicated between brackets

Table 4: Line least square means (±SE) for each of the performance variables

line	mortality[1,2]	total egg production		BW (kg)		growth (19-51 wks)	feather damage (63 wks)
		hen-day	hen-housed	19 wks	51 wks		
B1	0.39^{ab} (0.13)	278 ± 3.4^{ab}	262 ± 4.8^{ab}	1.54 ± 0.01^{ab}	1.90 ± 0.02^{c}	0.36 ± 0.02^{a}	1.94 ± 0.08^{bc}
B2	0.28^{ab} (0.07)	272 ± 3.5^{abc}	253 ± 5.0^{abc}	1.46 ± 0.01^{c}	1.97 ± 0.02^{c}	0.51 ± 0.02^{b}	1.76 ± 0.09^{c}
B3	0.18^{b} (0.04)	280 ± 3.5^{a}	275 ± 5.0^{a}	1.55 ± 0.01^{ab}	2.09 ± 0.02^{b}	0.54 ± 0.02^{b}	2.24 ± 0.09^{ab}
BA	0.34^{ab} (0.08)	273 ± 3.7^{abc}	258 ± 5.3^{abc}	1.56 ± 0.01^{ab}	2.19 ± 0.02^{a}	0.64 ± 0.02^{c}	2.01 ± 0.10^{abc}
BB	0.36^{ab} (0.13)	265 ± 3.4^{bc}	251 ± 4.8^{bc}	1.51 ± 0.01^{b}	1.94 ± 0.02^{c}	0.43 ± 0.02^{a}	1.88 ± 0.08^{c}
BE	0.53^{a} (0.18)	264 ± 3.4^{c}	241 ± 4.8^{c}	1.58 ± 0.01^{a}	2.06 ± 0.02^{b}	0.49 ± 0.02^{b}	2.32 ± 0.08^{a}

1Least square means within one column lacking a common letter superscript differ at $P<0.05$ after Bonferroni adjustment

2For each line, the probability of at least one case of mortality within one cage and the individual mortality rate (between brackets) are presented

Correlations

Higher feather damage scores over lines weakly correlated with lower BW at 51 (-0.17; P = 0.00) and 69 wks of age (-0.21; P =0.00) and with lower growth rates between 19 and 51 wks of age (-0.18; P = 0.00). Correlations within lines corresponded with correlations over lines. Higher feather damage scores correlated with lower growth rates between 19 and 51 wks in line B3 (-0.33; P = 0.03) and in line BE (-0.34; P = 0.01). In line BB, higher feather damage scores correlated with lower BW at 69 wks (-0.30; P = 0.02), lower growth rates between 51 and 69 wks (-0.29, P = 0.02), higher hen-day egg production at the start (0.33; P = 0.01), in the middle (0.38; P = 0.00), at the end (0.40; P = 0.00) and higher total hen-day egg production (0.44; P = 0.00).

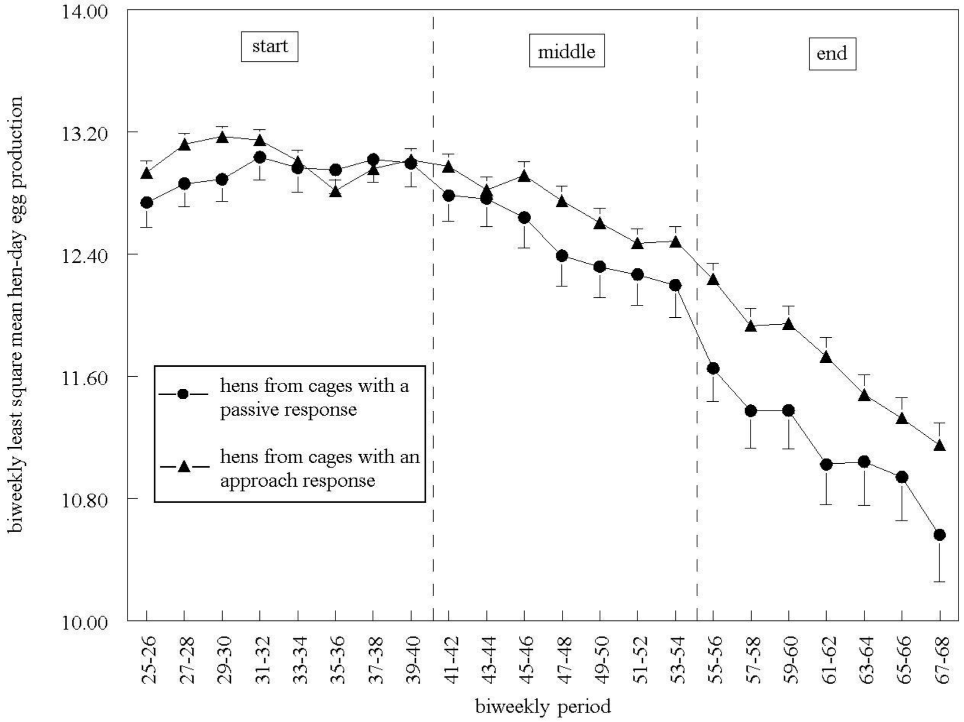

Figure 1: Hen-day egg production (indicated by least square means adjusted for line effects; bars indicate standard errors) for the passive and approach response towards the novel object in each consecutive fortnight period between 25 and 68 wks of age.

Discussion

Our objective was to investigate the association between the behavioural response to a novel object and subsequent performance. By measuring the behavioural response early in the laying period, its possible association with subsequent performance is assumed not to be caused by specific environmental factors that could simultaneously affect behaviour and performance. Results from our study indicate an association of the NOT response with egg production. Marin et al. (1999) and Marin et al. (2002) found a comparable, negative impact of a higher, early fear response on later performance in broilers and Japanese quail respectively. The slightly lower mortality in hens from cages with an approach response (30% of the cages had least one case of mortality; 0.42 cases of mortality per cage) compared with hens from cages with a passive response (35% of the cages had least one case of mortality; 0.44 cases of mortality per cage) probably caused the difference between both response groups in hen-housed egg production to be greater than their difference in hen-day egg production (Table 3). No other NOT effects on performance were found. Egg weight and egg mass were also not affected by NOT response (data not shown). This indicates that the higher egg production for hens from cages with an approach- as compared with hens from cages with a passive response was not accompanied either by lower egg weights or by lower egg mass.

When egg production was analyzed as a single variable by regression of hen-day egg production on consecutive biweekly periods during the laying cycle, effects of NOT responses on egg production were similar to those from model 1 (results not shown). This indicates that NOT effects on egg production from model 1 are not merely coincidental due to repetitive testing. The lack of interaction effects in the additional analyses of lines B1 and B3 indicate that the association of NOT response with subsequent performance could be general for hens from different genetic backgrounds.

Interpretation of Novel Object Response

Cages with hens showing the ‘fearful’ response were excluded from the analysis, because of their small numbers (n=3). This complicates the interpretation of our results, because the passive response may reflect both indifference and arousal as reaction to the test situation, without engaging in either avoidance or approach of the rod (Uitdehaag et al., 2008). Behavioural inactivity in both tonic immobility (Jones, 1985) and open field tests (Candland and Nagy, 1969) are, however, thought to reflect higher fear levels. The passive response towards the novel object in our study is therefore assumed to reflect a more fearful state than the approach response.

Hens from cages with an approach response were found to have more feather damage due to severe feather pecking than hens from cages with a passive response (same data as used by Uitdehaag et al., 2008). In their study it was argued that hens from cages with an approach response will have a strong tendency to show explorative pecking. In absence of an appropriate substrate, they will redirect their pecking towards plumage of cage mates and, as consequence, have more feather damage than hens from cages with passive response. This indicates that the approach and passive response could also reflect a difference in tendency to explore the environment. The approach rather than the passive response will then reflect a higher tendency to show exploratory behaviour. The contradicting associations of NOT with either egg production or feather damage suggests a trade-off between behavioural response and these performance variables in Rhode Island Red hens housed in cages.

Although alternative interpretations may be valid, it is assumed that the passive response reflects higher fear than the approach response. This is supported by the similarity with inactivity in the tonic immobility and open field test. In cage housing, a lower fear response may be favorable over a higher fearful response. Strong stressors may induce a state of fear in which individual hens respond by standing still or even by moving away from the stressor (i.e. the rod in our study). If specific, environmental conditions, such as cage housing, prevent hens from creating sufficient distance with the stressor according to their own, internal standard ('Sollwert'), it may be left in a continued state of high fearfulness. The NOT in our study might have identified hens from cages with high underlying fearfulness, predisposing them to respond fearfully to potential stressors occurring throughout the laying period. High fearfulness generally correlates with higher corticosterone levels in laying hens (Beuving and Vonder, 1978). When inducing a series of high fear responses, corticosterone levels may remain at a high level, which can have damaging effects on general functioning (Jones, 1996) and on egg production (Hemsworth and Barnett, 1989; Petitte and Etches, 1991). The association of higher fear at early age and lower subsequent egg production may be less likely to occur in floor housing systems, because these systems do provide the space for hens to move away from the stressor.

Correlations

Correlations of feather damage with egg production, BW and growth rates across lines were low and were generally in agreement with the correlations found within each individual line (except for the correlation between increased feather damage and decreased egg production in line B2). Correlations indicated that increased feather damage is associated with increased egg production, which was also found by Hughes (1980) in individually housed hens. Decreased BW at 51 wks and decreased growth were correlated with increased feather damage, which can be explained by an increased maintenance requirement (Hughes, 1980; Herremans and Decuypere, 1986). The correlations confirm the suggested trade-off between egg production and feather damage and together with the effect of early fear response on both egg production and feather damage imply that the early fear response is one of the factors influencing this trade-off.

Conclusion

Behavioural responses towards a novel object measured early in the laying period are associated with egg production per hen-present as well as per hen-housed in adult Rhode Island Red hens housed in cages. The predisposition to adopt a specific behavioural response early in the laying period may therefore be predictive for subsequent performance. Hens from cages with an approach response had more feather damage due to severe feather pecking than hens from cages with a passive response, which suggests a trade-off between plumage condition on the one hand and NOT response and egg production on the other. Causal mechanisms that could explain the association between behavioural response and performance remain to be investigated.

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Chapter 5:

Effects of mixed housing of birds from two genetic lines of laying hens on open field and manual restraint responses

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Abstract

Birds from Rhode Island Red origin (RIR) show a lower fear response and less feather pecking than birds from White Leghorn origin (WL). This study investigated whether responses in fear eliciting tests were affected if RIR and WL birds were housed together. Experimental groups contained either birds from one line only ('pure' groups) or an equal number of RIR and WL birds ('mixed' groups). These arrangements were maintained from hatch onwards, throughout the rearing and laying period. Birds were subjected to open field tests at 5-6 weeks and 17-18 weeks of age and to manual restraint tests at 7-8 weeks and 24 weeks of age. RIR birds were more active in both open field tests and in the manual restraint test at 24 weeks of age as compared with WL birds. RIR birds from pure groups were more active in the open field test at 17-18 weeks and in the manual restraint test at 24 weeks of age than RIR birds from mixed groups. These results suggest that otherwise low fearful RIR birds may adopt a higher fear response if they are housed together with more fearful conspecifics. These effects do not emerge until after 8 weeks of age.

Introduction

Fear responses should function to facilitate adaptation, but are undesirable in terms of animal welfare if they are excessive and accompanied by negative subjective states (Jones, 1996). In domestic chicks, fear responses start to develop early in life (Rogers, 1995) under the influence of both genetic and environmental factors (Boissy, 1995). These environmental factors may include the presence of group mates with specific behavioural characteristics. Social interactions between group mates are a powerful influence on chicken behaviour. The performance of established behaviours such as pecking and foraging is influenced by the presence of other birds performing the same behaviour, via social facilitation (Tolman, 1968), whilst the development of new behaviours such as 'key-pecking' and feather pecking can be acquired via observational learning (Nicol and Pope, 1994; Zeltner et al., 2000). It is therefore possible that social interactions between chicks with different behavioural characteristics could influence the development of fear related behaviour. Previous studies have investigated the effect of social interaction of broilers with both young laying hen chicks (Gvaryahu et al., 1996) and junglefowl (Zulkifli et al., 1998) on general activity, growth and feeding behaviour. In laying hens, the tonic immobility response, an anti-predatory reaction (Jones, 1996) at 20 weeks of age was affected by intermingling birds from two different breeds (Campo et al., 2005).

It is unknown, however, if such effects are already established earlier in life and if they can be detected with other tests, such as the open field test or the manual restraint test. Responses in the open field test reflect a conflict between fearfulness and motivations to reinstate social contact and to explore the novel environment (Rushen, 2000). The manual restraint test, on the other hand, is more similar to the tonic immobility test and is assumed to be a measure of stress-responsiveness (Van Hierden et al. 2004).

Many of the marked behavioural differences between commercial, genetically pure-bred lines of laying hens can be attributed to their origin, i.e. Rhode Island Red (RIR) or White Leghorn (WL). Rhode Island Red birds are, in general, more docile, less fearful and show less severe feather pecking than White Leghorn birds (Uitdehaag et al., 2008). In the current experiment, two different social environments were created by keeping birds from a RIR and WL line in either ‘pure’ (birds from one line only) or ‘mixed’ groups (birds from both lines). The main objective was to investigate whether responses in fear-eliciting test situations were affected by these social environments and at what age these effects could possibly occur. It was hypothesized that the response of RIR and WL birds which were housed together, would become more similar to the response of their group mates from the other line, assuming that both lines were equally susceptible to changes in their social environment. The ages at which a possible effect of social environment and at which the previously found difference between RIR and WL birds would occur, were also investigated.

Material and Methods

Animals

Adult birds from two genetic lines of laying hens, both with white feathers, were previously found to differ in feather damage due to severe feather pecking and in fear response towards a novel object (Uitdehaag et al., 2008). Birds from these same lines were used in the current study and were labelled as either from Rhode Island Red origin (low feather pecking and low fear response towards novel object; ‘RIR’) or from White Leghorn origin (high feather pecking and high fear response towards novel object; ‘WL’). In the current experiment, groups consisted either of birds from one line (pure groups) or of an equal number of RIR and WL birds (mixed groups) from hatching onwards.

Housing conditions

Rearing period. During the rearing period, a total of 325 birds with intact beaks was housed in battery rearing cages, which were of similar size (42 cm height x 65 cm depth x 96 cm width) providing on average 288 cm^2 of space per bird. These cages either contained pure groups with, on average, 23 RIR birds (3 cages) or 20 WL birds (3 cages), or contained mixed groups with 11 RIR and 10 WL birds (9 cages; Figure 1). Each group was randomly allocated to one of 15 cages from three stacked tiers of 5 adjacent cages. The upper tier was used to house the birds until 5 weeks of age. Groups were formed immediately after hatching and were kept together throughout the rearing and laying period.

A standard commercial rearing diet was provided ad libitum by a 96 cm food trough (cage width) and water was provided through three drinking nipples per cage. During the first week chicks received 23 hours of light per day (23L : 1D light scheme), which was reduced to a 19L : 5D light scheme in the second and third week. Between the fourth and 17th week, the light regime was gradually changed into 11L : 13D, reducing the diurnal light period with 1.14 hours between subsequent fortnights. Chicks were fed standard commercial rearing diets and treated with routine vaccinations.

Laying period. During the laying period (from 18 weeks onwards), a total of 160 birds was housed in 40 four-bird battery cages for laying hens, keeping group mates from the rearing period together. Each cage was of similar size (46 cm height x 39 cm depth x 44 cm width) providing on average 429 cm^2 of space per bird. The cages either contained pure groups with four RIR (15 cages) or four WL birds (12 cages), or contained mixed groups with two RIR and two WL birds (13 cages; Figure 1). Each group was randomly allocated to one of 75 cages from three stacked tiers of 25 adjacent cages. The remaining 35 cages from these three tiers were used for birds that were only included in the experiment during the rearing period.

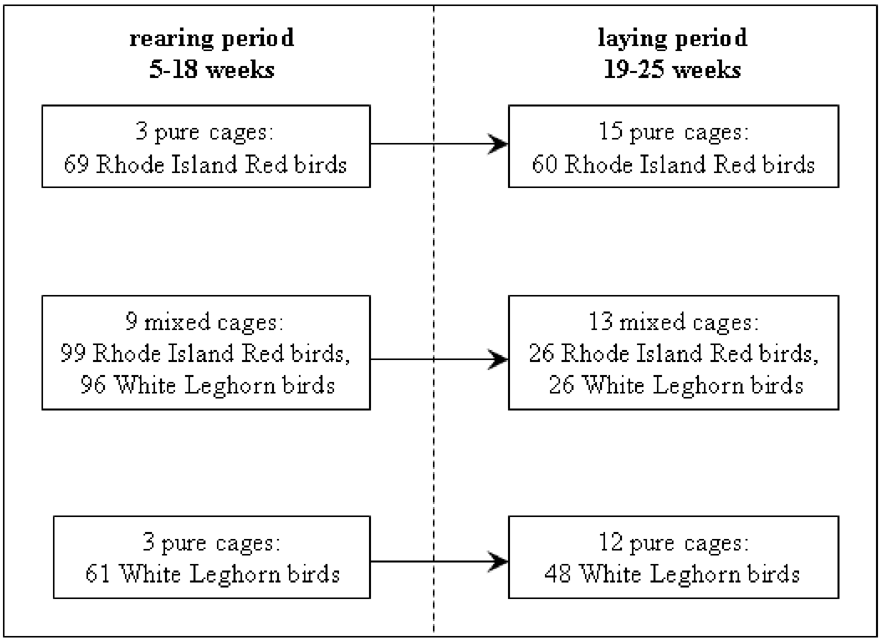

Figure 1: The experimental design indicating the number of animals at the start of the rearing and laying period.

A standard commercial laying diet was provided ad libitum by a 44 cm food trough (cage width) and water was provided through two drinking nipples per cage. After arriving at the laying facility at 18 weeks of age, the light schedule was 10L : 14D, which gradually changed to a final 16L : 8D scheme from 20 weeks of age onwards.

The experiment was carried out by licensed and authorized personnel under approval of Institut de Sélection Animale B.V.

Observations

Open field. After being randomly picked from their home cage, each individual bird was placed in the middle of an open field at 5-6 and at 17-18 weeks and its response was recorded for ten minutes using continuous focal sampling. The open field pen measured 1.25 x 1.25 m with a Perspex wall in front, allowing video observations, and was located in a room separate from the battery rearing cages. Lights in the open field room were switched on at the start of each test. Responses were scored by one person using The Observer 5.0 (Noldus Information Technology b.v., Wageningen, The Netherlands) and an ethogram that included latency to first step, frequency of stepping, defecating and jumping, and durations of standing, sitting, walking and pacing. Birds in consecutive tests were not taken from the same home cage. The open field test at 5-6 weeks was carried out during ten days (two consecutive five-day working weeks) between 8.00 and 21.45 h (split into five time blocks) and at 17-18 weeks during nine days (two consecutive weeks of five and four days) between 8:00 and 17.30 h (split into four time blocks; balancing over line and social environment).

Manual restrain. At 7-8 and at 24 weeks of age, each individual bird was taken out of its home cage and manually restrained on its side for five minutes. The bird was placed on one of two boards which were located within visual and auditory range of other birds, on one end of the 5-cage row (at 7-8 weeks of age) or on both ends of the 25 cage-row (at 24 weeks of age). The test person used one hand to restrain the legs, while the other hand was used to restrain the upper part of the body of the tested bird. The hand restraining the legs mainly functioned to prevent the birds from escaping when struggling, whereas the other hand put mild pressure on the upper body part when a bird was not struggling. After each struggle, birds were gently brought back in their original position. Observations on bird responses during the manual restraint included the latency to first struggle and frequency of struggles. Birds were tested in random order and were not taken from the same home cage in consecutive tests. Immediately after each test, a blood sample from the wing vein was taken for corticosterone analysis. Corticosterone concentrations were determined from the plasma samples using a radioimmunoassay kit (IDS, Inc., Bolton, UK) as described previously (Buyse et al. 1987). Each manual restraint test was performed by one of two persons on four consecutive days between 7.30 and 16.00 at 7-8 weeks and on five consecutive days between 7.30 and 16.00 (divided into 3 consecutive time blocks) at 24 weeks of age (balancing over line and social environment).

Statistical Analyses

The effects of genetic line, social environment and their interaction on open field responses at 5-6 weeks ($n = 318$) and 17-18 weeks of age ($n = 274$) and on manual restraint responses at 7-8 weeks ($n = 285$) and 24 weeks of age ($n = 150$) were tested separately for each test at each age in a GLM procedure. Responses were adjusted for possible effects of cage- (nested within genetic line- social environment interaction, which was not included in the open field test at 5-6 weeks of age), date-, time- (sequence of testing in manual restraint test at 6 weeks was included as continuous covariate) and test person effects, according to the following model:

y = intercept + line + social environment + line *social environment +
cage(line*social environment) + date + time + test person + error,

Before the analysis, responses recorded as duration and latency were expressed as a ratio of the total observation time after which they were transformed with an arcsin square root transformation to reach normality. A square root transformation was applied to responses recorded as frequency. Data are presented as back transformed least square means with confidence limits.

Another model, including social environment-, cage- (nested within social environment), date-, time- and test person effects, was applied to each line separately to obtain residuals for the dependent variables. These residuals were used to estimate Spearman's correlation coefficients. Correlations were estimated between latency to first step, duration of standing and duration of sitting from both open field tests and between latency to first struggle, frequency of struggling and corticosterone response from both manual restraint tests. The open field tests at 5-6 weeks and 17-18 weeks of age were also correlated to the manual restraint tests at 7-8 weeks and at 24 weeks of age respectively (resulting in a total of 36 correlations for each line). Fisher's Z transformation was applied to these correlations and it was tested if the transformed correlations differed between pure and mixed groups within each line. Pooled correlations were estimated if correlations were found equal ($P > 0.05$) for pure and mixed groups.

It was additionally tested whether the variability of the open field responses used to estimate the correlations differed between pure and mixed groups (Levene's test for homogeneity of variance). It was found that, in both RIR in WL birds, the variability of latency to step, duration of standing and of duration of sitting did not differ ($P > 0.05$) between pure and mixed groups at either 5-6 weeks or 17-18 weeks of age.

Results

Line differences

RIR birds showed more activity in the open field test at 5-6 weeks of age than WL birds. This was reflected by their shorter latency to first step ($P < 0.001$; Table 1), their higher frequency of stepping ($P < 0.001$), their longer duration of walking ($P = 0.00$), standing ($P < 0.01$) and pacing ($P < 0.001$) and their shorter duration of sitting ($P < 0.001$). RIR birds had a lower corticosterone response in the manual restraint test at 7-8 weeks of age than WL birds ($P < 0.05$; Table 2), but RIR and WL birds did not differ significantly ($P > 0.10$) in their latency and frequency of struggling.

RIR birds showed more activity in the open field test at 17-18 weeks of age than WL birds. This was reflected by their shorter latency to first step ($P < 0.001$, Table 1), their higher frequency of stepping ($P < 0.001$), their longer duration of walking ($P < 0.01$) and standing ($P < 0.001$) and their shorter duration of sitting ($P < 0.001$). RIR birds also showed more activity in the manual restraint test at 24 weeks of age than WL birds. This was reflected by their shorter latency to struggle ($P < 0.001$, Table 2) and their higher frequency of struggling ($P < 0.001$). RIR and WL birds did not differ significantly in their corticosterone response at 24 weeks of age.

Effects of social environment

Open field tests. Social environment had no effect on open field responses at 5-6 weeks of age in either RIR or WL birds (Table 1a). It seemed that the difference between RIR and WL birds from mixed groups in open field response at 17-18 weeks became smaller than the difference between RIR and WL birds from pure groups, although not significantly for each response. RIR birds from mixed groups showed less open field activity at 17-18 weeks of age than RIR birds from pure groups. This was reflected by their shorter duration of standing (significant interaction between line and social environment; $P < 0.05$, Table 1b) and their longer duration of sitting (significant interaction between line and social environment; $P < 0.05$) as compared with RIR birds from pure groups. WL birds from mixed groups defecated more than WL birds from pure groups (significant interaction between line and social environment; $P < 0.05$).

Table 1a: Back transformed least square means of the open field responses at 5-6 weeks of age for Rhode Island Red and White Leghorn birds in pure and mixed groups (95%-confidence intervals between brackets).

type	response	Rhode Island Red birds		White Leghorn birds	
		pure	mixed	pure	mixed
frequency	stepping	19^{a}[1] (14-26)	16^{a} (12-22)	0.63^{b} (0.00-2.33)	0.85^{b} (0.10-2.33)
	jumping	0.3 (0.12-0.55)	0.21 (0.09-0.39)	0.25 (0.09-0.49)	0.29 (0.14-0.50)
	defecating	1.32^{b} (0.93-1.77)	0.96^{b} (0.68-1.29)	0.53^{a} (0.29-0.85)	0.58^{a} (0.36-0.84)
duration (s)	standing	82^{a} (46-125)	96^{a} (63-135)	165^{b} (114-221)	127^{b} (89-170)
	walking	1.1^{b} (0.5-1.9)	0.81^{b} (0.37-1.43)	0.00^{a} (0.00-0.15)	0.01^{a} (0.00-0.14)
	sitting	175^{a} (103-257)	145^{a} (87-212)	317^{b} (228-405)	340^{b} (266-412)
	pacing	7.6^{b} (3.3-13.7)	8.2^{b} (4.2-13.5)	0.01^{a} (0.00-1.24)	0.00^{a} (0.00-0.64)
latency (s)	stepping	310^{a} (233-387)	288^{a} (222-355)	542^{b} (486-581)	516^{b} (464-557)

*$P<0.05$, **$P<0.01$, ***$P<0.001$

[1] Values within the same row lacking a common letter superscript differ significantly at $P < 0.05$.

Table 1b: Back transformed least square means of the open field responses at 17-18 weeks of age for Rhode Island Red and White Leghorn birds in pure and mixed groups (95%-confidence intervals between brackets).

		Rhode Island Red birds		White Leghorn birds	
type	response	pure	mixed	pure	mixed
frequency	stepping	2.94^{b} (1.67-4.56)	2.57^{b} (1.55-3.83)	0.18^{a} (0.00-0.76)	0.58^{a} (0.14-1.32)
	jumping	0.03^{a} (0.00-0.09)	0.02^{a} (0.00-0.06)	0.06^{b} (0.02-0.14)	0.14^{b} (0.07-0.23)
	defecating	0.67^{c} (0.45-0.94)	0.59^{c} (0.41-0.80)	0.06^{a} (0.01-0.16)	0.22^{b} (0.11-0.37)
duration (s)	standing	484^{c} (418-538)	385^{b} (321-445)	48^{a} (14-101)	85^{a} (42-140)
	walking	0.79^{b} (0.26-1.62)	0.38^{b} (0.09-0.88)	0.00^{a} (0.00-0.17)	0.07^{a} (0.01-0.37)
	sitting	78^{a} (33-139)	187^{b} (129-253)	541^{c} (482-581)	506^{c} (447-553)
	pacing	0.00^{ab} (0.00-0.04)	0.06^{b} (0.01-0.15)	0.00^{a} (0.00-0.03)	0.00^{a} (0.00-0.03)
latency (s)	stepping	449^{a} (388-502)	485^{a} (439-526)	561^{b} (520-588)	558^{b} (522-584)

*P<0.05, **P<0.01, ***P<0.001

[1] Values within the same row lacking a common letter superscript differ significantly at P < 0.05.

Manual restraint tests. Social environment had no effect on manual restraint responses at 7-8 weeks of age in either RIR or WL birds. Social environment tended to affect the frequency of struggling at 24 weeks of age ($P < 0.10$) and the interaction between line and social environment tended to affect the latency of struggling at 24 weeks of age. Examination of the corresponding least square means revealed that BB birds from mixed groups had a lower frequency of struggling ($P < 0.05$, Table 2) and a longer latency to struggle ($P < 0.05$) than RIR birds from pure groups. Corticosterone responses at 7-8 weeks and at 24 weeks of age were not affected by social environment in either RIR or WL birds (Table 2).

Correlations

Several correlations in RIR and WL birds were different for birds from pure and mixed groups. In RIR birds from pure groups, significant correlations were found between latency to first step at 5-6 weeks and either frequency of struggling at 7-8 weeks of age ($r = -0.30$; $P < 0.05$) or latency to first step at 17-18 weeks of age ($r = 0.51$; $P < 0.001$) and between duration of standing at 5-6 weeks and latency to first step at 17-18 weeks of age ($r = -0.29$; $P < 0.05$). These correlations differed significantly ($P < 0.05$) from the same correlations in RIR birds from mixed groups ($r = 0.16$; $P > 0.10$, $r = 0.01$; $P > 0.10$ and $r = 0.09$; $P > 0.10$ respectively). In WL birds from pure groups, significant correlations were found between latency to first step at 5-6 weeks and corticosterone response at 7-8 weeks of age ($r = -0.32$; $P < 0.05$) and between duration of sitting at 5-6 weeks and latency to first step at 17-18 weeks of age ($r = 0.35$; $P < 0.05$). These correlations differed significantly ($P < 0.05$) from the same correlations in WL birds from mixed groups ($r = 0.04$; $P > 0.10$ and $r = -0.02$; $P > 0.10$ respectively). Correlations in RIR and WL birds between manual restraint responses at 7-8 weeks and 24 weeks of age were not different for pure and mixed groups.

In RIR birds, pooled correlations were found significant between duration of standing at 5-6 weeks and corticosterone response at 7-8 weeks of age ($r = -0.25$; $P < 0.01$) and between latency to first step at 17-18 weeks and corticosterone response at 24 weeks of age ($r = 0.29$; $P < 0.05$). In WL birds, pooled correlations were found significant between latency to first step at 5-6 weeks and corticosterone response at 7-8 weeks of age ($r = -0.32$; $P < 0.05$) and between duration of sitting at 17-18 weeks of age and corticosterone response at 24 weeks of age ($r = -0.30$; $P < 0.05$).

Discussion

Interpretation of open field responses

Open field responses in laying hens are assumed to be under influence of fear elicited by both human handling and exposure to a novel environment, which conflict with motivations to reinstate social contact and to show explorative behaviour (Rushen, 2000). Pharmacological studies showed that open field activity in domestic chicks was reduced after administration of an anxiogenic agent (Moriarty, 1995; Marín et al., 1997). High open field activity is thus generally interpreted as an indication of low fear (Jones, 1996) but this may, however, depend on the age of the tested birds (Candland and Nagy, 1969; Rodenburg et al., 2004) and the extent of involvement of other motivations. In this experiment, for example, the open field arena provided birds with more space than their home cages, so some open field activity may have reflected a 'rebound' in spatially-restricted behaviours (Nicol, 1987). In this experiment the suite of behaviours observed in the open-field was not entirely consistent with the view that high activity reflects low fear as, for example, greater defaecation rates were observed in the RIR than in the WL birds.

If, however, it is assumed that open field activity does reflect fear, then RIR birds appear to be less fearful than WL birds as they showed more open field activity. This could already be observed at 5-6 weeks of age, which corresponds with a previous study (Jones et al., 1995). Similar to Hocking et al. (2001), RIR and WL birds both showed a decrease in open field activity between 5-6 and 17-18 weeks of age. This suggests that birds became more fearful with age. Habituation to the test procedure might have also altered open field activity in both lines. These habituation effects could, however, be of minor importance, because of the long time period in between both open field tests.

Table 2: Back transformed least square means of the manual restraint responses at 7-8 and 24 weeks of age for Rhode Island Red and White Leghorn birds in pure and mixed groups (95%-confidence intervals between brackets).

age	response	Rhode Island Red birds		White Leghorn birds	
		pure	mixed	pure	mixed
7-8 wks	corticosterone (ng/ml)	8.68^{a1} (7.39-9.98)	8.64^{a} (7.34-9.94)	9.53^{b} (8.15-10.90)	10.68^{b} (9.39-11.97)
	latency of struggling (s)	238 (207-263)	228 (198-254)	243 (212-270)	236 (204-261)
	frequency of struggling	0.88 (0.50-1.35)	0.86 (0.50-1.32)	0.88 (0.49-1.42)	0.79 (0.44-1.25)
24 wks	corticosterone (ng/ml)	11.35 (9.87-12.84)	10.00 (7.56-12.43)	9.39 (7.80-10.99)	9.54 (7.09-11.99)
	latency of struggling (s)	169^{a} (133-204)	236^{a} (187-274)	299^{b} (289-299)	298^{b} (280-297)
	frequency of struggling	1.35^{b} (0.94-1.82)	0.55^{b} (0.21-1.04)	0.02^{a} (0.00-0.10)	0.00^{a} (0.00-0.10)

†P<0.10, *P<0.05, **P<0.001.

[1] Values within the same row lacking a common letter superscript differ significantly at $P < 0.05$.

Interpretation of manual restraint responses

Identification of the possible motivations underlying the manual restraint response strictly requires the test to be correlated to behavioural responses obtained from a variety of other tests (Mignon-Grasteau et al., 2003; Beaumont et al., 2005; Forkman et al., 2007). The consistent line difference in open field and manual restraint response and the (weak) correlations in RIR birds between the open field and the manual restraint test, do, however, suggest that high activity in the manual restraint could reflect low fear. It is therefore hypothesized that struggling in the manual restraint test is influenced by fear elicited by human handling, which conflicts with the tendency to escape from the manual restraint (and to a lesser extent with the tendency to reinstate social contact). The lack of other correlations between open field and manual restraint tests may depend on age and genotype of the tested birds or on the low response variability in at least the open field at 17-18 weeks and manual restraint test at 24 weeks of age in WL birds. The variability between birds in the manual restraint test might be increased by extending the duration of the test, which appears most useful in WL birds, or by including observations on vocalizations.

RIR birds showed more activity than WL birds in the manual restraint test at 24 weeks of age, but not in the manual restraint test at 7-8 weeks of age. This could mainly be attributed to a decrease in activity between consecutive manual restraint tests in WL, but not in RIR birds. Duration of tonic immobility at 5 weeks of age also did not differ between a low and high feather pecking line (Jones et al., 1995). If habituation had similar effects on struggling in RIR and WL birds, then fear responses towards humans may have increased with age in WL but not in RIR birds, as has been reported for fear responses towards a novel object in adult birds of these same lines (Uitdehaag et al., 2008). As for the open field tests, habituation effects could be of minor importance, because of the long time period in between both manual restraint tests.

Correlations indicated that high corticosterone response after manual restraint related to lower activity in the open field in RIR birds, but to higher activity in the open field in WL birds (correlations in WL birds are similar to Japanese quail; Jones et al., 1992a, 1992b; Jones and Satterlee, 1996). It therefore seems that the apparent complex interaction between the behavioural and physiological response to fear-eliciting situations is influenced by genetic factors. Corticosterone response may have different roles in each line. For instance, in RIR birds, it might mainly have a stress-mediating function to potentiate and consolidate the state of fear (Korte, 2001), whereas in WL birds, corticosterone response might mainly have a gluconeogenic function to provide energy for behavioural activity (Davis et al., 2000).

Effects of social environment on Open Field and Manual Restraint responses

RIR birds from mixed groups were less active in the open field at 17-18 weeks (and tended to be less active in the manual restraint at 24 weeks of age) than RIR birds from pure groups, which could indicate that they became more fearful. Fear related behaviour has been suggested to be transmitted between group mates (Mills and Faure, 1990; Jones, 1996). High fear responses of WL birds from mixed groups elicited by occasional, stressful events during the rearing period, could therefore have influenced their RIR group mates to respond more similar to their WL group mates in fear-eliciting situations. Several correlations between activity in the open field at 5-6 weeks and 17-18 weeks of age were found in RIR as well as in WL birds from pure, but not from mixed groups. That the variability of the individual responses in estimation of these correlations were equal for pure and mixed groups, suggests that social environment affected a trait underlying these individual open field responses, i.e. fearfulness. Differences in other motivations could also be involved. RIR birds from pure groups might, for instance, be more motivated to reinstate social contact with their group mates than RIR birds from mixed groups.

As fearfulness is a trait which is only expressed after occasional, stressful events occurring throughout the rearing and laying period, a possible role for social facilitation in the transmission of fear responses seems unlikely. Foraging behaviour, which is closely related to explorative behaviour, one of the other motivations that underlie open field activity, can possibly be transmitted from bird to bird by social facilitation (Tolman, 1968; Noble et al., 1993; Nicol and Pope, 1999). Transmission of behaviour by social facilitation is, however, most likely to occur from dominant to subordinate birds (Nicol and Pope, 1994). In the current study, however, the behaviour of heavier and therefore assumed more dominant, RIR birds (Cloutier and Newberry, 2000) was mostly affected. RIR birds may also have experienced mixed groups as being more stressful than pure groups, because these RIR birds were exposed to the specific behavioural characteristics of WL birds (high feather pecking and high fear responses). An individual bird may, in general, experience more stress if its group mates simply have different behavioural characteristics (Albentosa et al. 2003). Irrespective of these possible manners in which behaviour is assumed to be transmitted, specific genotype- by social environment interactions may as well influence the individual susceptibility to social stress (Noble et al., 1993; Mahagna et al., 1994; Cheng et al., 2002).

The current study did not provide evidence that social environment affected fear responses of birds in the early open field test at 5-6 weeks or in the early manual restraint test at 7-8 weeks of age. It might be that fear related responses were not yet sufficiently differentiated between lines or that time was too short to establish an effect of social environment. Younger birds might also have been less susceptible to changes in their social environment than older birds, although social pecking at conspecifics, which is related to gentle feather pecking, already occurs in the first days of life (Roden and Wechsler, 1998; Riedstra and Groothuis, 2002).

In conclusion, RIR birds were confirmed to respond less fearful in fear-eliciting situations than WL birds, which was already observed in the open field test at 5-6 weeks of age. RIR birds from mixed groups became less active in the open field and manual restraint test than RIR birds from pure groups, adopting a response more similar to those of their WL group mates. This effect could only be observed after 8 weeks of age. Responses also seemed affected in WL birds from mixed groups, as correlations between responses at different ages were found different for pure and mixed groups in RIR as well as in WL birds. These results suggest that responses to fear-eliciting situations are influenced by genetic factors as well as by interaction with group mates. The influence of these group mates depends on their behavioural characteristics, which are partly genetically determined.

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Chapter 6:

Mixed housing of different genetic lines of laying hens negatively affects feather pecking and fear related behaviour

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Abstract

Adult laying hens from Rhode Island Red (RIR) origin both express lower levels of feather pecking and lower fear responses towards a novel object than laying hens from White Leghorn (WL) origin. The present study investigated whether mixed housing of RIR and WL laying hens would affect their behaviour in both an open field (at 17-18 weeks of age) and manual restraint test (at 24 weeks of age) and their feather damage due to severe feather pecking. In experiment A, 'pure' groups contained birds from one line only throughout the rearing and laying period. 'Mixed' groups contained an equal number of RIR and WL birds. Pure and mixed groups contained four birds, which were housed in battery cages. It was found that RIR birds were more active in the open field and manual restraint test than WL birds, although RIR birds from mixed groups became less active in the open field test than RIR birds from pure groups. This would indicate that RIR birds were less fearful than WL birds, but that they became more fearful in presence of these WL birds. In experiment B, RIR and WL birds were only housed together during the laying period, in varying ratios. It was found that WL birds from mixed groups had more feather damage due to severe feather pecking than WL birds from pure groups, whereas no effect of mixing was found in RIR birds. RIR birds from mixed groups therefore appeared to have developed relatively high levels of feather pecking, targeted at WL birds. This would indicate that, together with results from experiment A, fearful RIR birds from mixed groups were at higher risk to develop feather pecking than less fearful RIR birds from pure groups. This study clearly demonstrates that social factors have a strong influence on the development of feather pecking and related behavioural characteristics.

Introduction

Feather pecking is a maladaptive behaviour occurring in adult laying hens and is a major welfare problem in the current poultry industry. There are two forms of feather pecking: gentle and severe feather pecking. Gentle feather pecking does not lead to feather damage and is mostly ignored by the recipient. Severe feather pecking does lead to feather damage (Savory, 1995; Bilčík and Keeling, 1999) and victims of severe feather pecking may even suffer from tissue damage and cannibalistic death (Savory, 1995). Here we focus on severe feather pecking as it is most detrimental for animal welfare.

Feather pecking was originally thought to be promoted when expression of foraging (Blokhuis and Arkes, 1984), or dustbathing behaviour (Vestergaard and Lisborg, 1993) was blocked by environmental restrictions. A later study emphasized that feather pecking should be considered redirected foraging instead of redirected dustbathing behaviour (Huber-Eicher and Wechsler, 1997). However, a recent study showed that severe feather pecking in adult birds was not a substitute for foraging, since it was observed next to increased levels of foraging behaviour (Newberry et al., 2007).

These results still do not answer the question why some birds develop feather pecking, and other birds do not. There are indications that individual bird characteristics, such as their fearfulness, are related to the propensity to develop feather pecking behaviour. Fearful birds could have more problems with adapting to their environment (Jones, 1996) and may therefore be more likely to develop maladaptive behaviours. This is supported by other studies (Jones et al., 1995; Rodenburg et al., 2004) reporting more feather pecking in adult birds that showed high fear responses in an open field test at young age. Fear responses of laying hens already start to develop in early life and can be influenced by their social environment (Rogers, 1995). It has been suggested (Nicol, 1995; McAdie and Keeling, 2002) that the presence of docile birds with a low propensity to develop feather pecking reduces fear and possibly also feather pecking behaviour in groups of laying hens.

Many of the behavioural differences observed between commercial, genetically pure-bred lines of laying hens can be attributed to their origin, i.e. Rhode Island Red (RIR) or White Leghorn (WL; Uitdehaag et al., 2008). Rhode Island Red birds are, in general, more docile and less fearful and show less feather pecking than White Leghorn birds, which are more flighty and more fearful and show more feather pecking. The present study was designed to investigate whether mixed housing of RIR and WL birds would reduce both feather damage due to severe feather pecking and fear related behaviour. Fear related behaviour was assessed in an open field and in a manual restraint test. Part of the results with respect to these tests have been described elsewhere (Uitdehaag et al. submitted). In the present study, these behavioural tests were used to characterize both performers and victims of feather pecking. Results show that, contrary to the proposed hypothesis, RIR birds became more fearful in presence of WL birds and started to develop feather pecking, which was targeted only at WL birds.

Material and Methods

Birds from Rhode Island Red (RIR) origin show less feather pecking behaviour and a lower fear response towards a novel object than birds from White Leghorn (WL) origin (Uitdehaag et al., 2008). The current study comprised two parallel experiments A and B (Fig. 1) using birds of two genetic lines, both with white feathers, from either RIR (line BB in Uitdehaag et al., 2008) or WL origin (line W1 in Uitdehaag et al., 2008). In experiment A (325 birds), experimental groups consisted of either birds from one line only ('pure' groups) or an equal number of RIR and WL birds ('mixed' groups). These groups were kept together throughout the rearing (5-18 weeks of age) and laying period (19-58 weeks of age). Other experimental groups included birds that were kept in mixed groups during the rearing, but in pure groups during the laying period. In experiment B (877 birds), birds were kept in pure groups during the rearing period. At 18 weeks of age, birds were randomly taken from different groups to form experimental groups: one, two, three or four birds from one genetic line with three, two, one or zero birds from the other genetic line

(1:3, 2:2, 3:1 and 4:0 for each line respectively). The experiment was carried out by licensed and authorized personnel under approval of Institut de Sélection Animale B.V.

Housing conditions

Rearing period. Birds from experiments A and B were housed in two neighboring blocks within the same row of a battery cage rearing system. The first block (experiment A; 15 cages) comprised three stacked tiers of five adjacent cages. These cages either contained pure groups with, on average, 23 RIR birds (3 cages) or 20 WL birds (3 cages), or contained mixed groups with 11 RIR and 10 WL birds (9 cages; Figure 1).

The neighboring block (experiment B, 42 cages) comprised three stacked tiers of 14 adjacent cages. These cages either contained groups with, on average, 22 RIR birds (23 cages) or 19 WL birds (18 cages; Figure 1), leaving one out of 42 cages unoccupied. experiment. Each cage was of similar size (42 cm (height) x 65 cm (depth) x 96 cm (width)) and only the upper tier was used to house the birds until 5 weeks of age, after which birds were allocated to the cages in all three tiers.

In both experiments, feed was provided ad libitum by a 96 cm food trough (cage width) and water was provided through three drinking nipples per cage. During the first week chicks received 23 hours of light per day (23L : 1D light scheme), which was reduced to a 19L : 5D light scheme in the second and third week. Between the fourth and 17th week, the light regime was gradually changed into 11L : 13D, reducing the diurnal light period with 1.14 hours between subsequent fortnights. Chicks were fed standard commercial rearing diets and treated with routine vaccinations.

Laying period. From 18 weeks onwards, birds from experiments A and B were housed in two neighboring blocks within the same row of a battery cage laying system. The first block (experiment B; 150 cages) comprised three stacked tiers of 50 adjacent cages. These cages either contained pure groups with four RIR (30 cages) or four WL birds (25 cages), or contained mixed groups with one (27 cages), two (30 cages) or three (30 cages; Figure 1) RIR birds, which were housed together with three, two or one WL bird(s) respectively.

The neighboring block (experiment A, 75 cages) comprised three stacked tiers of 25 adjacent cages. These cages either contained pure groups with four RIR (15 cages) or four WL birds (12 cages), mixed groups with two RIR and two WL birds (13 cages) or ‘change’ groups (birds that were housed in mixed groups during the rearing-, but in pure groups during laying period) with four RIR (14 cages) or four WL birds (13 cages; Figure 1).

Experimental groups were randomly allocated to the cages within each experiment. Each cage was of similar size (46 cm (height) x 39 cm (depth) x 44 cm (width)). Remaining cages within each experiment were used for birds that were not included in the experiment.

In both experiments, a standard commercial laying diet was provided ad libitum by a 44 cm food trough (cage width) and water was provided through two drinking nipples per cage.

After arriving at the laying facility at 18 weeks of age, the light schedule was 10L : 14D, which gradually changed to a final 16L : 8D scheme from 20 weeks of age onwards.

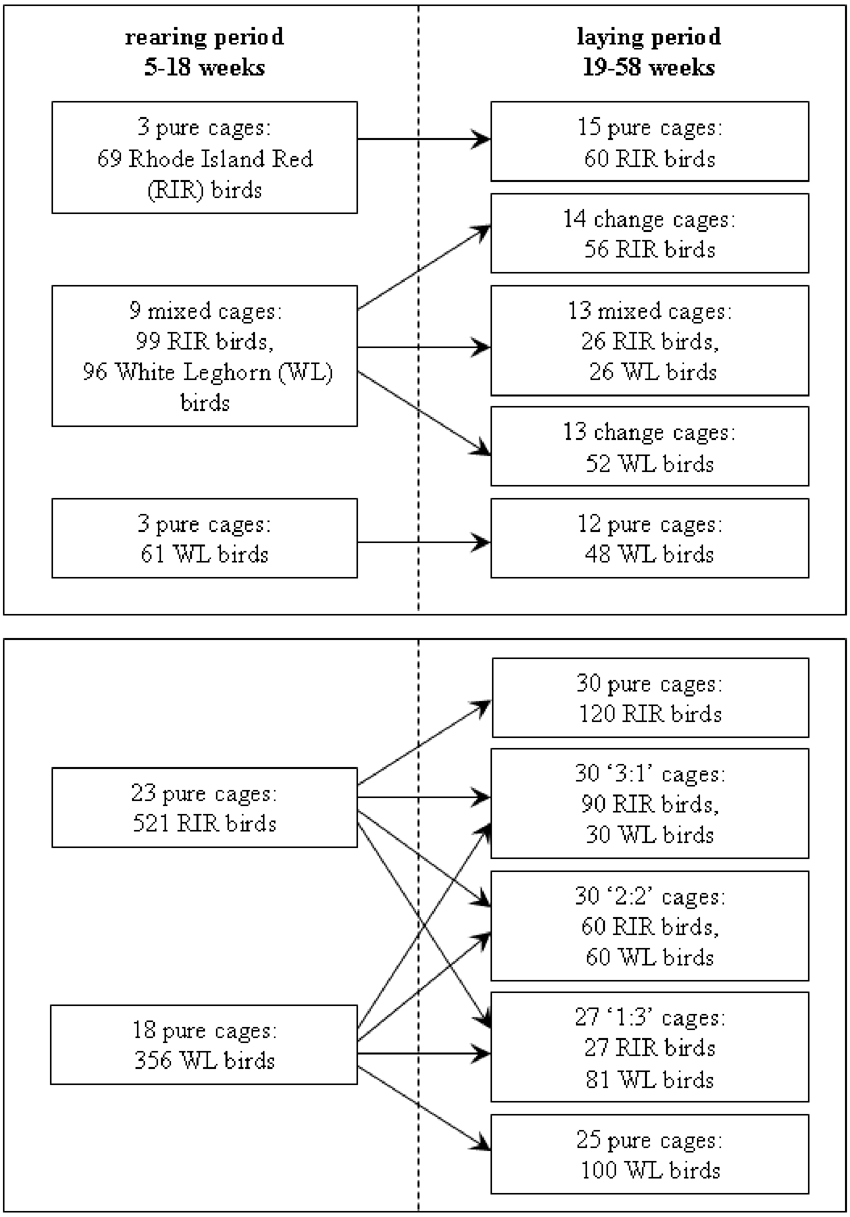

Figure 1: Experimental design for experiments A (top panel) and B (bottom panel).

Observations

Feather pecking damage (both experiments). Feather damage at back and rump, an indicator for damage due to severe feather pecking, was scored (Bilčík and Keeling, 1999) on each individual bird at 19, 24, 30, 35, 39 and 46 weeks. Additional observations were done at 58 weeks in experiment B.

Open field test (experiment A). After being randomly picked from their home cage, each individual bird was placed in the middle of an open field at 17-18 weeks (rearing period) and its response was recorded for ten minutes using continuous focal sampling. The open field pen was located in a separate room and measured 1.25 x 1.25 m with a Perspex wall in front allowing video observations. Responses were scored using The Observer 5.0 (Noldus Information Technology b.v., Wageningen, The Netherlands) using an ethogram that included latency to first step, number of steps, number of defecations and number of jumps, duration of standing, duration of sitting, duration of walking and duration of pacing. Lights in the open field room were switched on at the start of each test. Birds were tested in random order and were not taken from the same home cage in consecutive tests. The open field test was carried out during nine days (two consecutive five- and four-day working weeks) between 8:00 and 17.30 h (split into four time blocks).

Manual restraint test (experiment A). At 24 weeks, each individual bird was taken out of its home cage and manually restrained on its side for five minutes on one of two horizontal surfaces which were located on both ends of the cage-row. The test person used one hand to restrain the legs, while the other hand was used to restrain the upper part of the body of the tested bird. The hand restraining the legs mainly functioned to prevent the birds from escaping when struggling, whereas the other hand put mild pressure on the upper body part when a bird was not struggling. After each struggle, birds were gently brought back in their original position. If subsequent struggles occurred within five seconds then they were recorded as a single struggle, otherwise they were recorded as separate struggles. Observations on bird responses during the manual restraint included the latency to struggle and frequency of struggles. Immediately after each test, a blood sample from the wing vein was taken for corticosterone determination. Two ml of blood was collected in EDTA coated tubes and was stored at 4 °C. From these blood samples, plasma was recovered and stored at -20 °C. Corticosterone concentrations were determined from the plasma samples using a radioimmunoassay kit (IDS, Inc., Bolton, UK) as described previously (Buyse et al., 1987). Birds were tested in random order and were not taken from the same home cage in consecutive tests. Each manual restraint test was performed by one of two persons, on five consecutive days between 7.30 and 16.00 (divided into 3 consecutive time blocks).

Statistical methods

Feather pecking damage (both experiments). Feather pecking damage in both experiments was analyzed in the same way. After summing individual back and rump scores into a combined score for feather pecking damage (FPD), FPD means of line conspecifics within a cage were calculated at each time point. These FPD means were analyzed in a mixed model (using SAS version 9.1.3), which included social group, age and their interaction term as fixed effects and was run separately for each line in each experiment. Dependency of FPD at consecutive ages was accounted for by modeling an unstructured (co)variance matrix for repeated measurements on FPD cage means, which significantly improved the model fit ($P < 0.001$) for each line in experiments A and B.

Open field and manual restraint test (experiment A). Effects of social environment (pure vs. mixed groups) on individual open field and manual restraint responses were tested separately for each line in a GLM procedure, while adjusting for possible effects of cage (nested within social environment), date-, time- and test person effects. Line differences were tested with a similar model by replacing the social environment- with the line effect, using only observations from pure groups. Before these analyses, responses recorded as duration and latency were expressed as a ratio of the total observation time after which they were transformed with an arcsin square root transformation. A square root transformation was applied to responses recorded as frequency. Data are presented as back transformed least square means.

Factor analysis of open field responses (experiment A). A factor analysis, refer to Manly (1986) for a description of this statistical method, was performed separately for each line (using an orthogonal varimax rotation), to identify common factors that would comprehensively describe open field responsiveness. The loadings (ranging from -1 to 1) of the individual variables on the factors determine how these variables are assigned to these factors. In an ideal situation, a single variable will strongly load on one factor (loading close to -1 or 1), whereas it will not load on the other factors (loading close to 0). In our case, if the loading was smaller than -0.50 or bigger than 0.50, then a variable was assigned to a specific factor. Each bird was then assigned a score for each identified factor and the effect of social environment on these scores was tested in a GLM procedure, while adjusting for possible effects of cage (nested within social environment), date-, time- and test person effects.

Correlations of open field and manual restraint responses with FPD (experiment A). Duration of standing and sitting, factor scores and manual restraint responses (all adjusted for cage-, date-, time- and test person effects) were correlated with FPD (adjusted for cage effects) at 39 and 46 weeks. These Spearman correlations were calculated separately for each line using only records from individual birds in pure groups.

Results

Feather pecking damage (experiment A)

The development of FPD in the different groups with age is shown in figure 2. WL birds that were kept in mixed groups throughout the rearing and laying period had more FPD than WL birds in the other experimental groups (social environment effect: $F_{2,35} = 4.10$; $P < 0.05$). There was no such effect in the RIR birds ($F_{2,39} = 1.03$; $P > 0.05$). FPD significantly increased with age in both the RIR ($F_{5,39} = 4.79$; $P < 0.001$) and WL birds ($F_{5,35} = 12.00$; $P < 0.001$). The interaction between social group and age was significant in WL ($F_{10,35} = 2.63$; $P < 0.05$), but not in RIR birds ($F_{10,39} = 0.73$; $P > 0.05$). The interaction effect in WL birds indicated that the difference in FPD between pure and mixed groups became greater with age.

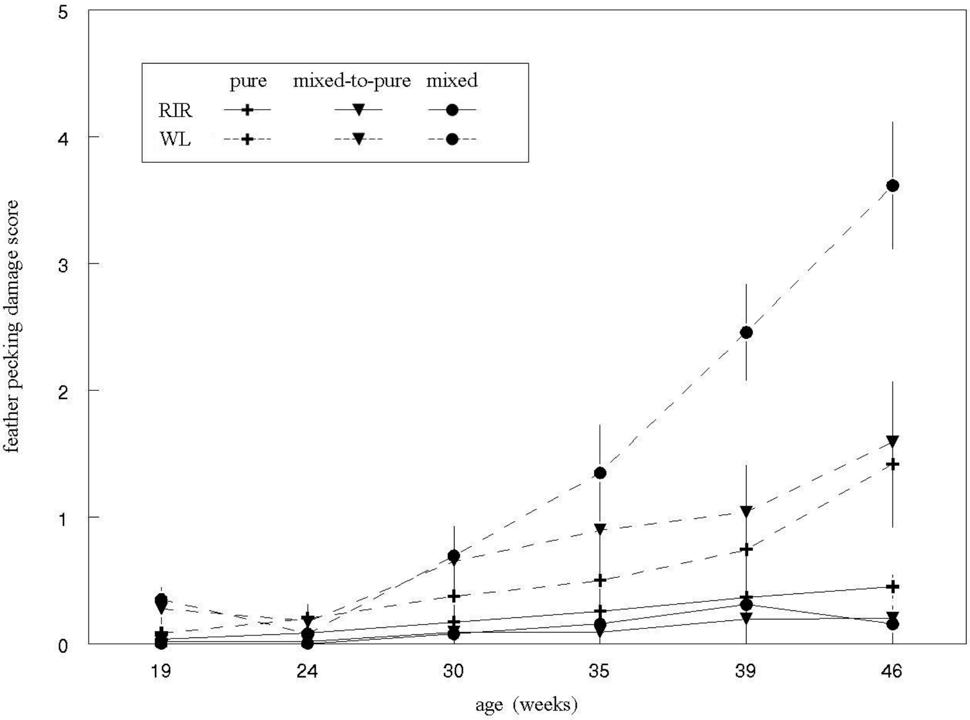

Figure 2 : Development of FPD least square means with age for different social groups in the Rhode Island Red and White Leghorn line in experiment A.

Feather pecking damage (experiment B)

The development of FPD in the different groups with age is shown in figure 3. WL birds that were kept in mixed groups only during the laying period had more FPD at 46 (3.00 ± 0.25 vs.1.88 ± 0.46; $P < 0.05$; least square mean ± SE) and at 58 weeks (4.43 ± 0.30 vs. 2.58 ± 0.54; $P < 0.001$) than WL birds from pure groups (social group by age interaction-effect: $F_{18,108} = 2.32$; $P < 0.001$). No main effect of social group on FPD was detected in either RIR ($F_{3,113} = 0.28$; $P > 0.05$) or WL birds ($F_{3,108} = 1.03$; $P > 0.05$). FPD did significantly increased with age in both RIR and WL birds ($F_{6,\ 113}$ = 22.12; P < 0.001 and $F_{6,\ 108}$ = 38.51; P < 0.001 for RIR and WL birds respectively). The interaction of social group with age in RIR birds was significant ($F_{18,\ 113}$ = 2.21; P < 0.05), but did not reveal a clear pattern.

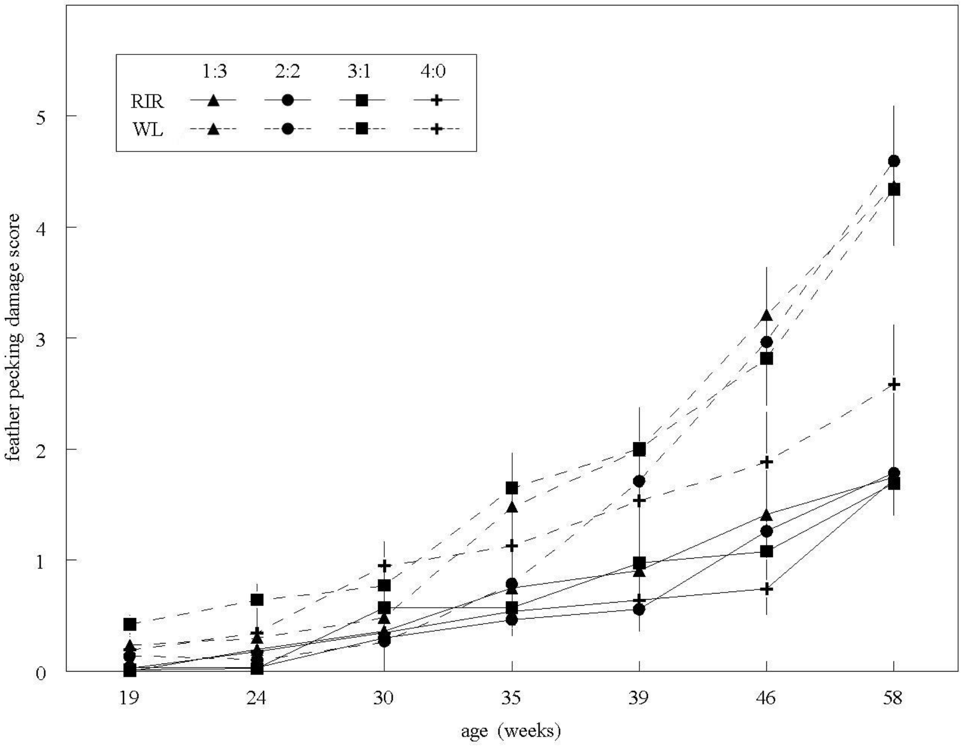

Figure 3: Development of FPD least square means with age for different social groups in the Rhode Island Red and White Leghorn line in experiment B.

Line differences in open field and manual restraint responses

Line differences between pure groups in open field responses were all significant (P < 0.01), except for frequency of jumping and duration of pacing. RIR birds showed more activity in the open field test than WL birds, which was reflected by their shorter latency to first step ($F_{1,\ 96}$ = 9.53; P < 0.001, Table 1), their higher frequency of stepping ($F_{1,\ 96}$ = 10.66; P < 0.001), their longer duration of walking ($F_{1,\ 96}$ = 4.44; P < 0.05) and standing ($F_{1,\ 96}$ = 12.78; P = 0.00) and their shorter duration of sitting ($F_{1,\ 96}$ = 15.39; P < 0.001). A similar pattern was found in the manual restraint test, where RIR birds struggled sooner ($F_{1,\ 47}$ = 41.98, 319 vs. 596 s; P < 0.001) and more ($F_{1,\ 47}$ = 37.74, 1.27 vs. 0.01 times; P < 0.001) than WL birds. Corticosterone response after manual restraint was higher in RIR birds, but did not statistically differ between RIR and WL birds ($F_{1, 47} = 2.42$; $P > 0.05$, 11.62 ± 0.92 vs. 9.55 ± 0.93 ng/ml).

Effects of social environment on open field and manual restraint responses

Open field responses of birds from mixed groups seemed to become more similar to that of their group mates from the other line (significant for two responses in RIR birds and for one response in WL birds; Table 1). RIR birds from mixed groups had a shorter duration of standing ($F_{1, 124} = 4.10$; $P < 0.05$; Table 1) and a longer duration of sitting ($F_{1, 124} = 5.50$; $P < 0.05$) as compared with RIR birds from pure groups. WL birds from mixed groups, on the other hand, had a higher frequency of defecating ($F_{1, 104} = 5.80$; $P < 0.05$) as compared with WL birds from pure groups.

Factor analysis revealed two underlying factors for open field response in RIR birds, which together accounted for (71% + 26% =) 97% of the variance. Duration of walking (0.67) and latency (-0.80) and frequency (0.93) of stepping loaded on the first factor (loading should be smaller than -0.50 or bigger than 0.50 for response to be assigned to that factor). Factor 1 scores did not differ ($F_{1, 135} = 0.02$; $P > 0.05$) between RIR birds from pure (0.01 ± 0.13) and mixed groups (-0.01 ± 0.11). RIR birds from mixed groups did have a significantly ($F_{1, 135} = 4.24$; $P < 0.05$) lower score for the second factor (-0.16 ± 0.11) than RIR birds from pure groups (0.20 ± 0.14). Based on the positive loading for standing (0.97) and negative loading for sitting (-0.95) on the second factor, this would indicate that RIR birds were less active in mixed than in pure groups.

Duration of standing (0.89) and sitting (-0.89), latency (-0.81) and frequency of stepping (0.83) and frequency of defecating (0.60), loaded on the first and only factor in WL birds. This factor accounted for 81% of the variance. Factor scores did not differ ($F_{1, 115} = 1.63$; $P > 0.05$) between WL birds from pure (-0.13 ± 0.14) and mixed groups (0.12 ± 0.13).

Latency and frequency of struggling as well as corticosterone response after manual restraint were not affected by social environment in RIR birds. WL birds from mixed groups tended ($F_{2,51} = 2.81$; $P < 0.10$) to struggle sooner (284 s, range: 264 – 297 s) than WL birds from pure groups (298 s, range: 293 – 300 s) and than WL birds that changed from mixed groups during rearing to pure groups during the laying period (300 s, range: 294 – 300 s). Frequency of struggling and corticosterone response were not affected by social environment in WL birds.

Table 1: Social environment-, cage-, date- and time effects on open field responses at 17 weeks of age separately for each line. Back transformed least square means (95%-confidence intervals between brackets) are presented for both the pure and mixed

line	type	response	social environment		significance			
			pure	mixed	soc. env.	cage	date	time
RIR	frequency	stepping	2.93 (1.44-4.94)	2.37 (1.21-3.91)	NS	NS	†	NS
		Jumping	0.03 (0.00-0.08)	0.02 (0.00-0.05)	NS	NS	NS	NS
		defecating	0.67 (0.42-0.97)	0.57 (0.37-0.81)	NS	*	*	NS
	duration	standing	486 (413-543)	386 (315-452)	*	**	NS	NS
		walking	0.78 (0.15-1.89)	0.32 (0.02-0.98)	NS	NS	NS	NS
		sitting	77 (29-145)	188 (123-261)	NS	NS	NS	NS
		pacing	0.00 (0.00-0.06)	0.06 (0.00-0.19)	*	**	NS	NS
	latency	stepping	453 (389-509)	495 (446-537)	NS	**	NS	*
WL	frequency	stepping	0.22 (0.03-0.57)	0.57 (0.25-1.03)	NS	†	†	NS
		jumping	0.06 (0.01-0.15)	0.14 (0.06-0.25)	NS	†	NS	NS
		defecating	0.06 (0.01-0.15)	0.22 (0.12-0.34)	NS	NS	NS	NS
	duration	standing	46 (14-94)	82 (42-133)	*	*	*	NS
		walking	0.00 (0.00-0.05)	0.06 (0.01-0.17)	NS	NS	NS	NS
		sitting	544 (492-580)	509 (456-552)	†	†	NS	NS
		pacing	0.00 (0.00-0.00)	0.00 (0.00-0.00)	.	.	.	.
	latency	stepping	.558 (520-585)	556 (522-581)	NS	NS	NS	NS

^{NS}P>0.10, †P<0.10, *P<0.05, **P<0.01.[1]Line differences between pure groups were all significant (P<0.01), except for frequency of jumping and duration of pacing.

Correlations of open field and manual restraint responses with FPD

Correlations of open field behaviour and manual restraint responses with FPD in pure groups are shown in table 2. Lower frequency of struggling in the manual restraint test significantly correlated with increased FPD at 46 weeks in RIR birds (r = -0.45; P < 0.001). Lower factor 1 scores (r = -0.27), longer latency of struggling in the manual restraint test (r = 0.27) and higher corticosterone responses after manual restraint (r = 0.28) tended to correlate (P < 0.10) with increased FPD at 46 weeks in RIR birds. Higher corticosterone responses after manual restraint also tended to correlate with increased FPD at 39 weeks in RIR birds (r = 0.26; P < 0.10). These correlations indicate that lower activity in the manual restraint test (and to a lesser extent in the open field test) was associated with increased FPD in RIR birds from pure groups. No such correlations were found in WL birds, although interpretation of these correlation was difficult because of their low activity in both open field and manual restraint tests.

High factor 1 scores, reflecting more activity in the open field in both RIR and WL birds, correlated with low corticosterone response in RIR (r = -0.30; P < 0.05), but with high corticosterone response after manual restraint in WL birds (r = 0.34; P < 0.05).

Discussion

The differences between RIR and WL birds in FPD, open field and manual restraint responses in the current study correspond well with earlier found characteristics of these same lines by Uitdehaag et al. (2008). Low feather pecking birds from RIR origin, show more open field activity and struggle sooner and more as compared with high feather pecking birds from WL origin. RIR birds also showed lower fearfulness in response to a novel object than WL birds (Uitdehaag et al., 2008). High open field activity in laying hens could reflect low fearfulness (Jones, 1996) and has already been associated with decreased feather pecking (Jones et al., 1995; Rodenburg et al., 2004).

It was hypothesized that the presence of RIR birds either during rearing or in the laying period would reduce fear and subsequent damage due to severe feather pecking (FPD) in WL birds. However, contrary to our hypothesis, WL birds from mixed groups in both experiments had increased FPD as compared with WL birds from pure groups. Results from experiment A show that presence of RIR birds in only the rearing period had no effect on FPD, whereas presence of RIR birds in both the rearing and laying period, increased FPD in WL birds. Results from experiment B show that presence of either one, two or three RIR birds in the laying period always increased FPD in WL birds, whereas the number of WL birds did not affect FPD in RIR birds. The FPD of the WL birds housed with three RIR birds was similar to the FPD of WL birds in the other mixed groups, whereas FPD of RIR birds was unaffected by their social environment. This strongly suggests that in each mixed group, WL birds became preferred victims of feather pecking by RIR birds and that RIR birds in mixed groups also showed relatively high levels of feather pecking. Combining results from experiments A and B, further indicates that the unfavourable effect of mixed housing on FPD in WL birds, will only occur earlier (before 46 weeks of age) when mixing is already applied during the rearing period.

RIR birds were heavier than WL birds (1.78 ± 0.01 vs. 1.52 ± 0.01 kg at 39 weeks). The higher bodyweight and a possibly associated higher social rank (Cloutier and Newberry, 2000) of RIR as compared with WL birds might have contributed to their increased expression of feather pecking (Bessei, 1984; Jensen et al., 2005). Lighter birds have been found to be more vulnerable to cannibalistic attacks (Yngvesson and Keeling, 2001; Cloutier and Newberry, 2002). Distinguishing between FPD as either the cause or consequence of lower body weight is, however, difficult. The current data did not reveal any correlations between body weight and feather damage within pure cages of RIR birds (data not presented). This suggests that RIR birds did not prefer light over heavy birds as their feather pecking victims, unless expression of feather pecking was triggered only after reaching a threshold difference in body weight between the RIR performer and its victim.

In a study comparable with experiment A, McAdie and Keeling (2002) investigated the effect of mixed housing of two White Leghorn lines differing in feather pecking behaviour. Birds from the high feather pecking line were also found to have more feather pecking damage at 12 and 17 weeks when mixed with birds from a low feather pecking line. However, birds from the low feather pecking line showed more severe feather pecking in both pure and mixed groups as compared with birds from the high feather pecking line. Environmental factors, other than mixing, may have obscured the relatively small genetic difference in feather pecking between the lines used by McAdie and Keeling (2002). Effects of mixing were more likely to be detected in the current study, as we used genetic lines from RIR and WL origin that display marked differences in feather pecking and other behaviours. Albentosa et al. (2003) used laying hens from one commercial line and found that groups of birds with variable responses to a novel object showed more feather pecking than groups of birds with similar response to a novel object. Mixing birds with different behavioural characteristics does therefore not seem favourable for reduction of feather pecking.

Open field and manual restraint responses of WL birds from mixed groups were hardly affected by the presence of their RIR cage mates (except for the effect on defecating and the non-significant effect on latency of struggling), whereas RIR birds from mixed groups showed fear responses more similar to that of their WL cage mates. The small effect of social environment on fear responses in WL birds could be explained by the insufficiency of the open field test to discriminate between the relatively high fear response of WL birds in general. Extension of the open field or manual restraint test or performing a more discriminating fear test (for instance by presenting a novel object in the open field) might increase response variability and subsequently facilitated better interpretation of behaviour in laying hens.

Table 2: Spearman correlation coefficients between open field responses and factors, manual restraint responses and feather damage at 39 and 46 weeks for birds from Rhode Island Red (above diagonal) and White Leghorn origin (below diagonal) in pure groups of experiment A.

	open field				manual restraint			feather damage	
	sitting	standing	factor1	factor2	struggling (frequency)	struggling (latency)	corticosterone	39 weeks	46 weeks
sitting	-	-0.93***	-0.16	-0.76***	0.12	0.06	0.04	0.03	0.06
standing	-0.94***	-	-0.01	0.80***	-0.23	0.03	0	-0.01	0
factor1	-0.62***	0.62***	-	-0.14	0.14	-0.17	-0.30*	-0.06	-0.27†
factor2	.	.	.	-	-0.17	0.15	0.06	-0.05	0.12
struggling (frequency)	-0.03	-0.1	-0.03	.	-	-0.75	-0.08	-0.23	-0.45**
struggling (latency)	0	0.12	0.06	.	-1.00***	-	-0.06	0.15	0.27†
corticosterone	-0.11	0.12	0.34*	.	0.04	-0.01	-	0.26†	0.28†
feather damage (39 weeks)	0.02	-0	0	.	0.26	-0.24	0.2	-	0.44**
feather damage (46 weeks)	0.03	-0	0.03	.	0.23	-0.21	0.01	0.53**	-

†P<0.10, *P<0.05, **P<0.01, ***P<0.001

That RIR birds did respond more to the presence of WL birds than vice versa, might also be due to individual characteristics of these birds. High open field activity, high corticosterone response and high serotonin as well as high dopamine turnover at brain level have all been associated with low expression of feather pecking, which provides evidence for existence of two distinct personality types (coping styles) in laying hens (Jones et al., 1995; Korte et al., 1997; Hierden et al., 2002; Buitenhuis et al., 2004; Rodenburg et al., 2004). Their underlying personality type might have predisposed RIR to be more responsive towards changes in their social environment, as has been suggested in studies with other species (Benus et al., 1989; Benus et al., 1990; Hessing et al., 1994; Bolhuis et al., 2004; Bolhuis et al., 2005).

Corticosterone response after manual restraint was higher, but not significant, in RIR than in WL birds, which has also been reported in previous studies (Korte et al., 1997; Hierden et al., 2002). The corticosterone response in the current study corresponded with the response found previously (Beuving and Vonder, 1978) after immobilization of 33 to 37 week old birds for six minutes by hand (11.62 and 9.55 vs. 10 ng/ml respectively). However, the negative correlation of lower open field activity with higher corticosterone response in RIR birds, is opposite to what could be expected (Hierden et al., 2002; Rodenburg et al., 2004) and suggests a more complex relationship between corticosterone response and personality types in laying hens.

Correlations of open field and manual restraint responses with FPD in RIR birds from pure groups might give further insight in why RIR birds from mixed groups started pecking WL birds. In RIR birds lower open field and manual restraint activity correlated with increased FPD at 46 weeks. This would suggest that fearful birds, assuming that open field activity reflects fearfulness, are preferred victims of feather pecking by RIR birds. In mixed groups, RIR birds might therefore have predominantly pecked at WL birds, because WL birds are more fearful.

High fearfulness, reflected by low open field activity, as found for RIR birds from mixed as compared with RIR birds from pure groups, has also been associated with increased tendency to express feather pecking (Jones et al., 1995; Rodenburg et al., 2004). High fearfulness may therefore be unfavourable for both the expression of feather pecking as well as for the probability to be victimized by feather pecking. Assuming that birds from the same genetic background adopt a similar fear response, effects of victim characteristics in previously found genetic line differences in feather pecking (Jones et al., 1995; Kjaer, 2000; Hocking et al., 2004; Uitdehaag et al., 2008) should not be underestimated. Selection for low fear responses in an open field might be a possible way to reduce the damaging effect of feather pecking by decreasing its expression as well as by improving victim characteristics.

In conclusion, presence of RIR birds throughout the rearing and laying period increased damage due to severe feather pecking in WL birds. This feather pecking damage is assumed to be caused by higher expression of feather pecking by RIR birds, which also became more fearful in an open field after mixed housing with WL birds. This suggests that individual susceptibility to develop feather pecking is affected by social environment and that this can be quantified by assessing open field activity. The high fear response of WL birds might have predisposed them to become preferred victims of feather pecking by RIR birds. Fearfulness is therefore a victim characteristic that could be considered when evaluating measures to reduce the damaging effects of feather pecking.

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Chapter 7:

Effects of mixing two genetic lines of laying hens with different tendencies to show feather pecking on behavioural, serotonergic and dopaminergic responses

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Abstract

Feather pecking is a behavioural problem in laying hens. Previous work showed that birds of Rhode Island Red (RIR) genetic origin which generally show low levels of this behaviour, displayed a profound increase in feather pecking if they were housed together with birds from a White Leghorn (WL) genetic origin. It is, however, unknown, whether this effect is accompanied by changes in stress-related behaviour and neurophysiological activity, which are assumed to be associated with feather pecking. The objective of this study was therefore to investigate the effects of genetic origin (WL or RIR), social environment and their interaction on behaviour and brain serotonin (5-HT) and dopamine (DA) turnover. Experimental birds, either housed with other birds from the same genetic origin (pure groups) or with both RIR and WL birds (mixed groups) from hatching onwards, were subjected to a manual restraint test at 47 weeks of age. High feather pecking WL birds showed less escape behaviour during restraint and higher DA and 5-HT turnover levels after restraint than RIR birds, which disagrees with earlier studies reporting opposite relationships between 5-HT and DA turnover and feather pecking propensity. WL birds showed higher levels of platelet 5-HT uptake than RIR birds. Although the social environment or its interaction with genetic origin did not affect behavioural-, brain 5-HT and DA activity, reversed correlations between the neurophysiological parameters and behaviour were found in RIR birds from mixed versus pure groups.

Introduction

Feather pecking is a behavioural problem in laying hens, which causes feather and tissue damage and may result in mortality due to cannibalism (18). Birds at risk to develop feather pecking are assumed to have specific, predisposing, behavioural and physiological characteristics. Birds from a high feather pecking line, for instance, showed less open field activity (9) and lower corticosterone responses (10, 24) than birds from a low feather pecking line. In other studies, birds from these same lines did, however, not differ in their open field activity (27). In another study, birds from the high feather pecking line were even found to express less feather pecking than birds from the low feather pecking line (12). These inconsistencies can be due to the environmental differences between studies which may obscure the genetic differences in the propensity to display feather pecking and other behaviours between these lines. Comparing lines from a Rhode Island Red (RIR) and a White Leghorn (WL) background revealed consistent differences in feather pecking and fear-related behaviour (21, 22, 23). Rhode Island Red birds showed less feather pecking and a more active response towards a novel object, in an open field and in a manual restraint test as compared with WL birds (21, 22, 23). Also differences in corticosterone response were found, which seemed to be age-dependent (22).

In other studies, birds from a high and low feather pecking line showed different brain serotonin (5-HT) and dopamine (DA) activity (24, 26, 27). In these studies, low 5-HT turnover was associated with a high frequency of feather pecking (25, 26). Based on these results, it was hypothesized that low brain 5-HT neurotransmission is a causative risk factor in the development of feather pecking, although it is unknown if this relationship exists in other genetic lines.

Recently, it was shown that the social environment of laying hens may also affect their tendency to develop feather pecking. Rhode Island Red birds showed profoundly more feather pecking if they were housed together with WL birds than in pure groups with only RIR birds (23). It is unknown whether this relative increase in feather pecking of RIR birds in mixed groups is accompanied by neurophysiological changes, such as a decrease in 5-HT and DA turnover.

The objective of this study was to investigate the effect of genetic origin (either RIR or WL origin), social environment (pure groups with only RIR or WL birds and mixed groups with both RIR and WL birds) and their interaction on behaviour, corticosterone concentrations and brain 5-HT- and DA turnover in laying hens.

Behavioural activity was assessed in a manual restraint test, which has commonly been used to characterize high and low feather pecking birds in terms of behavioural and physiological stress responsiveness (10, 26). Correlations were calculated between behaviour and brain 5-HT- and DA turnover for each genetic line in each social environment. In addition, correlations between whole blood 5-HT concentration, 5-HT uptake by platelets and brain 5-HT activity were calculated to investigate the possible use of blood and platelet 5-HT measurements to indicate brain 5-HT activity.

Materials and Methods

Animals

Adult birds from two genetic lines of laying hens, both with white feathers, were previously found to differ in feather damage due to severe feather pecking and in fear response towards a novel object (21). Birds from these same lines were used in the current study and were either from Rhode Island Red origin (less feather damage due to severe feather pecking and short latency to approach novel object; 'RIR') or from White Leghorn origin (more feather damage due to severe feather pecking and long latency to approach novel object; 'WL'). In the present experiment, animals were housed in groups consisting of either birds from one line (pure groups) or of an equal number of RIR and WL birds (mixed groups) which were kept in these arrangements from hatching onwards.

Housing conditions

During the laying period (from 18 weeks onwards), 80 birds were housed in 20 four-bird battery cages. Each cage was of similar size (46 cm (height) x 39 cm (depth) x 44 cm (width)) providing on average 429 cm^2 of space per bird. The cages either contained pure groups with four RIR (5 cages) or four WL birds (5 cages), or contained mixed groups with two RIR and two WL birds (10 cages). Each group was randomly allocated to one of 75 cages from three stacked tiers of 25 adjacent cages. The remaining 55 cages from these three tiers were used for birds that were not included in the current experiment.

A standard commercial laying diet was provided ad libitum by a 44 cm food trough (cage width) and water was provided through two drinking nipples per cage. After arriving at the laying facility at 18 weeks of age, the light schedule was 10L : 14D, which gradually changed to a final 16L : 8D scheme from 20 weeks of age onwards.

The experiment was carried out by licensed and authorized personnel under approval of Institut de Sélection Animale B.V.

Manual restraint test

At 47 weeks of age, each individual bird was taken out of its home cage and manually restrained on its side for five minutes. The bird was placed on one of two boards which were located within visual and auditory range of other birds, on both ends of the 25 cage-row. The test person used one hand to restrain the legs, while the other hand was used to restrain the upper part of the body of the tested bird. The hand restraining the legs mainly functioned to prevent the birds from escaping when struggling. Thumb and index finger were used to loosely restrain both legs. The other hand put mild pressure on the upper body part when a bird was not struggling. After each struggle, birds were gently brought back in their original position. Observations on bird responses during the manual restraint included the latency to first struggle and frequency of struggles. Birds were tested in random order and were not taken from the same home cage in consecutive tests. Each manual restraint test was performed by one of two persons on three consecutive days between 7.30 and 12.00h, during two consecutive time blocks of 120 min with a break of 30 min in between (balanced over line and social environment). After the manual restraint test, blood samples were drawn and birds were sacrificed by rapid decapitation.

Physiological parameters

Corticosterone. For assessment of basal corticosterone concentrations, blood samples were drawn at the beginning of each time block on the three experimental days (balanced over line and social environment). Individual birds were taken out of their home cage and, within two minutes, a blood sample was collected from the wing vein. Blood collection of birds from the same home cage within the same time block and day was avoided and samples were not collected from birds that already had been subjected to the manual restraint test on that same day.

Immediately after each manual restraint test, blood samples were collected for assessment of corticosterone, whole blood 5-HT and platelet 5-HT uptake velocity. Plasma samples for corticosterone analysis were stored at -20 °C until analysis. Corticosterone concentrations were determined using a radioimmunoassay kit (IDS Inc., Bolton, UK) as described previously (3).

5-HT in whole blood. Whole blood samples (1 ml), collected in EDTA-containing tubes, were placed on ice and stored at -70 °C until analysis. The 5-HT concentration in whole blood was determined by a fluorescence assay, based on the protocol of Yuwiler et al. (1970) for assessing 5-HT in human blood. Briefly, 1 ml of blood was pipetted in 50-ml centrifuge tubes, and 2 ml of 0.9% NaCl solution, 1 ml of an ascorbic acid solution (3% in distilled water, saturated with KCl and EDTA) and 5 ml of a phosphate buffer (2 M K_2HPO_4, saturated with KCl and adjusted to pH 10 with KOH) was added, followed by 20 ml of n-butanol. The tubes were shaken thoroughly for 5 min and centrifuged at 895 g for 15 min. Fifteen ml of the butanol layer was transferred to a second tube containing 2 ml of 0.1 M HCl and 25 ml of cyclohexane and tubes were shaken for 20 sec and centrifuged for 4 min at 895 rpm. The butanol-cyclohexane layer was removed and 1 ml of the acidic phase was pipetted in a tube containing 0.3 ml of 12 M HCl and vortexed for 3 sec. Fluorescence was determined in a Perkin-Elmer 2000 Fluorescence spectrophotometer at 283 and 540 nm. A standard curve was prepared by taking 0.1, 0.2, 0.3, 0.4 and 0.5 ml of serotonin hydrochloride (Sigma-Aldrich) dissolved in Krebs-Ringer-phosphate buffer (0.2755 μmol/ml), to a volume of 1 ml with 0.9% NaCl solution and subsequently the procedure as described above was followed.

Platelet 5-HT uptake. 5-HT uptake by platelets was determined on the day of sampling. Three to 4 ml of whole blood was used to prepare platelet rich plasma (PRP) by low speed centrifugation for 1 min at 378 g. Within 2 hours after blood collection PRP samples were analysed for 5-HT uptake using an assay (7, 20). Briefly, triplicates of 100 μl PRP in Krebs-phosphate-buffer without $CaCl_2$ (pH=7.4) were incubated at a concentration of 5 μM 5-HT in the presence of $[^{14}C]$-5-HT for 1 h at 37 °C. Because of the large volume of blood that would be required for incubation at different concentrations of 5-HT to make Eadie-Hofstee plots, only a single point assay at 5 μM 5-HT was performed. Platelets were collected by filtration on Skatron Filter Mat 11731 filters with a Skatron instruments 12 wells cell Harvester and counted for radioactivity in a Packard Tri-Carb 2200 CA Liquid scintillation analyzer. Passive diffusion of $[^{14}C]$-5-HT was determined by incubating blanks for 1 h at 0 °C and subtracted from the total uptake values at 37 °C to yield the values of active uptake. Uptake velocity was expressed as nmol 5-HT per 10^9 platelets per hour. For that purpose, the number of lymphocytes plus thrombocytes in PRP was counted in a Sysmex F-820**,** and subsequently a blood smear was made to determine the proportion of thrombocytes.

Brain 5-HT and DA turnover. The brains were removed within 1 min after decapitation, immediately frozen in *n*-heptane cooled with dry ice and stored at -70 °C until analysis. Brains were cut transversally as described previously (24) and rostral brain sections were used for the measurement of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) and DA and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) by high-pressure liquid chromatography with electrochemical detection (carried out by Brains-On line, Groningen). The turnover rates of DA and 5-HT were calculated as the proportion of DOPAC to DA and 5-HIAA tot 5-HT, respectively.

Statistical Analyses

For continuous response variables, like corticosterone concentrations, the mean per pen per line was calculated (one mean for pure groups and two means for mixed groups). These means were analysed by a mixed analysis of variance model. Dependence between means from the same mixed group was accounted for by a random group effect. A different residual variance was introduced for means from pure and mixed groups. Fixed effects in the model comprised main effects and interaction for genetic origin (factor G, with levels RIR or WL) and social environment (factor S, with levels pure or mixed). The analysis combined results from two separate and independent analyses: (1) an analysis of means from pure groups (one-way analysis of variance with factor L), and (2) an analysis of means from mixed groups (mixed analysis of variance with random group effect and factor L). The three components of variance were estimated from the group and residual sums of squares from the two analyses. Contrasts for interaction (G x S) and main effects (G and S) were estimated by combination of means from the two analyses. Approximate degrees of freedom for t-tests, e.g. for the hypothesis of no G x S interaction, were calculated by equating the first two moments of the estimated variance of the associated contrast to those of a multiple of a chi-square distribution.

For count data, like frequency of struggles, for the two separate independent analyses, generalized linear models (GLM) were used. These GLMs comprised a logarithmic link function, Poisson variance function and separate multiplicative dispersion parameters for totals from pure (one total) and mixed groups (two totals). To avoid the complexities of a heterogeneous generalized linear mixed model, group effects were introduced as fixed effects, i.e. non-random effects, for totals from the same mixed group. From these analyses, tests for interaction G x S and main effect G were derived. Inference was performed by maximum quasi-likelihood (13). Appropriate statistical weights for an overall analysis of all means were derived from the dispersion parameters that were estimated from Pearson chi-square statistics from the separate analyses. Significance tests were based on the maximum quasi-likelihood test. For the main effect S, the two totals from a mixed group were replaced by one total of the whole group. The analysis for the mixed groups was replaced by a GLM for these overall totals. Again, statistical weights were derived from estimated dispersion parameters for an overall analysis with a single mean for each group (pure or mixed). A combined factor for genetic origin and social environment (GS) with 3 levels was introduced: mixed, pure WL and pure RR origin, say GS1, GS2 and GS3 respectively. The main effect for S was evaluated as the contrast between GS1 and the average of GS2 and GS3 (on the log scale). A significance test for this contrast was derived from the Wald test.

Pearson correlation coefficients were calculated between individual observations on struggling behaviour, plasma corticosterone, whole blood 5-HT concentration, 5-HT uptake by platelets, and brain 5-HT and DA parameters. These correlations were calculated to orientate on the possible relation between the variables. Correlations were calculated for both lines in each social environment and, because residuals were used, in fact indicate pooled correlations within cages.

Pooled correlations over social environments within each line were calculated if correlations did not differ between social environments ($P > 0.05$; assessed by comparison of Z-transformed correlations). Pooled correlations over lines were subsequently calculated if these correlations also did not differ between lines ($P > 0.05$).

The individual observations that were used for calculation of these correlations were obtained as residuals from a model which included social environment and cage (nested within social environment). This model was run for each dependent variable, separately for each line.

Results

Rhode Island Red birds were more active in the manual restraint test than WL birds, which was indicated by their shorter latency to first struggle ($P < 0.001$; Table 1) and their higher frequency of struggling ($P < 0.001$). No effects of genetic line on baseline or stress-induced corticosterone concentrations were found.

Platelet 5-HT uptake velocity was lower ($P < 0.05$), and whole blood 5-HT concentration ($P < 0.001$) was higher in RIR as compared with WL birds. At brain level, 5-HIAA concentration ($P < 0.05$) and [5-HIAA]/[5-HT] ($P < 0.001$) were lower, whereas 5-HT tended ($P < 0.10$) to be higher in RIR as compared with WL birds. Similarly, DOPAC ($P < 0.001$) and [DOPAC]/[DA] ($P < 0.001$) were lower, whereas DA ($P < 0.01$) was higher in

RIR as compared with WL birds. No effects ($P > 0.10$) of social environment or of the interaction between line and social environment were found (Table 1).

Table 1: Least square means (± s.e.) of struggling, corticosterone and neuro-endocrine parameters for each line in each social environment.

variable	type	Rhode Island Red		White Leghorn	
		pure	mixed	pure	mixed
struggling	latency (s)	136 ± 20^{a}	197 ± 29^{a}	244 ± 20^{b}	263 ± 29^{b}
	frequency	1.9 ± 0.3^{a}	1.4 ± 0.4^{a}	0.7 ± 0.3^{b}	0.4 ± 0.4^{b}
cortico-sterone (ng/ml plasma)	baseline	3.0 ± 0.7	3.0 ± 0.5	3.2 ± 0.7	3.4 ± 0.5
	stress-induced	8.2 ± 1.1	7.5 ± 0.8	6.5 ± 1.1	6.3 ± 0.8
sero-tonin	[5-HT] (µg/ml blood)	11.0 ± 0.3^{b}	10.9 ± 0.4^{b}	7.8 ± 0.3^{a}	7.8 ± 0.3^{a}
	[5-HT] uptake (nmol per 10^9 platelets per h)	16.8 ± 2.6^{a}	19.9 ± 3.3^{a}	22.2 ± 2.6^{b}	24.8 ± 3.3^{b}
	brain [5-HT][1] (ng/mg brain tissue)	1.08 ± 0.04^{y}	1.07 ± 0.05^{y}	0.99 ± 0.04^{z}	1.00 ± 0.05^{z}
	[5-HIAA] (ng/mg brain tissue)	0.06 ± 0.01^{a}	0.05 ± 0.01^{a}	0.07 ± 0.01^{b}	0.07 ± 0.01^{b}
	[5-HIAA]/[5-HT] (ng/mg brain tissue)	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.07 ± 0.01^{b}	0.06 ± 0.01^{b}
dopa-mine (ng/mg brain tissue)	[DA]	0.68 ± 0.03^{b}	0.65 ± 0.02^{b}	0.56 ± 0.03^{a}	0.59 ± 0.02^{a}
	[DOPAC]	0.03 ± 0.06^{a}	0.03 ± 0.04^{a}	0.05 ± 0.06^{b}	0.04 ± 0.04^{b}
	[DOPAC]/ [DA]	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.09 ± 0.01^{b}	0.07 ± 0.01^{b}

Means within the same row lacking a common letter superscript differ, $P < 0.05$ (a,b) or tend to differ, $P < 0.10$ (y,z).

A significant correlation (pooled over lines and social environments; n = 69) was found between latency to first struggle and frequency of struggling (r = -0.88; P < 0.001). Because of this high correlation, it was decided to only correlate one of the behavioural responses (latency to first struggle) to 5-HT and DA-activity. A significant correlation (pooled over lines and social environments; n = 69) was found between latency to first struggle and DOPAC (r = 0.28; P < 0.05; Table 2).

In RIR birds from pure groups (n = 18), latency to first struggle was positively correlated with brain 5-HT (r = 0.51; P < 0.05), 5-HIAA (r = 0.66; P < 0.01), [5-HIAA]/[5-HT] (r = 0.58; P < 0.01) and with DA (r = 0.62; P < 0.01). In RIR birds from mixed groups (n = 19), on the other hand, latency to first struggle was negatively correlated with brain 5-HT (r = -0.77; P < 0.001) and DA (r = -0.51 P < 0.05; Table 2).

Correlations between whole blood- and brain serotonin

Significant correlations (pooled over lines and social environments; n = 69) were found between whole blood 5-HT and brain 5-HT (r = 0.34; P < 0.01), HIAA (r = 0.56; P < 0.001) and [HIAA]/[5-HT] (r = 0.57; P < 0.001). In WL birds (n = 31), significant correlations were found between platelet 5-HT uptake and both blood 5-HT (r = 0.36; P < 0.05) and [HIAA]/[5-HT] (r = 0.37; P < 0.05).

Discussion

Behavioural activity, plasma corticosterone concentrations (baseline and stress-induced) and blood and brain 5-HT and DA activity were measured in RIR and WL birds housed in pure (single line) or mixed groups.

Effects of genetic line

The present study revealed clear differences between RIR and WL birds in behaviour, 5-HT and DA activity. RIR birds were more active in the manual restraint test, had higher brain 5-HT, and lower 5-HIAA and DOPAC concentrations, accompanied by lower 5-HT and DA turnover rates than WL birds. The direction of these differences between RIR birds, generally characterized as low feather peckers, and high feather pecking WL birds, was, however, not consistent with other studies on other genetic lines differing in feather pecking behaviour. Previous studies reported a higher activity in the manual restraint test, higher brain 5-HT and lower brain 5-HIAA and DOPAC concentrations and lower 5-HT and DA turnover rates in birds from a high as compared with a low feather pecking line (24, 25, 26). These studies reported that particularly low 5-HT turnover rates in young birds were related with feather pecking behaviour, and suggested that a low 5-HT neurotransmission might trigger feather pecking in laying hens (25, 26). The fact that adult RIR birds in our study showed both a lower 5-HT turnover rate and lower levels of feather pecking than WL birds indicates, however, that low brain 5-HT turnover cannot be indisputably used to identify birds at risk to develop feather pecking. This means that either feather pecking is not (always) related to low serotonergic neurotransmission (contra 25, 26) or that despite higher 5-HT turnover rates in WL birds, activity of other components of the 5-HT system, such as receptors or uptake by transporters may have affected serotonergic signaling.

Table 2: Correlations between latency to first to struggle and whole blood serotonin, brain serotonin and dopamine responses in chickens from Rhode Island Red and White Leghorn genetic origin in pure or mixed social environments. Pooled correlations are presented if correlations did not differ ($P > 0.05$) for birds from pure and mixed groups or, subsequently, for birds from RIR and WL origin.

genetic origin	social environment	platelet 5-HT uptake	blood [5-HT]	brain [5-HT]	brain [5-HIAA]	brain [5-HIAA]/[5-HT]	brain [DA]	brain [DOPAC]	brain [DOPAC]/[DA]
Rhode Island Red	pure	0.081	0.11	_0.51*_	_0.66**_	_0.58**_	_0.62**_	0.28*	0.21[†]
	mixed	0.08	0.11	_-0.77***_	_-0.23_	_-0.22_	_-0.51*_	0.28*	0.21[†]
White Leghorn	pure	0.08	0.11	-0.2	0.29	0.38*	-0.04	0.28*	0.21[†]
	mixed	0.08	0.11	-0.2	0.29	0.38*	-0.04	0.28*	0.21[†]

[†] $P < 0.10$, *$P < 0.05$, **$P < 0.01$, ***$P<0.001$

[1] Equal correlations within the same column indicate pooled correlations, whereas correlations in italics indicate that these differed between origins or social environments

The action of the 5-HT system is terminated by a single type of 5-HT transporter which is a major determinant of 5-HT neurotransmission (11). It was found in the present study that platelet 5-HT uptake in RIR birds was lower than in WL birds, indicating that 5-HT neurotransmission may be terminated less rapidly in RIR than in WL birds. As such, the present results do correspond with previous results (24, 26), which have associated high 5-HT neurotransmission could be related with low feather pecking. The actions of 5-HT and DA systems are mediated by different types of receptors and differences in 5-HT receptor functioning have in a recent study been related to feather pecking (6). Feather pecking in this study was found to be regulated by different 5-HT receptors in birds selected for high and low group productivity and survivability. Potential differences between RIR and WL birds in 5-HT and DA receptor functioning rather than in 5-HT and DA activity might also explain their difference in feather pecking behaviour.

It remains to be investigated whether brain 5-HT turnover (or, in general, brain 5-HT and DA activity) in laying hens undergo changes with age. 5-HT turnover rates in WL birds in mixed groups at an adult age seemed, however, not affected by the high levels of feather pecking to which these birds were exposed during their lifetime.

The role of the 5-HT system in the development of feather pecking could also differ between ages as feather pecking at a young and later age appear to be uncorrelated (14, 17).

Similar to brain 5-HT, blood 5-HT in the present study was higher in RIR than in WL birds. These results disagree with a previous study (4), in which blood 5-HT was lower in birds selected for high- than in birds selected for low group productivity and survivability. Birds selected for high group survivability and productivity show less mortality due to cannibalism and feather damage than birds selected for low group survivability and productivity (5) and are in this sense comparable with RIR birds.

Effects of social environment

Rhode Island Red birds showed profoundly more feather pecking if they were housed together with WL birds than in pure groups with only RIR birds (23). The present study shows that this effect of social environment on feather pecking in RIR birds was not accompanied by changes in the physiological variables measured. Remarkably, however, correlations between behaviour in the manual restraint test and brain 5-HT and DA were reversed in RIR birds in mixed groups as compared with those in pure groups.

The correlation between latency to first struggle and DOPAC pooled over lines and between latency to first struggle and brain 5-HT, 5-HIAA and DA in RIR birds from pure groups, indicate that increased activation of the 5-HT and DA system is associated with decreased behavioural activity in the manual restraint test. The correlations between latency to first struggle and brain 5-HT and DA in RIR birds from mixed groups, however, indicate that increased activation of the 5-HT and DA system is associated with increased behavioural activity during manual restraint in birds from these groups. In chickens, brain 5-HT and DA activity have been related with both increased and decreased duration of the tonic immobility response (8). These contrasting results can be due to a different sensitivity of 5-HT and DA receptors to particular concentrations of 5-HT and DA (8). In rodents, it was found that socially isolated mice had a lower expression of 5-HT receptor genes as compared with grouped mice. Chronically altered corticosterone levels may affect 5-HT receptor functions and subsequently influence expression of 5-HT mediated behaviours (1, 19), like struggling in the manual restraint. Although there was no effect of social environment on corticosterone responses in the present study, elevated 5-HT and DA levels after manual restraint could accordingly have led to different behavioural responses.

Correlations between platelet and brain 5-HT
Correlations (pooled over lines; varying from 0.34 to 0.57) were found between blood 5-HT and brain 5-HT, 5-HIAA and 5-HT turnover rates. These results suggest that peripheral 5-HT variables may partly reflect brain 5-HT activity, which is in accordance with studies on rodents (2) and humans (16).The use of the platelet 5-HT system as a peripheral model for central 5-HT system activity and its relationship with the development of feather pecking merits further research.

In conclusion, RIR and WL birds differed in the behavioural response to manual restraint, 5-HT and DA turnover and 5-HT uptake by platelets, but the direction of these differences was not consistent with earlier reports on genetic lines differing in feather pecking behaviour. Low brain 5-HT turnover, therefore, can therefore not indisputably be used to identify birds at risk to develop feather pecking. Although no effects of social environment (pure vs.mixed groups) or its interaction with genetic origin were found, the reversed correlations between behaviour and brain 5-HT and DA turnover in RIR birds from pure and mixed groups, suggest that the social environment may have modulated aspects of 5-HT and DA signalling in these birds.

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Chapter 8:
General Discussion

The experiments presented in this thesis focused on genetic and behavioural risk factors for the development of feather pecking, a behavioural disorder occurring in laying hens. These experiments had three main objectives: i) to investigate the genetic variation in feather pecking and in response towards a novel object among 12 pure-bred lines from either Rhode Island Red (RIR) or White Leghorn origin (WL; chapter 3), ii) to investigate the use of the novel object test to predict performance in birds from RIR origin (chapter 4) and iii) to investigate the effects of mixed rearing and / or housing of birds from RIR and WL origin on feather pecking and related behavioural and physiological characteristics (chapters 5, 6 and 7). birds for the experiments in chapters 4, 5 and 6 were selected from pure-bred lines, based on the results from chapter 4. In all experiments, birds were housed in battery cage-systems.

Chapters 3 and 4 describe clear differences between pure-bred, genetic lines of laying hens in feather damage due to severe feather pecking and in behavioural response towards a novel object. Throughout the laying period, birds from RIR origin showed a lower fear response towards a novel object and less feather damage due to severe feather pecking as compared with birds from WL origin. Chapters 5, 6 and 7 show that expression of the genetic predisposition to show feather pecking and behavioural responses is influenced by group mates. Rhode Island Red birds generally showed a lower fear response in open field- and in the manual restraint test as compared with WL birds. Birds from RIR origin showed higher fear responses and started to develop more feather pecking when they were housed together with WL birds.

In the following, it will be discussed how behavioural differences could have affected the way feather pecking developed in RIR and WL birds. Furthermore, it will be discussed how artificial selection for production efficiency could have lead to RIR and WL birds with such clear behavioural differences.

Theories on development of feather pecking

Theories on the development of feather pecking are various (Rodenburg et al., 2004b; Sedlačková et al., 2004). It was proposed that feather pecking would develop as redirected behaviour when birds were unable to express ground pecking as component of either foraging (Blokhuis and Arkes, 1984; Blokhuis, 1986) or dustbathing behaviour (Vestergaard and Lisborg, 1993). These theories have provided insight in environmental restrictions that may lead to feather pecking. They did, however, not consider that birds, independent of their environment, might be differentially predisposed to develop feather pecking. More recently, studies that focused on bird characteristics have demonstrated that fearful chicks were more likely to develop feather pecking at an older age (Jones et al., 1995; Rodenburg et al., 2004a). The theories in which feather pecking either develops as redirected behaviour or will most likely develop in more fearful birds, are not necessarily mutually exclusive: fear can reduce the potential to cope with stress by blocking the expression of these normal behaviours (Jones, 1996). When birds are unable to express normal behaviour which is required for adaptation, maladaptive behaviour like feather pecking can more easily develop.

The tests that were carried out in this thesis pointed out that birds from RIR and WL origin have distinct behavioural characteristics, which could be related to the way they develop feather pecking: it is hypothesized that in a poor environment, RIR birds will develop feather pecking because of their high, but unsatisfied motivations to show normal pecking behaviour. White Leghorn birds, on the other hand, will develop feather pecking because their (low) motivation to show normal pecking behaviour is easily redirected towards conspecifics, a process which is influenced by their high fearfulness.

Fearfulness

Fearfulness is defined as the predisposition of an individual to respond similarly to a variety of anxiety-inducing, environmental stressors (Boissy, 1995). The state of anxiety will affect the mode and or intensity of the observed behavioural response. Successful adaptation to the environment will partly depend on the adequacy of the observed fear response towards environmental stressors. In captivity, high fear responses can be maladaptive, and lead to the expression of abnormal behaviour like feather pecking (Jones, 1996). Genetic differences in fear related behaviour indicate that birds have different, innate tendencies to show fearful behaviour (i.e. the different responses of RIR and WL birds in the novel object-, open field- and manual restraint test).

Pecking behaviour

Pecking is another prominent component of laying hen behaviour, and is expressed in relation to foraging, explorative and dust bathing behaviour. From this point of view, theories that related feather pecking to absence of appropriate substrate to either perform foraging (Blokhuis and Arkes, 1984; Blokhuis, 1986) or dust bathing behaviour (Vestergaard and Lisborg, 1993), can be generalized based on the common factor (ground) pecking. The motivation to peck is therefore assumed to reflect the joint motivation to show one or more of these behaviours. The risk to develop feather pecking will depend on how the motivation to perform pecking behaviour is satisfied by the functional expression of pecking behaviour. Genetic differences in expression of ground pecking (Rodenburg et al., 2003), foraging (Klein et al., 2000) and dust bathing behaviour (Gerken and Petersen, 1992) indicate that birds may have different, innate motivations to show pecking behaviour.

Identifying the components which underlie behaviour in test-situations

The behavioural response in a test-situation results from interaction between several underlying components, which is a complex mechanism (Rushen, 2000). Disentangling this complex mechanism of interacting components involved in a specific test may even remain too complicated. Specific experimental designs can, however, give some insight in the influence of different components on established behaviour. Presence of conspecifics in fear-eliciting situations, for instance, can provide information on motivation to reinstate social contact in such situations (Jones, 1982). Administration of anxiogenic agents, on the other hand, can provide information on the role of anxiety in such situations (Moriarty, 1995; Marín et al., 1997). Divergent selection for assumed fear responses in a specific test was found to similarly affect responses in other tests (Jones et al., 1982; Jones et al., 1994), indicating that fear was at least one of the underlying components in these tests.

Multivariate analyses have shown that within populations of cattle (Van Reenen et al., 2004; Van Reenen et al., 2005) and fowl (Rodenburg et al., 2004a), behaviour in several test-situations was controlled by at least two independent components. These components were interpreted as the induced state of fear (either high or low) after a stressor, and the tendency to show either 'active' or 'passive' coping behaviour (Van Reenen et al., 2005). Such a model would give rise to the existence of four different behavioural extremities. In this model, a high state of fear can either lead to a flight/fight or to an immobility response in 'active' or 'passive' animals respectively. Alternatively, (in)activity in low fearful animals can be interpreted as their motivation to explore a novel environment. Another study (Jensen et al., 1997) found that open field activity of heifer calves was determined by two factors, which were interpreted as motivations to show fearful and exploratory behaviour. Fear and the motivation to express pecking behaviour as part of exploratory behaviour are two important components which underly behaviour of laying hens in test-situations.

Identification of behavioural components by factor analysis

Factor analysis is a statistical method that can be used to identify common factors which explain more variation than the original, individual variables. The analysis is based on the correlation matrix between the original, individual variables. The true biological relation between these original variables is of interest. Before calculating the correlation matrix, it is therefore of importance to adjust for any structural influences of, for instance, genetic background, home-cage etc. Alternatively, apart from their means, correlations between original variables can also differ for animals from, for instance, different backgrounds. The correlation matrix will then be different for these animals. Separate factor analysis for animals from different backgrounds should then be conducted, in this case, for RIR and WL birds (chapter 6).

Components which underlie behaviour in Rhode Island Red birds

Factor analysis in chapter 6 indicated that two factors explained most variation (70 and 26% respectively) in open field responses at 17 weeks in RIR birds. This indicates the involvement of at least two components which underlie open field activity of RIR birds. The variables walking and stepping mostly loaded on the first factor and this factor could be related to movement in order to explore the open field. The variables standing and sitting mostly loaded on the second factor and this factor could be related to the degree of fearfulness after initial awareness in the open field test situation. Recovery from the state of anxiety after start of the open field test could be associated with a positional change from sitting to standing. Low open field activity is generally associated with high fearfulness in laying hens (Forkman, 2007).

Components which underlie behaviour in White Leghorn birds

In WL birds, factor analysis only revealed one factor underlying open field activity at 17 weeks of age, indicating that one component was responsible for most of the variation. Each of the variables stepping, standing and sitting loaded on this factor, which indicated that birds either remained sitting inactive or stood up and slightly moved around in the open field. As WL birds hardly walked in the open field, it could be that this only factor was related to the degree of fear after initial awareness in the open field test situation. There was, however, little variation in responses of WL birds obtained from the open field as well as from the novel object and manual restraint test, which complicates identification of other components.

Feather pecking in Rhode Island Red birds

In RIR birds, an approach/ low fear response towards a novel object in the home pen was positively associated with subsequent feather damage due to severe pecking (chapter 3). Another study using ISA brown hens from comparable genetic background, however, did not find such an association (Albentosa et al., 2003). Importantly, housing conditions (i.e. possibilities to perch and to forage) in the study by Albentosa et al. (2003) did allow birds to express normal pecking behaviour, whereas in our study a cage-environment hampered expression of such pecking behaviour. Responses in the novel object- and open field test could indicate that pecking is an important behaviour in RIR birds. Redirection of pecking behaviour towards plumage of other birds could occur if the motivation to peck is left unsatisfied in a stimulus-poor environment. Conversely, an environment which includes appropriate substrate to peck at, would decrease the risk of RIR birds to develop feather pecking.

In chapter 6, RIR birds which were housed together with WL birds in mixed groups, were less active in the open field test and developed more feather pecking than RIR birds, which were housed with only other RIR birds in pure groups. Mean scores for the second factor (indicative for fear, as previously argued) were also lower for RIR birds from mixed than for RIR birds from pure groups (chapter 6). This would then agree with the hypothesis (Rodenburg et al., 2004a) that fearful young birds are at risk to develop feather pecking. In this sense, fearfulness and the motivation to show pecking behaviour seem independent risk factors in the development of feather pecking.

Feather pecking in White Leghorn birds

Results from the novel object-, open field- and manual restraint test, indicate that WL birds have a strong predisposition to experience a range of test-situations as stressful. This complicates the identification of other components which underlie their behaviour, as these may simply not be expressed. White Leghorn birds will generally show a withdrawal response to an environmental stressor (e.g. a stockperson), which seems ineffective in a space-restricted environment. In a battery cage, a bird is not likely to experience the created distance between itself and the stressor as sufficient. The resulting prolonged state of anxiety will increase the risk of WL birds to develop feather pecking (Jones, 1996). The situation can be different if housing conditions allow WL chicks to develop environmentally oriented behaviour (Keeling and Jensen, 1995; Rodenburg et al., 2004; Jensen et al., 2005). Some studies have indeed indicated that in a floor housing system, birds from comparable WL origin develop less feather pecking than birds from comparable RIR origin (Kjaer, 2000; Kjaer and Sørensen, 2002).

Lower open field activity at young age, reflecting higher fear, has been related with higher risk to develop feather pecking at older age in WL birds (Rodenburg et al., 2004a). At a later age, however, birds that were more active in the open field (Rodenburg et al., 2004a; Jensen et al., 2005) or in their home pen (Keeling and Jensen, 1995) had higher risk to develop feather pecking. Within populations of WL origin, birds characterized as feather peckers therefore appear to become more active with age, whereas birds characterized as non-feather peckers become less active with age. White Leghorn chicks might eventually fail to adopt an adequate, environmentally-oriented behavioural repertoire because of their high fearfulness. They subsequently develop feather pecking behaviour, which requires active approach of inactive victims.

Mixed housing of Rhode Island Red and White Leghorn birds

The results from chapter 6 indicate that, if housed together, RIR birds develop feather pecking which is targeted at WL birds. Presence of WL birds therefore seemed to promote expression of feather pecking by RIR birds. The low levels of feather pecking observed in groups with only RIR birds may be explained by the specific characteristics of RIR birds. Rhode Island Red birds may be more active in their home-cage as they have a relatively high motivation to explore their environment, whereas inactive birds are the preferred victims of feather pecking (Riber and Forkman, 2007). A uniform group of active RIR birds will therefore not likely contain birds that are easily victimized by feather pecking. If RIR birds are housed together with WL birds, they will target their feather pecking towards inactive WL birds. This suggests that RIR birds do have a tendency to develop feather pecking, but that it will only be expressed in a specific social environment.

If feather pecking does occur in a group of RIR birds, plumage damage might be minimal, because moving birds do not provide easy targets (although these effects might be minimal in a restricted cage-environment). An aggressive response by a victimized RIR bird towards the performer of feather pecking might also reduce the effects of feather pecking on plumage quality. Exploratory and aggressive behaviour have indeed been found positively correlated in, for instance, great tits (Verbeek et al., 1996). White Leghorn birds, with a lower tendency to show exploratory behaviour will accordingly not easily show an aggressive response when victimized by feather pecking. In groups with inactive WL birds, the damaging effects of feather pecking will not be diminished by their behavioural characteristics as victims.

Domestication effects on laying hen behaviour

Consistent differences between individuals in their behavioural types and associated physiological responses have also been referred to as, amongst others, 'pro-active' and 'reactive', 'bold' and 'shy', and 'fast-' and 'slow-explorative'. These behavioural types show different responses both towards the physical and social aspects of their environment. In order to reduce feather pecking, defining an optimal physical and social environment will thus largely depend on the specific behavioural type to be used.

The fitness of an individual with specific behavioural characteristics will depend on the environment it is exposed to (Koolhaas et al., 2007). On population level, presence of different coping styles and their subsequent inheritance across generations enables wild species to buffer against fluctuations in environmental conditions (Koolhaas et al., 2007). In great tit, it was shown that fitness of either slow or fast-exploring behavioural types in three consecutive years depended on the differences in resource-availability in these three years (Dingemanse et al., 2004). From studies in feral mice selected for either long (LAL) or short attack latency (SAL) in a resident intruder test, it was hypothesized that the LAL mice were better in establishing new territory, whereas SAL mice would better thrive in a constant environment (Koolhaas et al., 2007). Natural selection is, however, a less important evolutionary process in domesticated livestock species (Price, 1984). Under domestication, the behavioural phenotype predominantly develops as a correlated response to artificial selection on production efficiency and, secondly as an environmentally-induced change during an animal's life-time (Price, 1999).

Two different correlated responses to artificial selection

The constant environment to which domesticated species are exposed, should have presumably led to the evolution of one single, optimally-adapted behavioural type. In RIR and WL lines, however, two distinct behavioural types seem to have evolved. Artificial selection, a process with the largest effects on an individual's fitness during domestication (Price, 1999), might have resulted in two different correlated responses in RIR and WL birds. Genetic processes as random drift and inbreeding could be other influential factors, assuming that different behavioural types were already present in the RIR / WL base population.

As a consequence of artificial selection on production efficiency, available resources will mostly be allocated to the reproduction system of laying hens. This would, according to the re-allocation theory (Beilharz et al., 1993), leave less resources available for other activities such as, for instance, expression of behaviour. The specific motivational system which is subsequently affected by the trade-off between production efficiency and behaviour, may vary between birds from different genetic origin. The behavioural difference between RIR and WL birds could be a consequence of a difference in correlated response to artificial selection. In other words, increased production efficiency in RIR and WL birds was achieved at the cost of different components which underlie feather pecking behaviour.

Several studies have investigated the effects of artificial selection for production efficiency on behavioural development by comparing differences between a WL hybrid and its wild ancestor, the Red Junglefowl (Schütz et al., 2001; Schütz and Jensen, 2001; Jensen and Andersson, 2005). Birds from WL origin showed less foraging-, exploratory-, social and anti-predatory behaviour than Red Junglefowl (Jensen, 2006). Genetic selection for production efficiency was therefore concluded to reallocate resources from high-energy-demanding behaviour towards traits such as egg production and egg weight. Following this line of reasoning, the more energy-demanding, active responses in the novel object, open field and manual restraint test of RIR birds, as compared with WL birds, should be accompanied by less (efficient) production. however, Flight/escape behaviour, which is most pronounced in WL birds, is considered to be at least as energy-demanding behaviour as, for instance, food pecking behaviour (Braastad and Katle, 1989) which is more pronounced in RIR birds. It can be speculated that in RIR birds, increased production efficiency is achieved at the cost of the motivational system related to fearfulness, whereas in WL birds, it is achieved at the cost of the motivational system related to pecking behaviour (Figure 1). Ultimately, a ‘passive’ behavioural type with low motivations to show pecking and fearful behaviour is most efficient (Figure 1). During domestication and selection, birds with energy-demanding, conflicting motivations to show both fearful and pecking behaviour were probably the first to be removed from RIR and WL breeding populations. Current breeding practices should be aimed at allowing livestock to express a behavioural repertoire with which they can actively adapt to the range of captive environments they are exposed to.

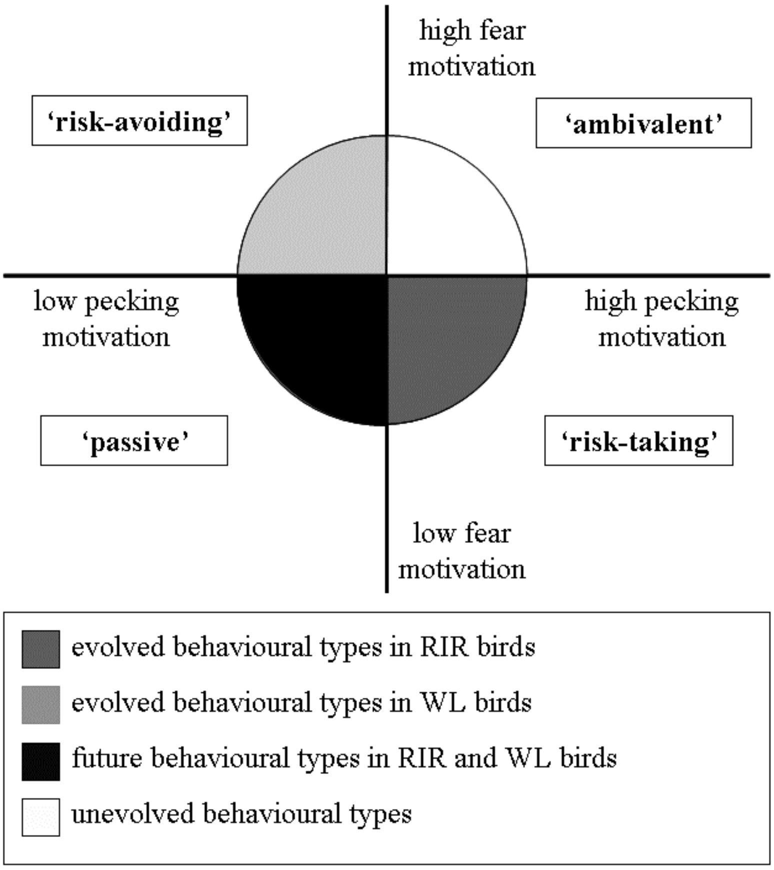

Figure 1: Schematic representation of the behavioural types in RIR and WL birds that have evolved as a correlated response to artificial selection on production efficiency.

Behavioural types

The behavioural types which have evolved as a correlated response to artificial selection in RIR and WL birds, show similarities with recently identified behavioural types occurring under natural conditions. Observed responses in the novel object, open field and manual restraint test could generally be interpreted as either risk-taking or risk-avoiding behaviour. These observed responses result from the trade-off between the way an animal perceives both the potential danger (fearfulness) and the potential benefit (exploratory) of a new environmental context. Recently, distinct fitness strategies of either early reproduction (requiring faster access to resources with high chance of mortality) or late reproduction (requiring low chance of mortality and non-immediate access to resources) have been related to risk-taking and risk-avoiding behaviour respectively (Bell, 2007; Wolf et al., 2007). Rhode Island Red birds appear less fearful in their response towards the novel object and showed more open field activity and more struggling behaviour in the manual restraint test as compared with WL birds. Rhode Island Red birds can therefore be considered to be representatives of the 'risk-taking' behavioural type, whereas birds from WL origin can be considered to be representatives of the 'risk-avoiding' behavioural type.

The distinction between different behavioural types becomes less clear when focus is changed from between population-level (i.e. RIR vs. WL) to within population-level (i.e. differences in pure-bred lines within RIR or WL). Different populations of the same species often clearly differ in their behaviour (e.g. in laying hens; Jones et al., 1995; Korte et al., 1997; Hierden et al., 2002), whereas individuals from the same population often show more similar behaviour. In order to distinguish between individuals within the same population, it is possible to use only animals with the most extreme responses in a behavioural test (Drent et al., 2003). Difficulties then arise in generalization of the results, because at both ends of the distribution animals could be more rigid, whereas 'intermediate' animals could be more flexible in their behavioural pattern. These 'intermediate' animals could be more susceptible to the environmental influences on their behavioural responses. This would explain why studies using all instead of only 'extreme' animals mostly failed to find strong correlations between different tests (Van Erp-Van Der Kooij et al., 2000; Miller et al., 2005; McCall et al., 2006).

Behavioural types and physiological characteristics

Difference in the serotonergic (5-HT) and dopaminergic (DA) neurotransmission systems have recently been associated with the proactive and reactive coping style of a high and low feather pecking WL line respectively (Van Hierden et al., 2002; Van Hierden et al., 2004; Van Hierden et al., 2005). The difference in these systems between the low feather pecking RIR and the high feather pecking WL line (chapter 7) were, however, in contrast with these earlier results. This would indicate that levels of, for instance, 5-HT, its metabolite or its turnover level, cannot directly be used to predict the coping style of an individual laying hen nor its tendency to develop feather pecking. A more complete characterization of the activity of, for instance, the 5-HT system would, however, also require measuring 5-HT uptake levels. Classifying all individuals within a population into only two groups, i.e. proactive and reactive, may be a too simple representation of all existing behavioural types. In chapter 5 and 7, it was found that the correlation between behaviour and corticosterone or brain 5-HT / DA systems, seemed to depend on the genetic background and social environment of the tested birds. The correlations between these characteristics should be consistent (that is, independent of genetic or social factors) if distinct coping styles were to exist. It can also be argued that the interaction between a bird's coping style and its social environment affect the way behaviour and brain 5-HT / DA systems are correlated.

Application and further research

According to the results from this thesis, laying hens should be kept in behavioural uniform groups to minimize the damage due to feather pecking. These groups would preferably consist of birds with similar behavioural types. Production in groups containing pigs with different behavioural characteristics seemed, however, improved as compared with groups containing pigs with similar behavioural characteristics (Hessing et al., 1994). In current poultry industry, most of the commercially kept laying hens originate from a three- or four-way line-cross. The genetic variation between pure bred- or crossbred lines (like commercially kept laying hens) is therefore greater than within pure bred- or crossbred lines. Laying hens are normally kept in groups with all birds originating from the same pure-bred or crossbred line. Such genetically similar birds will not likely represent different behavioural types. If feather pecking occurs in these groups, it is therefore probably not caused by the interaction between birds with different behavioural types. In order to further reduce the expression of feather pecking within RIR and WL groups, selection against high fearfulness seems a promising tool. This could also reduce feather damage due to feather pecking in groups of WL birds, as it could provide them with behavioural characteristics making them less susceptible to be victimized by feather pecking.

It remains, however, to be investigated whether the results from this thesis can be translated towards more extensive systems, such as floor-housing. The effects of the interaction between RIR and WL birds on feather pecking in a floor-housing system might be different from these effects in a battery-cage system. A floor-housing system, for instance, provides better opportunity for WL birds to escape when victimized by feather pecking.

In conclusion, birds from different pure-bred lines show differences in feather damage due to severe feather pecking, indicating that it is possible to select against feather pecking. Feather damage due to severe feather pecking and fear related behaviour were found to be affected by group mates: low-fearful birds became more fearful in presence of fearful birds. Fearful birds, on the other hand, showed more feather damage when kept together with low-fearful birds. This indicates that fearful behaviour predisposes birds to more easily develop and to be targeted by feather pecking. Reducing the expression of feather pecking could be achieved by breeding against expression of fearful behaviour.

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Summary

Woldwide, but especially in Europe, poultry husbandry will undergo significant changes due to the prohibition of both battery cage systems and beak-trimming. A robust laying hen should, under normal physical conditions, have the potential to keep functioning and take short periods to recover under these changing environmental conditions. In terms of behaviour, a robust laying hen is considered to show no feather pecking. Feather pecking is an abnormal behaviour occurring in laying hens, which is defined as the non-aggressive pecking towards the plumage of other birds.

The experiments as conducted in this thesis had three main objectives: i) to investigate the genetic variation in feather pecking and in response towards a novel object among 12 pure-bred lines from either Rhode Island Red (RIR) or White Leghorn origin (WL; chapter 2), ii) to investigate the use of the novel object test to predict performance in birds from RIR origin (chapter 3) and iii) to investigate the effects of mixed housing of birds from RIR and WL origin on feather pecking and related behavioural and physiological characteristics (chapters 4, 5 and 6).

The propensity to develop feather pecking has a genetic component and has been related to fear responses in laying hens. In chapter 3, it was investigated if a convenient test measuring the fear response could be used to identify hens with a stronger propensity to develop feather pecking. However, genetic origin and age can influence the relation between fear response and FP. Therefore, a novel object test in the home cage was used to predict feather pecking in 6 lines of adult laying hens from Rhode Island Red (RIR) origin, and in 6 lines from White Leghorn (WL) origin. Incidence of FP was assessed by scoring feather damage at back, rump and belly at 35, 43, 51, 63 and 69 weeks of age. A novel object test, measuring fear response to a rod, was performed at 23, 46 and 69 weeks of age. For RIR lines, a stronger fear response at 23 weeks was related to decreased feather damage at 51 weeks and for both RIR and WL lines stronger fear responses at 46 weeks were related to increased feather damage at 51 weeks. These results suggest that the relation between FP and fear response differs between ages and between Rhode Island Red and White Leghorn lines. Throughout the laying period, birds from RIR origin has less feather damage and showed lower fear responses towards the rod than birds from WL origin.

It is unknown whether the same response in the novel object test is also related to performance. The main objective in chapter 4 was therefore to investigate the association of the response towards a novel object at 23 weeks of age with subsequent performance A total of 1251 hens from six different pure-bred Rhode Island Red lines (on average 208 hens per line) were housed in 318 cages (on average 56 cages per line). Performance variables included egg production (both per hen-present and hen-housed), bodyweight at 19, 51 and 69 weeks of age, mortality and feather damage at 63 weeks. Hens from cages with an approach response realized a higher total egg production (hen-day and hen-housed) and a higher egg production in the middle (hen-housed) and end of the laying period (per hen-present and hen housed), as compared with hens from cages with a passive response towards the novel object. Approach responses were also associated with more feather damage, but not with bodyweight or mortality. Low correlations were found between higher egg production and more feather damage. These results suggest that an approach response towards a novel object measured early in the laying period is associated with better performance later in the laying period. The novel object test early in the laying period can be used to predict subsequent performance, although possible causal mechanisms for this association remain to be investigated.

In chapter 3 it was found that birds from RIR origin have a lower fear response and show less feather pecking than birds from WL origin. Chapter 5 investigated whether responses in fear eliciting tests were affected if RIR and WL birds were housed together. Experimental groups contained either birds from one line only ('pure' groups) or an equal number of RIR and WL birds ('mixed' groups). These arrangements were maintained from hatch onwards, throughout the rearing and laying period. Birds were subjected to open field tests at 5-6 weeks and 17-18 weeks of age and to manual restraint tests at 7-8 weeks and 24 weeks of age. Rhode Island Red birds were more active in both open field tests and in the manual restraint test at 24 weeks of age as compared with WL birds. Rhode Island Red birds from pure groups were more active in the open field test at 17-18 weeks and in the manual restraint test at 24 weeks of age than RIR birds from mixed groups. These results suggest that otherwise low fearful RIR birds may adopt a higher fear response if they are housed together with more fearful conspecifics. These effects do not emerge until after 8 weeks of age.

Open field activity at young age has been associated with feather pecking at adult age. The lower open field activity of RIR birds from mixed- as compared with RIR birds from pure groups as found in chapter 5, could accordingly be accompanied by an increase in feather pecking. In chapter 6, it was therefore investigated whether mixed housing of RIR and WL laying hens would feather damage due to severe feather pecking. In experiment A, 'pure' groups contained birds from one line only throughout the rearing and laying period. 'Mixed' groups contained an equal number of RIR and WL birds. In experiment B, RIR and WL birds were only housed together during the laying period, in varying ratios. It was found that WL birds from mixed groups had more feather damage due to severe feather pecking than WL birds from pure groups, whereas no effect of mixing was found in RIR birds. Mixed housing during rearing had no effects on feather damage due to severe feather pecking. Rhode Island Red birds from mixed groups appeared to have developed relatively high levels of feather pecking, targeted at WL birds. These results indicate that, together with results from chapter 5, fearful RIR birds from mixed groups were at higher risk to develop feather pecking than less fearful RIR birds from pure groups. This study demonstrates that social factors have a strong influence on the development of feather pecking.

In chapter 7, it was investigated whether the difference in feather pecking and fear response between RIR and WL birds and between different social environments were accompanied by differences in brain serotonin (5-HT) and dopamine (DA) activity. These neurotransmission systems have been associated with feather pecking. Experimental groups consisted either of birds from the same genetic origin (pure groups) or of an equal number of RIR and WL birds (mixed groups) which were kept in these groups from hatching onwards. Behavioural-, brain 5-HT and DA activity were assessed using a manual restraint test. Results indicated that genetic origin had profound effects, whereas social environment had no effects on behavioural-, brain 5-HT and DA activity. Except for a difference in platelet 5-HT uptake, the differences between RIR and WL birds were in contrast with earlier found differences between low and high feather pecking birds. These inconsistencies can be caused by the specific genetic lines under study, or by environmental and age-dependent changes in the 5-HT and DA systems. In conclusion, both 5-HT and DA activity can not indisputably be used to identify birds at risk to develop feather pecking. The role of serotonin uptake in the development of feather pecking does, however, require further attention.

Samenvatting

Het toekomstige verbod op de batterijkooi als huisvestingsysteem en op het snavelkappen van dieren heeft grote gevolgen voor de Europese pluimveesector. Een robuuste leghen zal, bij een normale fysieke gesteldheid, in staat zijn om te blijven functioneren en slechts korte perioden nodig hebben om te herstellen onder deze veranderende omstandigheden. Met het oog op gedrag wordt een robuuste leghen geacht geen verenpikken te vertonen. Verenpikken is een afwijkend gedrag dat onder meer voorkomt bij leghennen en wordt gedefinieerd als het niet-agressieve pikken gericht op het verenkleed van andere hennen.

De experimenten die in dit proefschrift worden beschreven hadden drie belangrijke doelstellingen: i) het onderzoeken van de genetische variatie in verenpikken en in reactie op een 'novel object' tussen 12 zuivere lijnen van leghennen met of een 'Rhode Island Red' (RIR) of een 'White Leghorn' achtergrond (hoofdstuk 3), ii) het onderzoeken van de voorspellende waarde van de 'novel object test' voor de latere prestatie van RIR leghennen (hoofdstuk 4) en iii) het onderzoeken van de effecten van het gemengd huisvesten van RIR and WL leghennen op verenpikken en gerelateerde gedrags- en fysiologische karakteristieken (hoofdstukken 5, 6 en 7).

De aanleg om verenpikken te ontwikkelen is deels genetisch bepaald en is gerelateerd aan de angstrespons in leghennen. In hoofdstuk 3 is onderzocht of een praktische test, zoals de 'novel object' test, gebruikt kan worden om hennen te identificeren met een verhoogd risico om verenpikken te ontwikkelen. Genetische achtergrond en leeftijd kunnen een effect hebben op de relatie tussen verenpikken en angstrespons. Een 'novel-object' test in de thuiskooi werd uitgevoerd op 23, 46 en 69 weken leeftijd om verenpikken te voorspellen in 6 zuivere lijnen met een 'RIR' en in 6 zuivere lijnen met een 'WL' achtergrond. Verenpikken werd gemeten door evaluatie van het verenkleed op de rug, onderrug en buik op 35, 43, 51, 63 en 69 weken leeftijd. Een sterkere angstrespons op 23 weken leeftijd in 'RIR' lijnen was gerelateerd aan minder veerschade op 51 weken leeftijd, maar een sterkere angstrespons op 46 weken leeftijd was, in zowel RIR als WL lijnen, gerelateerd aan meer veerschade op 51 weken. Deze resultaten geven aan dat relatie tussen verenpikken en angstrespons verschilt tussen RIR en WL lijnen en dat deze relatie ook afhangt van de leeftijd waarop de metingen worden gedaan. Gedurende de hele legperiode vertoonden de RIR lijnen minder veerschade en een minder sterke angstrespons dan de WL lijnen.

Het is onbekend of dezelfde angstrespons in de 'novel object' test ook een voorspellende waarde heeft voor de prestatie van leghennen gedurende een legperiode. De voornaamste doelstelling van hoofdstuk 4 was om de relatie tussen de 'novel object' test op 23 weken leeftijd en de prestatie hierna in de legperiode. De gebruikte 1251 leghennen van 6 verschillende zuivere RIR lijnen waren hiertoe gehuisvest in 318 batterijkooien (gemiddeld 208 dieren en 56 kooien per lijn). Eiproductie (per opgezette en per aanwezige hen) lichaamsgewicht op 19, 51 en 69 weken leeftijd, uitval en veerschade op 69 leeftijd werden gebruikt als indicatoren voor de prestatie. Hennen in kooien die het 'novel-object' benaderden realiseerden een hogere totale eiproductie (per opgezette en per aanwezige hen) en een hogere eiproductie in het midden van de legperiode (per opgezette hen) en aan het van de legperiode (per opgezette en per aanwezige hen) dan hennen die geen respons (d.w.z. noch benaderen noch terugtrekken) vertoonden in de 'novel object' test. Er werd ook een relatie aangetoond tussen benaderen van het 'novel object' en verhoogde veerschade, maar niet met veranderingen in lichaamsgewicht of uitval. Verhoogde eiproductie was gecorreleerd aan verhoogde veerschade, maar deze correlatie was niet

hoog. Deze resultaten geven aan dat het benaderen van het ‘novel object’ gerelateerd is aan een betere eiproductie later gedurende de legperiode. Een ‘novel object’ test in het begin van de legperiode zou daarom gebruikt kunnen worden om latere eiproductie te voorspellen, alhoewel de onderliggende mechanismen van deze relatie nog onduidelijk zijn.
Resultaten uit hoofdstuk 3 gaven aan dat hennen met een RIR achtergrond een minder sterke angstrespons en minder verenpikken vertonen dan hennen met een WL achtergrond. In hoofdstuk werd er onderzocht of het gedrag in zogenaamde ‘angst-inducerende’ testen werd beïnvloed door het samen huisvesten van RIR en WL hennen. Hiertoe werden hennen of samen gehuisvest met dieren van dezelfde genetische achtergrond (‘pure’ groepen met alleen RIR of alleen WL dieren) of samen met dieren met een andere genetische achtergrond (‘gemengde’ groepen met zowel RIR als WL dieren). Deze groepen werden gelijk na uitkomst uit het ei gevormd en gehandhaafd gedurende de opfok- en legperiode. Op 5-6 en op 17-18 weken leeftijd werden de dieren onderworpen aan een ‘open field’ test en op 7-8 en op 24-25 weken leeftijd aan een ‘manual restraint’ test. Hennen met een RIR achtergrond vertoonden actiever gedrag in beide ‘open field’ testen in de manual restraint test op 24-25 weken leeftijd dan hennen met een WL achtergrond. Hennen met een RIR achtergrond uit pure groepen vertoonden actiever gedrag in de ‘open field’ test op 17-18 weken en in de ‘manual restraint’ test op 24-25 weken leeftijd, dan hennen met een RIR achtergrond uit gemengde groepen. Dit kan betekenen dat hennen met een normaliter minder sterke angstrespons in aanwezigheid van angstigere hennen ook een sterkere angstrespons ontwikkelen. Deze effecten treden dan pas vanaf 8 weken leeftijd op.
Activiteit in de ‘open field’ test op jonge leeftijd is geassocieerd met een verhoogd risico op verenpikken op latere leeftijd. De verminderde activiteit van RIR hennen uit gemengde groepen ten opzichte van RIR hennen uit pure groepen, zoals werd gevonden in hoofdstuk 5, zou zo ook kunnen leiden tot een verhoogd risico op verenpikken. Het doel van hoofdstuk 6 was daarom om te onderzoeken of het mengen van RIR en WL hennen zou leiden tot een mindere bevedering als gevolg van beschadigend verenpikken. Gedurende de opfok- en legperiode bestonden pure groepen uit hennen met dezelfde genetische achtergrond (of RIR of WL) en gemengde groepen uit een gelijk aantal RIR en WL hennen (experiment A). In experiment B werden hennen met een RIR en een WL achtergrond alleen gedurende de legperiode in verschillende verhoudingen samen gehuisvest. Hennen met een WL achtergrond in gemengde groepen bleken meer veerschade als gevolg van verenpikken te hebben dan WL hennen in pure groepen. Mengen bleek geen effect te hebben op veerschade in RIR hennen. Het mengen alleen in de opfokperiode had ook geen effect op veerschade (noch in RIR noch in WL hennen). Deze resultaten impliceren dat RIR hennen uit gemengde groepen relatief veel verenpikken ontwikkelden, wat voornamelijk gericht was op WL hennen. Samen met de resultaten uit hoofdstuk 5, lijkt het er op dat relatief angstige RIR hennen uit gemengde groepen een verhoogd risico hebben om verenpikken te ontwikkelen, vergeleken met RIR hennen uit pure groepen.
In hoofdstuk 7 werd onderzocht of de effecten van mengen zoals gevonden in hoofdstukken 5 en 6 ook gekoppeld waren aan eventuele verschillen in serotonine (5-HT) en dopamine (DA) responsen op hersenniveau. Deze neurotransmissie-systemen zijn gerelateerd aan het optreden van verenpikken. Experimentele groepen bestonden of uit hennen met dezelfde genetische achtergrond (pure groepen met of RIR of WL) of uit een gelijk aantal hennen met een verschillende genetische achtergrond (gemengde groepen met RIR en WL dieren).

Dieren werden reeds als eendagskuiken aan hun experimentele groep toegewezen. Gedrags-, 5-HT-, en DA responsen (deze laatste twee op hersenniveau) werden verzameld in een zogenaamde ‘manual restraint’ test. Analyse van de gegevens gaf aan dat genetische achtergrond een sterk effect-, maar dat sociale omgeving (puur of gemengd) geen effect had op de gedrags-, 5-HT en DA responsen. Behalve wat betreft het verschil in 5-HT opname door bloedplaatjes, bleken andere verschillen tussen RIR en WL in 5-HT en DA parameters tegenovergesteld te zijn aan eerder gevonden verschillen tussen hennen met een hoog en laag risico om verenpikken te ontwikkelen. Dit kan het gevolg zijn van de specifieke genetisch lijnen die gebruikt zijn, maar ook van omgevings- en/of leeftijdsafhankelijke invloeden op het 5-HT and DA systeem. Serotonine en DA-responsen kunnen dus niet zonder meer gebruikt worden om hennen te identificeren die een verhoogd risico hebben om verenpikken te ontwikkelen. De rol van 5-HT opname door bloedplaatjes zou in deze zin echter wel nader onderzocht kunnen worden.

Curriculum vitae

Koen Anton Uitdehaag was born on the 8th of June (1980) in Roosendaal. He spent the first four years of his life in Roosendaal and Bergen op Zoom in the south of The Netherlands. Hereafter, he and his family moved to Druten in the middle of the Netherlands, where he graduaded from elementary- ('De Appelhof') and high school (VWO at 'Pax Christi College'). His subjects at high school included Dutch, English, Economics, Mathematics, Physics, Chemistry and Biology. After his graduation from high school in 1998, he started to study Animal Science (former 'Zoötechniek') at the Wageningen University and Research Centre that same year. He took specialization courses in Animal Breeding and Genetics and in Animal Behaviour. The topics he focussed on in the final stage included the genetics of feather pecking and related behavioural characteristics in laying hens (major thesis), the occurrence of savaging behaviour in sows (minor thesis) and the genetics of mothering behaviour, also in sows (practical period). After he obtained his MSc-degree in March 2004, he started his PhD-project in April 2004, also at the Animal Breeding and Genomics Centre of Wageningen University. Within this project, the effects of genetic background and social environment on feather pecking in laying hens were studied. After this project finished in May 2008, he started with his current job as poultry specialist at Vencomatic in Eersel.

List of Publications

Papers in refereed journals

Uitdehaag, K.A., Rodenburg, T.B., Komen, H., Kemp B., Arendonk, J.A.M. van. 2008. The novel object test as predictor of feather damage in cage-housed Rhode Island Red- and White Leghorn laying hens. *Applied Animal Behaviour Science 109: 292-305*

Uitdehaag, K.A., Rodenburg, T.B., Komen, H., Kemp B., Arendonk, J.A.M. van. The association of response to a novel object with subsequent performance and feather damage in adult, cage-housed, pure-bred Rhode Island Red laying hens. *Accepted for publication in Poultry Science.*

Uitdehaag, K.A., Rodenburg, T.B., Hierden, Y.M. van, Bolhuis, J.E., Toscano, M.J., Nicol, C.J., and Komen, H. Effects of mixed housing of birds from two genetic lines of laying hens on open field and manual restraint responses. *Behavioural Processes. DOI: 10.1016/j.beproc.2008.04.004.*

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Uitdehaag, K.A., Rodenburg, T.B., Reenen, C.G. van, Koopmanschap, R.E., De Vries Reilingh, G., Engel, B., Buist, W.G., Komen, H., Bolhuis, J.E. Effects of mixing two genetic lines of laying hens with different tendencies to show feather pecking on behavioural, serotonergic and dopaminergic responses. *Submitted to Physiology and Behaviour*

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Rodenburg, T.B., Koene, P., Bokkers, E.A.M., Bos, M.E.H., Uitdehaag, K.A., Spruijt, B.M. 2005. Can short-term frustration facilitate feather pecking in laying hens? *Applied Animal Behaviour Science 91 (1-2). - p. 85 - 101.*

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Conference proceedings/abstracts

Uitdehaag, K.A., Komen, H., Rodenburg, T.B. 2006. Plumage condition, fearfulness and their relation in four commercial lines of adult laying hens. *In: Book of Abstracts of XII European Poultry Conference, XII European Poultry Conference, Verona, Italy, 10 - 14 September, 2006. - World's Poultry Science Journal 62.Verona, Italy.*

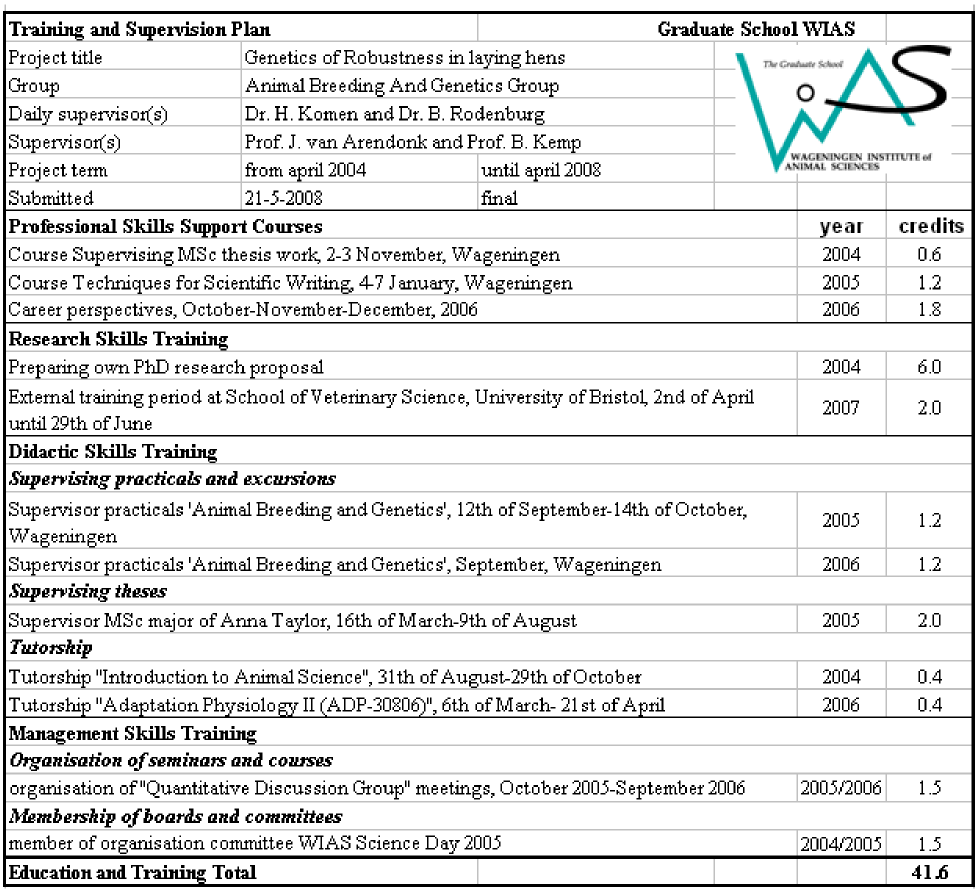
Uitdehaag, K.A., Rodenburg, T.B., Komen, H. 2007. Effect of mixed housing on feather pecking in laying hens. *In Proceedings of the 41st International Congress of the International Society for Applied Ethology* (ISAE), *Merida, Mexico, 30th of July – 3rd August*

Uitdehaag, K.A., Ekkel, E.D., Kanis, E., Knol, E.F. and Lende, T. van der. 2005. Relation between (the breeding value for) weaning survival and periparturient sow behaviour. *In Book of abstracts of the 56th Annual Meeting of the European Association for Animal Production, No.11, Uppsala, Sweden, 5-8 June 2005* (pp. 130).

Rodenburg, T.B., Buitenhuis, A.J., Ask, B., Uitdehaag, K.A., Koene, P., Poel, J.J. van der, Arendonk, J.A.M. van, Bovenhuis, H. 2003. Open-field activity at young age is genetically correlated with pecking behaviour at adult age. *In: Book of abstracts 3rd European Poultry Genetics Symposium, 17-19 September 2003.*

Training and Supervision Plan		**Graduate School WIAS**
Project title	Genetics of Robustness in laying hens	
Group	Animal Breeding And Genetics Group	
Daily supervisor(s)	Dr. H. Komen and Dr. B. Rodenburg	
Supervisor(s)	Prof. J. van Arendonk and Prof. B. Kemp	
Project term	from april 2004	until april 2008
Submitted	21-5-2008	final

EDUCATION AND TRAINING	**year**	**credits**
WIAS Introduction Course	2005	1.5
Course on philosophy of science and/or ethics	2005	1.5
Scientific Exposure		
International conferences		
56th Conference of European Association for Animal Production, Uppsala, 5-8 June	2005	1.1
12th European Poultry Conference, Verona, 10-14 September	2006	1.4
41st International Congress of the International Society for Applied Ethology, Merida, 30th of July - 3rd of September	2007	1.4
Seminars and workshops		
'Fokkerij en Genetica" Connection Day, 18-19 November, Vught	2004	0.6
'Breeding Healthy Working Dogs, 3rd of June, Wageningen	2004	0.1
Poultry Coordination Centre (PhD day), 16th of December, Wageningen	2004	0.1
WIAS Science Day, 17th of February, Wageningen	2005	0.3
WIAS Science Day, 9th of March, Wageningen	2006	0.3
'Fokkerij en Genetica" Connection Day, 16-17 November, Vught	2006	0.6
Robustness in Future Animal Production Systems: Stakeholder Visions, 23rd of January,	2007	1.5
Presentations		
56th Conference of the European Association for Animal Production, Uppsala, 5-8 June, oral	2005	1.0
12th European Poultry Conference, EPC, Verona, 10-14 September, oral	2006	1.0
41st International Congress of the International Society for Applied Ethology, Merida, 29 of July - 3rd of August, oral	2007	1.0
WIAS Science Day, 8th of March, oral	2007	1.0
In-Depth Studies		
Disciplinary and interdisciplinary courses		
Incorporation of Competitive Effects in Breeding Programs for Improved Performance and Well-being, 21-25 June, Wageningen	2004	1.4
Genes AND Environment- a PhD course on environmental sensitivity and genotype-environment interactions, 9-16 June, Uppsala	2005	2.0
Methodik der Nutztierethologie, 5-10 September, Grub	2005	1.4
Linear models in Animal Breeding, 2-6 July, Wageningen	2007	1.5
Advanced statistics courses		
Design of Animal Experiments, 21-23 September, Wageningen	2005	1.0



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Professional Skills Support Courses			year	credits
Course Supervising MSc thesis work, 2-3 November, Wageningen			2004	0.6
Course Techniques for Scientific Writing, 4-7 January, Wageningen			2005	1.2
Career perspectives, October-November-December, 2006			2006	1.8
Research Skills Training				
Preparing own PhD research proposal			2004	6.0
External training period at School of Veterinary Science, University of Bristol, 2nd of April until 29th of June			2007	2.0
Didactic Skills Training				
Supervising practicals and excursions				
Supervisor practicals 'Animal Breeding and Genetics', 12th of September-14th of October, Wageningen			2005	1.2
Supervisor practicals 'Animal Breeding and Genetics', September, Wageningen			2006	1.2
Supervising theses				
Supervisor MSc major of Anna Taylor, 16th of March-9th of August			2005	2.0
Tutorship				
Tutorship "Introduction to Animal Science", 31th of August-29th of October			2004	0.4
Tutorship "Adaptation Physiology II (ADP-30806)", 6th of March- 21st of April			2006	0.4
Management Skills Training				
Organisation of seminars and courses				
organisation of "Quantitative Discussion Group" meetings, October 2005-September 2006			2005/2006	1.5
Membership of boards and committees				
member of organisation committee WIAS Science Day 2005			2004/2005	1.5
Education and Training Total				**41.6**

Colophon

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