

The fearful feather pecker

Applying the principles to practice to
prevent feather pecking in laying
hens

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Het mooiste overkomt je, het minste is bedacht

Freek de Jonge

voor mijn zus...

ABSTRACT

This thesis aimed to find the risk factors in the development of severe feather pecking (SFP), a behavioural problem in laying hens. To that aim, experiments were conducted to understand the principles and those principles were, consequently, studied in the laying hen production chain. As SFP has a strong genetic component, laying hen lines differing in their tendency to develop SFP were compared in traits related to fearfulness, stress-sensitivity and the serotonergic and dopaminergic systems. Hens originating from a line selected against mortality in group housed hens had lower fearfulness and higher dopaminergic activity measured in a brain area related to fearfulness and motor control (arcopallium) compared to a control line. It was also found that having a fearful, highly active, bird in a group affected stress-sensitivity of the group members. Under practical conditions, it was recorded that the Dekalb White (DW) cross had higher levels of fearfulness and lower 5-HT peripheral levels than the ISA Brown (ISA) cross. In parental flocks, DW hens had higher feather damage levels than ISA hens. In parental flocks high levels of fearfulness related to lower levels of production, with specifically high corticosterone levels relating to low egg weight. For the DW cross, high levels of stress in the mother hens related to high fearfulness and SFP in the offspring. During rearing, feather damage and SFP significantly increased when foraging substrate availability was disrupted or limited before five weeks of age. These effects were strongest for the ISA cross. Furthermore, SFP at five weeks of age related to high levels of feather damage at 40 weeks of age, as did fearfulness during the rearing and laying period. If during the laying period, farmers provided a radio, housed roosters in the flock, and provided aerated pecking blocks then feather damage levels were reduced. This thesis showed that the tendency to develop SFP relates to high levels of fearfulness, stress-sensitivity, and activity of the dopaminergic and serotonergic systems. Even on a farm level, maternal stress can lead to SFP in offspring flocks. Risk factors for SFP and consequential feather damage are suboptimal substrate conditions during early life and high fearfulness throughout life. If all farmers in the chain provide undisrupted availability of foraging substrate and aim to reduce fearfulness in their flocks, SFP will likely be reduced.

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Chapter 1

General Introduction

1. INTRODUCTION

Many laying hens are kept worldwide for the production of eggs aimed for human consumption. In February 2014, there were 364 million laying hens in the USA, producing more than 95 billion eggs (NASS, 2014). The egg-laying industry of Europe consists of approximately 363 million laying hens (Eurostat, 2011), while in The Netherlands on average approximately 45 million laying hens were kept (CBS, 2013a). Globally, The Netherlands is the main exporter of eggs (FAOSTAT, 2012).

A major behavioural problem seen in laying hens is severe feather pecking (SFP), where hens peck and pull at feathers of flock-mates causing plumage and tissue damage (Savory, 1995; see Figure 1.1). As SFP inflicts pain in the victims (Gentle, 2011; Gentle and Hunter, 1990), it reduces the welfare of victim birds but also indicates welfare problems in the perpetrators (Rodenburg et al., 2013). On commercial laying hen farms, the mortality rate due to cannibalism or culling as a consequence of SFP can reach 20% depending on the housing system (% of mortality due to cannibalism in cage-housing: 4% (Sherwin et al., 2010) to 14% (Tablante et al., 2000), furnished cages: 5% (Fossum et al., 2009) to 9% (Sherwin et al., 2010), non-cage systems: 15-20% (Blokhuis et al., 2007; Rodenburg et al., 2008). In the UK, around 65%, and between 68 to 86% of free range laying flocks were severely affected by SFP in 2013 and 2010, respectively (Gilani et al., 2013; Lambton et al., 2010). In the Netherlands, 71% of organic flocks were severely affected by SFP in 2009 (Bestman et al., 2009). Thus, SFP occurs frequently on farms and compromises the welfare of a vast number of birds in the egg-laying industry. The urgency to find a practical solution to prevent SFP under commercial conditions has been amplified by the European

ban on conventional cage housing in 2012 (Council directive 2007/43/EC, 2007) and the ban on beak trimming in many European countries (van Horne and Achterbosch, 2008) which The Netherlands will follow in 2018 (Dijksma, 2013). As SFP shows to be, to some extent, socially transmittable between birds within a group (Zeltner et al., 2000; Riedstra and Groothuis, 2002), the risk of SFP affecting many birds in a group makes the control and prevention of SFP in non-cage systems a challenge. On top of that, a ban on beak trimming will most likely result in higher casualties due to augmented feather and tissue damage when SFP occurs (Blokhuys and van der Haar, 1989; Bolhuis et al., 2009). **An early detection of predisposing factors can help in controlling and possibly preventing the development of SFP. The research project which led to this thesis therefore aimed to find the risk factors of SFP in the laying hen production chain. To that aim, experiments were both conducted in small-scale controlled conditions and on commercial laying hen farms.**

Figure 1.1. Victims of feather pecking: see denuded body areas and feather damage as a result of severe feather pecking (SFP).



1.1. WHAT IS FEATHER PECKING AND WHAT IS IT NOT?

There are two distinct forms of bird-to-bird feather pecking; the gentle form and severe form (Rodenburg et al., 2013). Gentle feather pecking (GFP) is defined as a subtle form of feather pecking, seen as gentle pecking, nibbling and plucking at tips of the feathers and particles in the plumage, during which the recipient generally does not react (Savory, 1995). Severe feather pecking (SFP) is the severe form, where birds peck and pluck on feathers, mostly at the back area of the body and base of tail (Norgaard-Nielsen et al., 1993; Bilčík and Keeling, 1999). During SFP, feathers can be pulled out and, in some cases, be eaten (Harlander-Mataushek et al., 2006; 2007b). Especially, the downy back feathers are often ingested (Harlander-Mataushek et al., 2007b). While GFP is a behaviour performed by the majority of birds in a population, especially at a young age, SFP is initially performed by only a few individuals (Rodenburg et al., 2004b; Bilcik and Keeling, 2000) and typically peaks in adulthood (Bright, 2009). Gentle feather pecking does not necessarily develop into SFP (Lambton et al., 2007), neither on individual (Newberry et al., 2007; Hughes and Buitenhuis, 2010; Rodenburg et al., 2004b) nor on flock level (Lambton et al., 2010). The causal factors of GFP and SFP appear to be different, with GFP being suggested to derive from explorative social behaviour (Riedstra and Groothuis, 2002) and SFP from a redirected ground pecking behaviour (Blokhuys, 1989; Rodenburg et al., 2013). In extreme cases SFP can culminate into cannibalistic tissue pecking, leading to mortality of pecked birds. There are also other forms of cannibalistic tissue pecking: these are vent pecking and toe pecking (Savory, 1995; Nicol et al., 2013; Rodenburg et al., 2013). In vent pecking, pecks are directed at the vent and its surrounding area. Vent pecking occurs frequently during egg laying as hens are attracted to the red colour of the mucosa of the vent which is visible during egg laying (Potzsch et al.,

2001; Newberry, 2004). In toe pecking, pecks are directed at toes, substrate on toes, and lesions on toes (Glatz and Bourke, 2006; Rodenburg et al., 2009b). Feather pecking is different from aggressive pecking (Rodenburg et al., 2013). Aggressive pecks are most often directed at the comb and head region and occur during dominance interactions (Savory, 1995; Bilčík and Keeling, 1999). All aforementioned types of pecking behaviour are some form of allo-pecking (i.e. pecking another bird), while also self-directed pecking exists i.e. preening which is performed out in connection with maintenance and cleaning of the plumage (Sandilands and Savory, 2002). Preening consist of nibbling and pecking at own feathers and involves body movements such as shakes and stretches of the wings and tail (Sandilands and Savory, 2002). In this thesis I will refer to feather pecking (FP) consisting of both gentle (GFP) and severe feather pecking (SFP).

SFP is a welfare problem, because it inflicts feather and/or tissue damage and causes distress in the victims and because the behaviour indicates a bird's inability to cope with its environment.

1.2. WHY DO BIRDS FEATHER PECK?

The tendency to develop SFP can be influenced by a bird's individual characteristics and environmental conditions. Therefore, SFP is considered a multifactorial problem (Nicol et al., 2013; Rodenburg et al., 2013). Probably the most influential environmental condition affecting the development of SFP is the lack of suitable substrate for foraging and dust-bathing. SFP has been repeatedly defined as a redirected from of ground pecking either derived from dust bathing (Vestergaard and Lisborg, 1993; Vestergaard and Bildsoe, 1999) or foraging pecking (Blokhuys, 1986), and is as such influenced by availability of substrate for foraging or dust bathing (Huber-Eicher and Wechsler, 1998; Johnsen et al., 1998;

Nicol et al., 2001b). Supporting this redirected foraging theory of SFP is the finding that when diluting hens' food with insoluble feed substances which prolongs time spent on foraging behaviour, the onset of SFP can be delayed and feather damage can be diminished (Van Krimpen et al., 2005; 2009).

Specific housing conditions, such as a high light intensity (Kjær and Sorensen, 2002; Drake et al., 2010; Mohammed et al., 2010), a non-cage system (Rodenburg et al., 2005;; Sherwin et al., 2010; Blokhuis et al., 2007), a lack of available perches (Lay et al., 2011; Huber-Eicher and Audige, 1999; Gunnarsson et al., 2000) and the presence of chain feeders (Drake et al., 2010) can, in many cases, lead to aggravated SFP and feather damage (Nicol et al., 2013). Additionally, the social settings of a bird's housing conditions seem to contribute to the development of SFP (Rodenburg and Koene, 2007; Uitdehaag et al., 2009). For instance, a large group size and a high stocking density with more than >10 birds/m² (Huber-Eicher and Audige, 1999; Nicol et al., 1999; Bestman et al., 2009) can cause an additional risk of feather damage due to SFP. In addition to the aforementioned environmental conditions, a bird's tendency to develop SFP can be influenced by its inability to cope with fear and stress. Young laying hens which are highly fearful (Jones et al., 1995; Rodenburg et al., 2004a) and sensitive to stress as adults (Rodenburg et al., 2009a) have a high tendency to develop SFP (Rodenburg et al., 2013). How birds cope with fear and stress may be related to their serotonin (5-hydroxytryptamine: 5-HT) levels. It appears that low levels of both central (van Hierden et al., 2002) and peripheral 5-HT (Bolhuis et al., 2009; Rodenburg et al., 2009a) are associated with fearfulness and SFP. It is, however, unclear to what extent a bird's physical environment affects how it can cope with fear and stress, and consequently the tendency to develop SFP. **Further research**

is needed to understand the interplay between characteristics of birds and their environmental conditions in influencing the tendency to develop SFP.

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1.2.1 FEARFULNESS

Fearfulness at a young age has been phenotypically and genetically correlated with SFP (Rodenburg et al., 2004a). At a young age, chicks of lines with a high genetic propensity to develop SFP have repeatedly showed to respond more fearful to fear tests compared to chicks of lines with a low genetic propensity to develop SFP (Jones et al., 1995; Uitdehaag et al., 2008c). At an adult age, hens of lines with a high genetic propensity to develop SFP also react more fearful to stressful events, both behaviourally (Uitdehaag et al., 2008c; Bolhuis et al., 2009; Rodenburg et al., 2009b) and physiologically (Uitdehaag et al., 2008c; Rodenburg et al., 2009a; Uitdehaag et al., 2011). The consistency of this finding across different lines suggests that fearfulness underlies the potential risk to start SFP. However, why fearful birds have a higher tendency to develop SFP is still relatively unclear. Not surprisingly, high fearfulness can be an effect of SFP, as fearfulness and stress sensitivity increases when being a victim of SFP (Hughes & Duncan, 1972; Vestergaard et al., 1993) and high fearfulness in groups relates to higher levels of feather damage (Hughes and Duncan, 1972; Vestergaard et al., 1993; Uitdehaag et al., 2006; 2008b; Rodenburg et al., 2010). The social setting in which birds live can also influence fearfulness (Uitdehaag et al., 2009; 2011), stress sensitivity and dopaminergic and serotonergic parameters (Fahey and Cheng, 2008; Cheng and Fahey, 2009; Uitdehaag et al., 2011). **As laying hens live in groups it is relevant to assess the relationship between SFP and fearfulness in relation to their social setting i.e. on a group level, and in large flocks which are present on commercial laying hen farms.**

1.2.2. SEROTONIN AND DOPAMINE

Low levels of central serotonin (5-HT) and dopamine (DA) have been associated with high SFP (van Hierden et al., 2002; 2004a; Uitdehaag et al., 2011). However, differences between studies exist and this relationship appears to depend on the brain area and age when measured. At a young age, hens from lines with a high tendency to develop SFP showed low 5-HT and DA turnover (van Hierden et al., 2002; van Hierden et al., 2004a), while at an adult age SFP was related to high 5-HT and DA activity in specific brain areas (Kops et al., 2014a; 2013b; Uitdehaag et al., 2011). Furthermore, low levels of peripheral 5-HT, which is stored in the platelets and originates from the gut (Berger et al., 2009), were recorded in adult hens of lines with a high tendency to develop SFP and cannibalism (Buitenhuis et al., 2006; Bolhuis et al., 2009; Rodenburg et al., 2009a; Uitdehaag et al., 2011). As peripheral and central 5-HT appear correlated in laying hens (Uitdehaag et al., 2011), measurements of peripheral 5-HT could be informative of a bird's potential to develop SFP under conditions where central 5-HT levels cannot be measured (e.g. on-farm conditions).

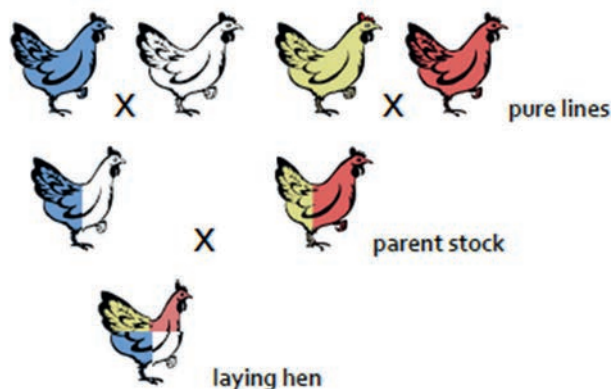
In several species including humans, low levels of 5-HT have been associated with high sustained fearfulness i.e. anxiety (Lesch et al., 1996) and forms of abnormal pecking and plucking behaviour such as trichotillomania (Bordnick, et al., 1994). Serotonin is involved in regulation of physiological processes such as sleep, growth and reproduction, and behavioural responses, such as impulsive and aggressive behaviour, and mood regulation (Angoa-Pérez et al., 2012; Dalley and Roiser, 2012; De Boer et al., 2009; Berger et al., 2009). Dopamine regulates reward related behaviour and plays a role in cognitive functions which depend on motivation and motor control (Kalenscher et al., 2006). **To understand the role of 5-HT and DA in the development of SFP, it is**

important to assess the involvement of the serotonergic and dopaminergic systems in different brain areas involved in fear and motor control. Knowledge on brain area specific modulation of 5-HT and DA involved in SFP would contribute to the recent findings indicating that SFP relates to both high fearfulness and high activity (Kjær, 2009; de Haas et al., 2010). Recent studies also show that the underlying factors of SFP are genetically predisposed (Brunberg et al., 2011; Flisikowski et al., 2009). It is thus relevant to compare lines which differ in potential predisposing traits for SFP.

1.3. THE LAYING HEN PRODUCTION CHAIN

Commercial laying hens are genetically selected for egg production (Muir et al., 2008). Hens in the laying hen production chain are hybrids of two or more genetic pure lines [see Figure 1.2]. The laying hen production chain consists of the parent stock farms, rearing farms and laying farms [see Figure 1.3].

Figure 1.2. A graphical representation of genetic crosses which lead to the commercial laying hen (four-way cross) used for egg production



Egg production intended for human consumption takes place on the laying farm. The laying hens on these farms originate from fertilized eggs produced by the parent stock i.e. flocks containing roosters and hens, and which have been reared on rearing farms.

Figure 1.3. The laying hen production chain: with the parent stock consisting of roosters and hens, the rearing flocks: offspring of parent stock and the laying flocks: offspring of parent stock, rearing flocks in adult life.



Pictures with courtesy of laying hen farmers of Ter Heerdt BV., taken by Elske de Haas (parent stock and rearing flock), and Nanda Ursinus (laying flock)

After incubation of the fertilized eggs at a commercial hatchery, day-old chicks are transferred to the rearing farms, where they stay until approximately 17 weeks of age. Hereafter the pullets are placed on the laying farm, where they will start laying eggs from approximately 20 weeks of age onwards until approximate 70 weeks of age. The parental, rearing and laying environment are thus completely separate from one another. **Certain factors, which predispose laying hens to develop SFP may derive from previous parts in the chain. An assessment of risk factors for SFP needs to take into account all parts of the**

laying hen chain in order to determine from where the risk to develop SFP derives from, and when it is highest.

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1.4. GENETIC BACKGROUND AND PREDISPOSITION

The tendency to develop SFP may derive from genetic selection for egg production traits. For example, commercial White Leghorn (WL) laying hen lines selected for diverging egg production traits also differed in FP tendencies, mainly GFP and to a lesser extent SFP (Blokhuis and Beutler, 1992; Blokhuis and Beuving, 1993; Johnsen and Vestergaard, 1996). These lines also differed in stress sensitivity, fearfulness and central 5-HT levels (Korte et al., 1997; 1999; van Hierden et al., 2002; 2004a; 2005). Both GFP and SFP are moderately heritable (Rodenburg et al., 2003) and genetic selection for and against SFP is feasible (Kjær et al., 2001; Buitenhuis and Kjær, 2008). Divergent selection on both GFP and SFP has yielded two lines, which distinctly differ in SFP and GFP (Kjær et al., 2001), production traits (Su et al., 2006), in coping with novel settings (de Haas et al., 2010), activity patterns (Kjær, 2009), peripheral 5-HT (Buitenhuis et al., 2006) and brain DA levels (Kjær et al., 2004b). Commercial laying hen strains derived from a White Leghorn (WL) and Rhode Island Red (RIR) origin, which are (among other production traits; van Sambeek, personal communication) selected on white and brown eggs respectively also differ in traits related to SFP. The WL hens, which are more flighty than RIR hens, are generally more fearful and stress-sensitive and have higher levels of feather damage (Uitdehaag et al., 2006; 2008ac). In addition, WL hens have lower levels of peripheral and central 5-HT levels and lower DA brain levels (Uitdehaag et al., 2011).

In selection for egg production traits, traditionally, the individual with the highest egg production is selected as parent for the next generation. Genetic

selection of certain individuals may invest in traits beneficial for the individual but, potentially, detrimental for its group mates (e.g. potentially selecting for a highly dominant or aggressive individual who monopolizes all resources). In a recently developed selection method, information on an individual's egg production and information on the mortality levels of the individual's group-housed siblings were combined (Bijma et al., 2006; 2007a; Ellen et al., 2007). By choosing hens with high individual production and low mortality levels in group-housed siblings, mortality due to cannibalism declined in subsequent generations (Bijma et al., 2006; Ellen et al., 2010). When comparing this low mortality line (LML) with the same line selected on individual performance only (control line: CL), birds from the LML had lower levels of feather damage (Bolhuis et al., 2009), toe damage, and comb lesions (Rodenburg et al., 2009b). Birds from the LML also had lower levels of fearfulness than birds from the CL in various test set-ups and a lower stress response and higher peripheral 5-HT levels than CL birds (Bolhuis et al., 2009; Rodenburg et al., 2009a; Nordquist et al., 2011). Until now however, it is unknown if LML and CL birds also differ in central levels of 5-HT and DA in brain areas involved in fearfulness and motor control. **These studies show that commercially selected laying hen lines differ substantially in their predisposition for SFP and that this is related to fearfulness, stress sensitivity and, serotonin and dopamine levels.**

1.5. MATERNAL EFFECTS

In addition to a genetic predisposition SFP and high fearfulness may be influenced by high levels of stress in the mother bird. In laying hens and quails, chicks from mothers with high levels of plasma corticosterone (CORT) due to living in an unpredictable environment show high levels of fearfulness (hens

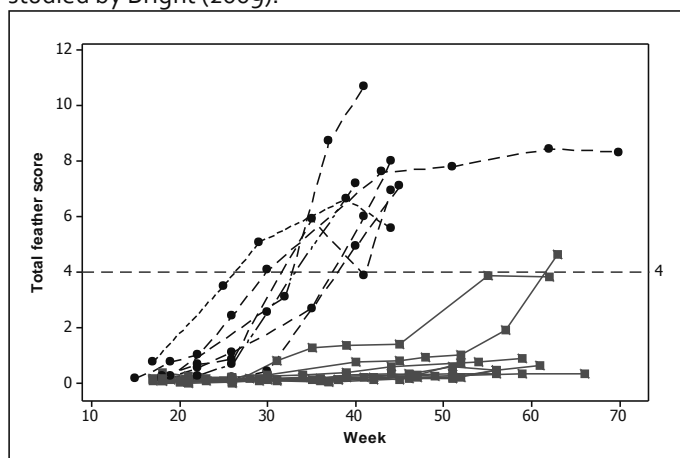
(Janczak et al., 2007b)) and emotional reactivity (quail (Guibert et al., 2011; 2012). Also egg weight can be affected by high CORT in the mother bird (Janczak et al., 2009; Henriksen et al., 2011b; 2013; Guibert et al., 2011). In a number of trans-generational studies in laying hens and quails there are indications that stress in the parental birds affects behaviour and stress sensitivity in the offspring (Lindqvist et al., 2007; Goerlich et al., 2012; Guibert et al., 2011; 2012). Offspring from stressed mothers showed similar behavioural and physiological responses to stress and similar expression of genes related to the stress response as their stressed parents (Lindqvist et al., 2007; Nätt et al., 2009; Goerlich et al., 2012). **Until now, the influence of maternal effects in the development of SFP, which relates to coping with fear and stress-sensitivity, has not been studied in the laying hen production chain.**

1.6. EARLY LIFE CONDITIONS

Early life conditions and experiences, combined with an animal's genetic and epigenetic background, can have substantial effects on its brain and behavioural development (Rogers, 1995; Holmes et al., 2005; Rodenburg et al., 2008a; 2009a; Nordquist et al., 2013). The chicken's brain continues to develop and mature over the course of ten weeks post hatch (Atkinson et al., 2008). During this period, the brain is particularly sensitive to environmental input (Rogers, 1994). For example, in absence of a suitable foraging substrate, chicks may develop a preference for pecking feathers. Several experimental studies have shown that absence or limitation of foraging substrate during early life can induce SFP in early life (Blokhuys and van der Haar, 1992; Huber-Eicher and Wechsler, 1997; 1998; Johnsen et al., 1998; de Jong et al., 2013). The early life conditions and

experiences can have a long-term effect, as it has been shown that lack of foraging substrate during rearing increases SFP during the laying period (Bestman et al., 2009; de Jong et al., 2013) although not all studies find similar results (Dixon and Duncan, 2010). **Until now it is not clear what the sensitive periods for the development of SFP during early life are, and how environmental factors may play a role in the development of SFP during early life.**

Figure 1.5. Feather damage score (0-12) by age in weeks for 18 laying hen flocks, studied by Bright (2009).



Dashed lines: flocks that developed feather pecking by 40 weeks ($n = 7$). Solid lines: flocks that had not developed feather pecking by 40 weeks ($n = 11$). Total feather score of 4 (severe feather damage) was used as a threshold for flocks that developed feather pecking. Figure from Bright (2009)

1.7. THE LAYING PERIOD

Generally, the highest occurrences of SFP and feather damage are recorded during the laying period (Bright, 2009) around peak of lay, i.e. around 30 weeks of age [see Figure 1.5]. Although early life conditions seem to play a role in the development of SFP, also factors during the laying period can affect SFP (Gilani et al., 2013; Bestman et al., 2009; Lambton et al., 2010). This raises the question

as to which environmental factors are crucial in the development of SFP under commercial conditions, and during which period. During the laying period, many housing factors can influence SFP of which especially availability of foraging substrate and social factors appear to be most influential (Nicol et al., 2013).

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1.7.1. FORAGING SUBSTRATE AVAILABILITY

Availability of a substrate to forage is important for laying hens, as adult laying hens spend approximately 90% of their active daytime on foraging behaviour (Dawkins, 1989). If hens are prevented from performing foraging or dust bathing pecks, a certain level of discomfort and frustration may evolve as their need to peck for foraging or dust bathing cannot be fulfilled (Zimmerman and Koene, 1998; Rodenburg et al., 2004b). As a result of frustration, birds may redirect their pecks to feathers of conspecifics. Experimental studies have shown that absence of a foraging substrate during the laying period (Nicol et al., 2001b; de Jong et al., 2013; Johnson et al., 1998) can initiate SFP and aggravate feather damage. These findings are supported by on-farm studies, where limited foraging substrate on the laying farm increased SFP and feather damage (Potzsch et al., 2001; Nicol et al., 2001b; Lambton et al., 2010; Bestman et al., 2009; Bestman and Wagenaar, 2003). **Foraging conditions thus seem pivotal in the development of SFP, both during the rearing and laying period.**

1.7.2. SOCIAL FACTORS

An important aspect in the housing conditions of group housed laying hens is the social environment. Laying hens naturally live in groups and use cues from conspecifics to obtain information on location and availability of food sources and predator threats (Nicol, 2004). Therefore, hens likely pay attention to their group mates and are influenced by their group mates' behaviour. Usually, SFP is

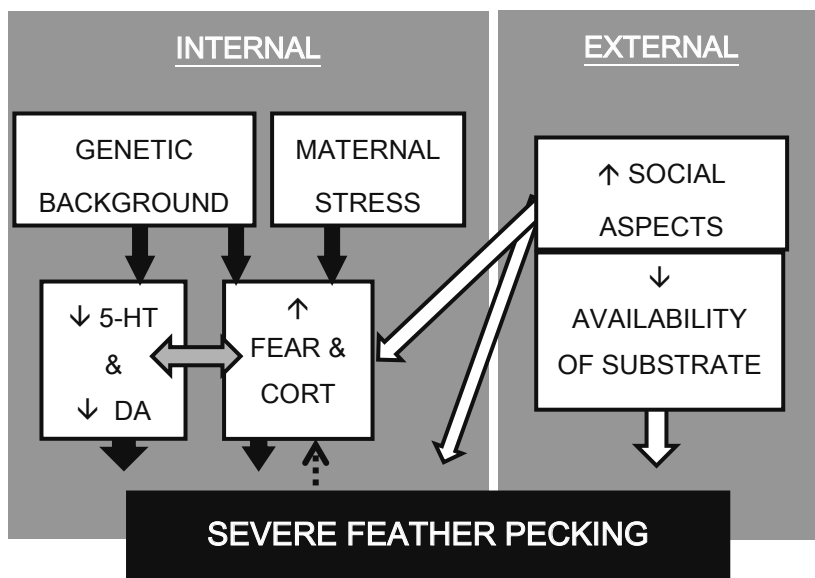
performed by a subset of individuals in a flock (Bilčík and Keeling, 1999; 2000; Rodenburg et al., 2004a), but may spread through a group either by means of social facilitation (suggested by Riedstra & Groothuis, 2002; Nicol, 1995; 2004) or because other birds, initially not involved in SFP, can become attracted to the ruffled/damaged feathers/denuded body areas and start SFP (McAdie and Keeling, 2000). These social factors may especially play a role during early life, where chicks use information from their conspecifics about what type of food to ingest (Nicol, 2004). Social factors such as stocking density, group size and the composition of the group can thus influence SFP directly or indirectly. A high stocking density can increase the risk of SFP (Huber-Eicher and Audige, 1999; Savory and Mann, 1997; Shimmura et al., 2008; Zimmerman et al., 2006), while some studies indicate that a large group size increases the risk of SFP (Shimmura et al., 2010, Nicol et al., 1999; Bilčík and Keeling, 2000). In a set space, stocking density and group size are, however, confounded. Studies attempting to disentangle effects of group size and stocking density indicate that a large group size does not always lead to more SFP (Savory and Mann, 1997; Zimmerman et al., 2006). A potential explanation for differences in social effects on SFP can be that hens of a different genetic origin respond differently to their social environment. For example, when housing RIR hens, which have a low SFP tendency, together with WL hens, which have a high SFP tendency, the RIR hens developed SFP directed at the WL hens (Uitdehaag et al., 2009) and became more fearful (Uitdehaag et al., 2008c; 2011). It appears that group composition may facilitate SFP either directly or via effects on fearfulness. **How group composition and group sizes exactly influence fearfulness and SFP in a group still remains to be elucidated.**

1.8. CONCLUSION

Severe feather pecking is a serious welfare problem in laying hens. Several laying hen lines which have a high genetic tendency to develop SFP, also exhibit high fearfulness as well as alterations in central and peripheral serotonin levels and central dopamine levels compared to lines with a low tendency to develop SFP. The internal state of a bird, partly due to genetics, may play a central role in development of SFP. Stress-sensitivity in mother birds may also influence SFP by influencing fear levels in the offspring. External conditions of a bird including aspects of the social group (group size, density and composition) and availability of foraging substrate can influence SFP both at young and adult age. Together, the internal and external conditions of a bird can thus affect development of SFP [Figure 1.6], but it is unknown if and how these factors interact.

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Figure 1.6. Theoretical model on the risk factors in the development of SFP based on the influence of the internal and external condition of the laying hen



The black arrows show potential genetic and maternal effects acting on internal levels of high fearfulness and high stress-sensitivity (CORT) and low serotonin (5-HT) and dopamine (DA) levels. The white arrows show the expected external effects on SFP and high fearfulness. The grey arrow show the relation between internal state of high fearfulness, stress-sensitivity and low levels of 5-HT.

1.9. AIM AND SCOPE OF THIS THESIS

The main objective of this thesis was to determine the risk factors in the development of SFP in the laying hen production chain pre- and post-hatch. First, two studies were conducted under experimental conditions to gain more insight into the principles of SFP with emphasis on why high fearfulness in young chicks may lead to development of SFP (chapter 2) and the involvement of 5-HT and DA levels in four brain areas of adult hens of the low mortality (LML) and control line (CL) (chapter 3). Hereafter, in chapters 4, 5 and 6, the contribution of fearfulness, stress sensitivity, and peripheral 5-HT levels were studied under on-farm conditions in all parts of the laying hen chain: parent stock, rearing flocks, and

1

laying flocks. These potential indicators or predictors of SFP were related to the genetic background (G) of the birds and the environmental conditions (E) on-farm, and when possible, the interactions between G*E. For this reason, we used two commercial hybrids - Dekalb White and ISA brown – of which the ancestor lines are known to differ in levels of fearfulness, stress sensitivity, 5-HT and DA parameters as well as the tendency to develop SFP (Uitdehaag et al., 2011). As maternal effects may play a role in the predisposition for developing SFP, the parental flocks were studied first (chapter 4). It was assessed how parental birds cope with fear and stress and how this relates to their production outcome. Subsequently, it was assessed whether stress in the parent stock plays a role in the development of fearfulness and SFP in their offspring, i.e. the rearing flocks (chapter 5). Also in chapter 5, the ontogeny of SFP was assessed in relation to the environmental conditions on the rearing farm and age of the chicks, in order to determine the sensitive or risk periods for the development of SFP during early life. In chapter 6, these rearing flocks were studied in the laying period. Here it was assessed which risk factors during the rearing period and which risk factors during the laying period would lead to high levels of feather damage (used as a proxy for SFP). In chapter 7, a synthesis of this thesis is given and the major findings are discussed.

The Principles



Chapter 2

The relation between fearfulness in young and stress-response in adult laying hens, on individual and group level

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ABSTRACT

Fearfulness of an individual can affect its sensitivity to stress, while at the same time the social situation in which an animal lives can affect its fear level. It is however unknown what the long-term effects of high fearfulness on sensitivity to stress are, on individual or group level in laying hens. We hypothesize that increased fearfulness at a young age results in increased sensitivity to stress at an adult age, and that this relation can differ between groups, due to differences in group composition. Therefore, we studied the relation between fearfulness in an Open Field (OF) test at six weeks of age and plasma-corticosterone (CORT) levels after a 5-min Manual Restraint test (MR) at 33 weeks of age, and assessed behaviour in the home pen. We used birds from a low mortality line, selected for four generations on low mortality due to feather pecking and cannibalism and a control line ($n=153$ in total, eight pens/line). These lines are known to differ in fearfulness and stress physiology. Chicks from the low mortality line were more active in the OF compared to chicks from the control line. Chicks that showed a fearful response (no walking, no vocalizing) in the OF test had higher CORT at 33 weeks of age than chicks that walked and/or vocalized in the OF test and had higher activity in the home pen as adults. On group level, a passive response in the OF was related to high CORT levels after MR. Presence of at least one fearful bird in a group led to higher CORT in the other group mates compared to birds from groups with no fearful birds present. Birds from groups in which more than 50% of birds had severe comb lesions had higher CORT levels compared to birds from groups with less than 50% of birds affected. High fearfulness of laying hen chicks can on individual level have a long-term effect on stress sensitivity. The presence of fearful birds in a group as well as signs of social instability in a group, indicated by comb lesions, can affect sensitivity to stress of birds from the same group. The mechanism by which this occurs can lie in social transmission of (fear related) behaviour, but this suggestion needs further investigation.

KEYWORDS Fear, Laying hens, Group selection, Corticosterone, Anxiety, Stress, Group behaviour, Social transmission

2.1. INTRODUCTION

Fear is an animal's state of apprehension to an imminent existing threat (Davis et al., 2010). An animal's fearfulness is therefore the likelihood of responding fearfully to numerous potentially dangerous stimuli (Jones, 1996a). In response to a threatening situation, a physiological stress response is initiated, consisting of activation of the hypothalamic–pituitary–adrenal axis with release of catecholamines ((nor)adrenaline) and corticosteroids (cortisol and/or corticosterone) (Cockrem, 2007). Behaviourally, animals become more alert, and they can either respond actively or passively in order to increase their survival (Koolhaas et al., 1999). An animal's fearfulness and its physiological stress-response are therefore, within a short time frame, related. In some species it has been found that a high sustained fear level causes an increased vulnerability to stress in later life (Sandi and Rose, 1994; Pawlak et al., 2008; Calandreau et al., 2011b).

Factors which can influence an animal's fear level can be its genetic make-up, unpredictable and uncontrollable (early) life experiences and associative learning experiences (Barlow, 2000). Furthermore, the social environment may also affect an animal's fear level (Clément and Chapouthier, 1998). Exposure to a stressed adult cage-mate can induce anxiety (perceiving a situation as threatening (Lang et al., 2000)) in young mice (Jacobson-Pick et al., 2011). When this occurs in early life, it can influence stress sensitivity (Anisman et al., 2003) and possibly also social behaviour (suggested by Sachser et al., 1998). In laying hens, fearful individuals may cause other hens to become more fearful (Uitdehaag et al., 2009). For example, it has been shown that housing birds from a fearful line together with birds from a non-fearful line, led to increased

fearfulness in the birds from the non-fearful line (Uitdehaag et al., 2008c). Others (Cheng et al., 2002) also found an effect of mixing birds from a fearful line with birds from a less fearful line on basal corticosterone levels. Irrespective of mixing laying hens in groups which can be stressful as such (Uitdehaag et al., 2008c) differences in fear level between stable groups of laying hens are known to exist (Vestergaard et al., 1993). Social interactions between animals with different behavioural characteristics could influence the development of fearfulness. As such, group composition may affect fear level within a group, and, potentially stress sensitivity.

Fearfulness in a group can also be affected by the method of breeding. Traditional breeding of laying hens consists of choosing the individuals with the highest egg-production. Individual egg production can only be measured in individually housed hens, and therefore no information on social behaviour is available from these individually housed hens. A recently developed breeding method enables one to select birds on the basis of a combination of individual and group performance (Ellen et al., 2007). Selection is still based on individual egg production, but with additional information on levels of mortality due to cannibalism and feather pecking of relatives living in a four-hen family cage (Ellen et al., 2007). This group selection method has been shown to reduce fear in the second generation of selection on low mortality (from now on labelled low mortality line) (Bolhuis et al., 2009). Chicks from the second generation on low mortality were more active in the Open Field test than chicks from a control line (selected on individual egg production only) (Rodenburg et al., 2009b). As adults, hens from the low mortality line had lower corticosterone levels after a restraint test (Rodenburg et al., 2009a) and higher whole-blood serotonin and lower serotonin-uptake (Bolhuis et al., 2009) than control hens. Serotonin is known to

be involved in fear, anxiety and stress (Lucki, 1998; Schwarting et al., 1998; Antoniou et al., 2000; Metzger et al., 2002; Pawlak et al., 2008). Thus, selection on group performance seemed to have reduced fearfulness and response to stress in comparison to selection on individual performance only.

The aim of this study was to assess the long-term effects of fearfulness. Therefore, we used lines which were known to differ in fearfulness and stress physiology (low mortality line versus control line). Our hypothesis was that increased fearfulness at a young age results in increased stress sensitivity at an adult age, but that this relation can differ between groups. Additionally, we wanted to determine if differences between groups in home pen behaviour could be affected by fear level of individuals within a group. As the low mortality line was selected on the basis of reduced levels of mortality due to cannibalism and feather pecking, we also assessed differences in body damage due to pecking.

2.2. MATERIAL AND METHODS

2.2.1. ANIMALS AND HOUSING

Two White leghorn lines from ISA BV, the layer breeding division of Hendrix Genetics, were used: a low mortality line and a control line. The low mortality line was selected for four generations on low mortality in group housing (Ellen et al., 2007). Selection candidates were housed individually, to enable recording of individual egg-production. Siblings of these selection candidates were housed in family groups and in those (non-beak trimmed) groups mortality was recorded. Only selection candidates with low mortality levels in the group of siblings and sufficient individual egg-production were selected as parents for the next generation of the low mortality line. In the control line, the standard commercial breeding program was implemented (focusing on individual egg-production).

Eggs from both lines were brooded at the experimental farm 'De Haar' (Wageningen University, the Netherlands). After hatching, chicks received a neck tag with a colour/number combination. Per line, 104 chicks were randomly assigned to one of eight 13-chick floor-pens measuring 1.9*1.2 m, where they had access to sand (1/3 of the surface), wood-shavings (2/3 of the surface), a 10 cm high perch (dividing both areas) and had ad libitum water and food. The pens were situated in two different rooms, with an equal number of pens per line within both rooms. A commercial mash diet was provided; a starter diet (weeks 1–5), a starter 2 diet (weeks 6–16) and a layer diet (from week 17 onwards). Throughout the experiment whole grains were scattered once a day around 8 a.m. in the sand area (particle size adjusted to bird age). At seven weeks of age a 50 cm high perch was added in the wood-shavings area and group size was reduced to ten birds per pen. At 17 weeks of age, a group nest was added in each of the pens. One hundred and fifty three hens were retained in the experiment (11 pens with 10 hens/pen, 3 pens with 9 hens/pen, and 2 pens with 8 hens/pen). This experiment was approved by the Institutional Care and Use Committee and in accordance with Dutch legislation on the treatment of experimental animals, in conformation with the ETS₁₂₃ (Council of Europe 1985) and the 86/609/EEC Directive.

2.2.2. BEHAVIOURAL OBSERVATIONS

Birds were individually subjected to two tests: an Open Field (OF) test at six-weeks of age and a Manual Restraint (MR) test at 33 weeks of age. Body damage was assessed at 30 weeks of age, and home pen observations took place between one and four weeks of age, and 30 and 33 weeks of age. Order of

testing/observations was balanced for lines, birds and pens. The experimenter was blind to the allocation of lines and designation of pens and birds within pens.

OPEN FIELD TEST

Each bird (n=153) was individually subjected to an Open Field for a duration of five minutes (see (Rodenburg et al., 2009b) for a detailed description of test set-up). Birds were tested between 8 a.m. and 4 p.m. over a 10-day period. A square barren observation pen measuring 1.25*1.25 m (4.1*4.1 ft.) operated as OF, with wire walls through which camera recordings were obtained. A chick was placed in the middle of the OF, which was kept dark until the start of the test. Behaviour was scored from a video-screen in an adjacent room by a single person using the Observer software package (Noldus Information Technology B.V., Wageningen, The Netherlands). Durations and latencies to walk, stand, sit and vocalize (distress calls (Collias, 1987)) were recorded, as well as the number of vocalizations. Chicks were transported to and from the test arena in a cardboard box.

BODY DAMAGE

Body damage was scored on each bird at 30 weeks of age, based on Welfare Quality (2009). Damage to neck, back and belly were used as indicators for severe feather pecking (Bilčík and Keeling, 1999). All regions were scored on a three point scale: intact/slight wear (a), moderate wear (b) and featherless areas (c), and summed to give a whole body index. The total score was either; zero (all regions had "a"), one (only one region with "b" led to a total score of one) or two (only one region with "c" led to a total score of two) based on Welfare Quality (2009). Comb lesions were scored on a three-point scale; zero (no lesions), one

(less than three lesions), two (more than three lesions) modified from (Tauson et al., 2005).

HOME PEN OBSERVATIONS

Home pen observations took place between one and four weeks of age, and 30–33 weeks of age, once a week between 9.30 a.m. and 4 p.m. alternating morning and afternoon in a balanced design (each pen was observed twice in the morning and twice in the afternoon) by the use of scan sampling (Martin and Bateson, 2007) with a duration of 20 min with four-minute interval at young age, while at adult age 1 h with three-minute interval was used (both leading to 18 s/bird observation time). Individual hen's behaviour was entered on a PSION hand-computer with a pre-programmed ethogram. The number of behaviour recordings (scans) was expressed as the proportion of behaviour performed in relation to the total observation time, averaged over all observation days by age (young and adult). We clustered behaviours related to activity (walking, dust-bathing, eating, foraging and drinking), non-active behaviour (standing, resting, preening, perching, and inside the nest-box), so as to have measurements on general activity in the pens.

MANUAL RESTRAINT TEST

At 33 weeks of age, each hen was individually subjected to a Manual Restraint (MR) test situated in a room adjacent to her home pen. A hen was placed on her right side on a table covered with cardboard, with the right hand of the experimenter covering the hen's trunk and the left hand gently stretching the hen's legs (Bolhuis et al., 2009). Hens were restrained in this position for 5 min. Consecutive struggles were scored as escape attempts, after which the hen was brought back in start position. Recording of struggles were measured as

frequency and binominal variable (yes/no). All hens were tested within 2 days by two observers. Distribution of hens over observers, time of the day (4 blocks of 2 h (approx. 20 hens per block) per test day) was balanced for pens and lines.

PLASMA CORTICOSTERONE POST MANUAL RESTRAINT

Immediately after the Manual Restraint (MR) test, 2 ml of blood was drawn from the bird's wing vein and collected in a four ml EDTA tube for corticosterone analysis. Samples were centrifuged at 5251 RCF for six min. Plasma was obtained and stored at -20 °C before analysis at the Laboratory of Physiology and Immunology of Domestic Animals in Leuven (Belgium). Plasma-corticosterone concentration was determined by a radioimmunoassay kit (IDS Inc., Bolton, UK), as described previously (Buyse et al., 1987). Post-MR corticosterone levels are hereafter labelled CORT.

2.3. STATISTICAL ANALYSIS

SAS Software 9.2 was used for statistical analysis. Effects of line on OF behaviour, MR behaviour and CORT were tested with a mixed model with line, observer (only for MR), and stable as fixed effects, and pen nested within line and stable as random effect. Effects of line on comb damage and feather damage scores and on occurrence of struggles in the MR test (expressed as binominal variable) were assessed with a Chi-square test of independence. Birds were categorized based on their OF response as: freeze (F) (i.e. no walking and no vocalizing); walking and vocalizing (WV), no walking but vocalizing (V) and walking but not vocalizing (W). Effects of OF category and body damage on corticosterone were assessed by a mixed model with line (low and control), rooms (1 and 2), comb lesions (0, 1, 2) and category (F, WV, V and W) in the OF

test and block (eight blocks, four blocks per test day, to assess effects due to diurnal rhythms of CORT (Beuving and Blokhuis, 1997)) as fixed effects and pen nested within line and stable as random effect. To correct for activity in the MR test, which could increase CORT as such (Koolhaas et al., 2011), the number of struggles during MR test was added as a co-variable. To correct for order of testing, sequence number was also added as a co-variable. To calculate correlations between OF response and CORT on pen level, data were averaged per pen. Pearson correlations were calculated on the residuals of a general linear model, correcting for the fixed effect of line. Further, it was tested how group composition affected CORT of individual birds within groups. We tested whether the absence or presence of F chicks in a pen and the level of comb lesions within a pen (more or less than 50% of the birds with score 2) affected individual CORT levels of the other (WV, V or W) birds within a pen. The mixed model for this comparison contained line, presence/absence of F birds, level of comb lesions, and their interaction as fixed effects and pen nested within line, presence or absence of F birds and level of comb lesions as random effect. As feather damage was very low in both lines we did not include it in the model. Home pen behaviour was analysed with fixed effects of line, room, comb lesions and OF category and pen nested within line and stable as random effect. Additionally, group composition effects on home pen behaviour were analysed with the same model used to study the effects of group composition on CORT.

2.4. RESULTS

2.4.1 LINE EFFECTS

For line effects, see Table 2.1. In the Open Field, chicks of the low mortality line vocalized more ($F_{1, 14}=7.67$, $P=0.02$), spent more time standing ($F_{1, 14}=4.76$,

$P=0.05$) and tended to have a shorter latency to stand ($F_{1, 14} = 3.64$, $P = 0.08$) and a shorter duration of sitting ($F_{1, 14} = 3.65$, $P = 0.08$) than chicks of the control line. Lines did not differ in walking duration, latency to vocalize, number of hens with different OF categories, level of comb lesions or feather damage, behavioural or CORT response to MR.

2.4.2. RELATION BETWEEN OPEN FIELD BEHAVIOUR, POST-MR CORTICOSTERONE AND HOME PEN BEHAVIOUR

Birds showing freezing (F) during the OF (i.e. no walking or vocalizing) at six weeks of age had higher CORT levels at 33 weeks of age than birds classified as WV (walking and vocalizing), V (only vocalizing) or W (only walking) ($F_{3, 123} = 2.57$, $P = 0.05$, see Figure 2.1A). Post-MR CORT increased when struggles in the MR test increased ($F_{1, 123} = 9.58$, $P < 0.01$), but were not affected by the level of comb lesions ($F_{2, 123} = 0.09$, $P = 0.9$). Open Field classification did not affect the behavioural response during MR [Figure 2.1B,C]. As adult – but not as young – proportion of time spent on active behaviour in the home pen tended to be higher for F birds than WV, V and W birds ($F_{3, 132} = 2.27$, $P = 0.08$, Figure 2.2) and for birds with no comb lesions ($F_{2, 132} = 2.74$, $P = 0.07$; no lesions: 51%, lesion score 1: 47% and lesion score 2: 45%).

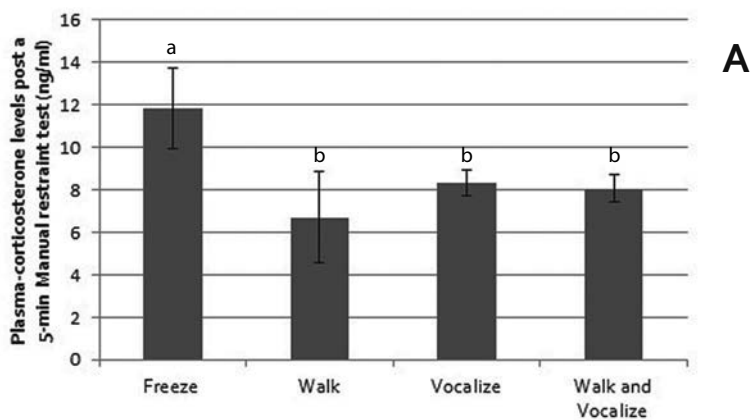
Table 2.1. Weights, Open Field behaviour and classification, comb and feather damage, and struggles during the Manual Restraint (MR) test, and post-MR corticosterone levels of hens from a low mortality and control line.

Variables	Low mortality	Control
<i>Weight</i>		
At hatch (wk. o) (g)	37.6±0.2	37.7±0.2
At 7 weeks	408±6.6	430±12.4
<i>Open Field behaviour</i>		
Walking duration (s)	21.0±4.5	19.2±4.0
Vocalization frequency	42.5±3.3 ^a	30.3±2.9 ^b
Vocalization latency (s)	95.6±11.8	122.9±12.0
Sitting duration (s)	110.0±11.3 ^y	147.7±10.7 ^z
Standing latency (s)	110.9±11.0 ^y	148.4±10.7 ^z
Standing duration (s)	160.9±10.5 ^a	126.4±9.4 ^b
<i>Classification based on Open Field behaviour</i>		
Freezing (Not Walk Not Vocalize) (F)	8(10%)	11(15%)
Walk (W)	2(2%)	3(4%)
Vocalize (V)	40(52%)	29(39%)
Walk and Vocalize (WV)	28(36%)	32(42%)
<i>Comb lesions</i> ¹		
0 (no lesions)	29	20
1 (<3 lesions)	28	24
2 (>3 lesions)	21	31
<i>Feather score</i> ²		
0 (no damage)	55	59
1 (one area with <5cm bald patches)	23	16
<i>Manual Restraint (MR) test</i>		
Number of struggles	0.5±0.1	0.7±0.2
Number of vocalizations	14.4±5.2	8.0±4.4
Latency to struggle (s)	274.2±6.2	272.0±7.1
Latency to vocalize (s)	251.5±8.8	264.6±7.7
Post MR corticosterone levels (ng/ml)	8.88±2.4	8.34±2.1

numbers with a different superscript differ ab(P<0.05), yz(P<0.1), ¹ based on Welfare Quality®, 2009,

² total feather score, based on damage to neck, back and belly region.

Figure 2.1. Post Manual Restraint plasma-corticosterone (ng/ml) at 33 weeks of age of birds which froze, only walked, only vocalized, or vocalized and walked in the Open-Field at six-weeks of age (A)



Means with different superscript differ ($p < 0.05$)

Figure 2.1. Latency to vocalize and struggle (B) and number of struggles and vocalization (C) during the MR test at 33 weeks of age of birds which froze, only walked, only vocalized, or vocalized and walked in the Open-Field at six-weeks of age

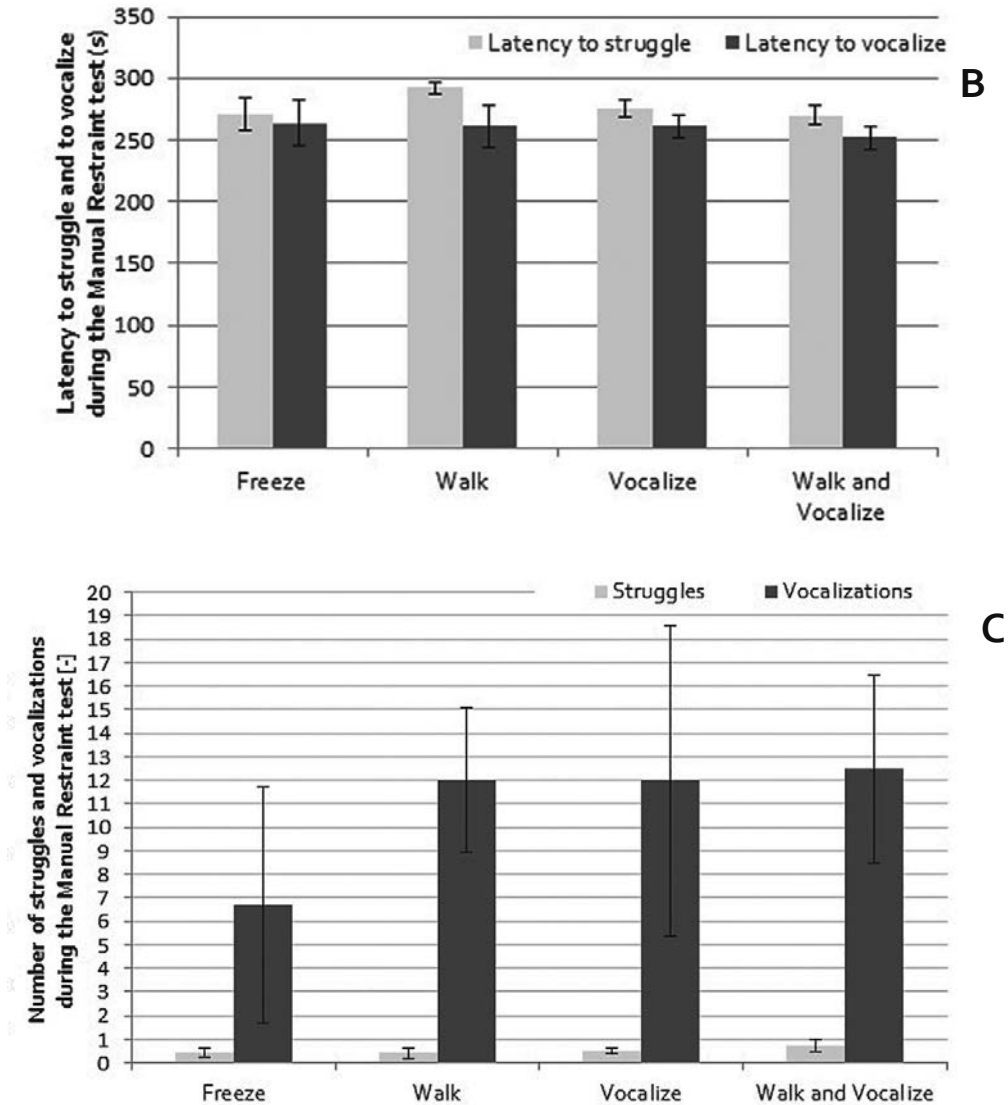
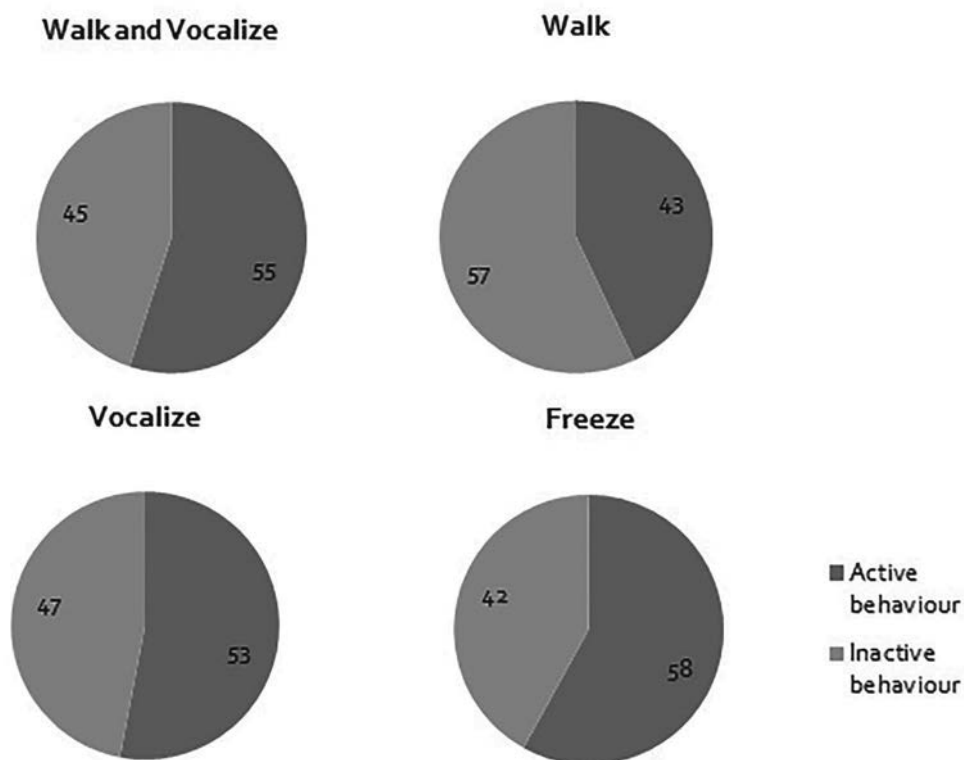


Figure 2.2. Proportion of time spent on active and inactive behaviour in the home pen between 30 and 33 weeks of age of birds which froze, only walked, only vocalized, and vocalized and walked in the Open-Field at six-weeks of age.



¹active behaviour comprises of sum of time spent on: walking, dust-bathing, eating, foraging and drinking in proportion of the total time spent on active and inactive behaviour ² Inactive behaviour comprises of sum of time spent on: standing, resting, preening, perching and inside the nest box in proportion of the total time spent on active and inactive behaviour

2.4.3. GROUP EFFECTS

On a group level, correlations were found between OF behaviour and CORT. The average latency to vocalize ($r = 0.57$, $P < 0.05$) and the average duration of sitting

($r = 0.64$, $P < 0.01$) were positively correlated with average CORT, while the average duration of walking was negatively correlated with average CORT ($r = -0.71$, $P < 0.01$), see Figure 2.3. The presence of F birds within a group affected group-average CORT ($F_{1, 11} = 6.14$, $P = 0.03$, F birds in group = 9.26 ± 0.58 , no F birds in group = 7.25 ± 0.58 ng/ml). But group-average CORT was not affected by the presence of F birds per se, as this effect could also be seen when the F birds were excluded from the analysis. Post-MR corticosterone levels of birds with classifications WV, V and W were higher if a F bird was present in the group ($F_{1, 11} = 7.70$, $P = 0.02$), if more than 50% of the birds within a group had severe comb lesions ($F_{1, 11} = 5.53$, $P = 0.02$) and tended to be affected by the interaction between the presence of F birds and comb lesions (interaction: comb lesions * presence/absence of F birds/group: $F_{1, 11} = 3.22$, $P = 0.07$), see Figure 2.4.

2.5. DISCUSSION

The aim of this study was to assess the relation between behaviour in an Open Field (OF) test at six weeks of age with post-Manual Restraint corticosterone (CORT) levels at 33 weeks of age, with the use of birds from a low mortality and control line. Chicks from the low mortality line were, as expected, more active in the OF than chicks from the control line. Birds that showed freezing behaviour in the OF, i.e. no walking or vocalizing, had higher CORT levels compared to chicks that walked and/or vocalized in the OF. We found correlations between pen-average OF behaviour and pen-average CORT. Post-MR CORT levels of birds were affected by the presence of fearful birds in the pen, and were related to proportion of birds with high levels of comb lesions in the pen.

Figure 2.3. Correlations(r) between group-averages of plasma-corticosterone levels post a 5-min Manual Restraint test at 33 weeks of age with group-average duration of walking (A), sitting (B) and latency to vocalize (C) in the Open Field test at six weeks of age, based on residuals of a model including effects of line ($n=8$ pens/line)

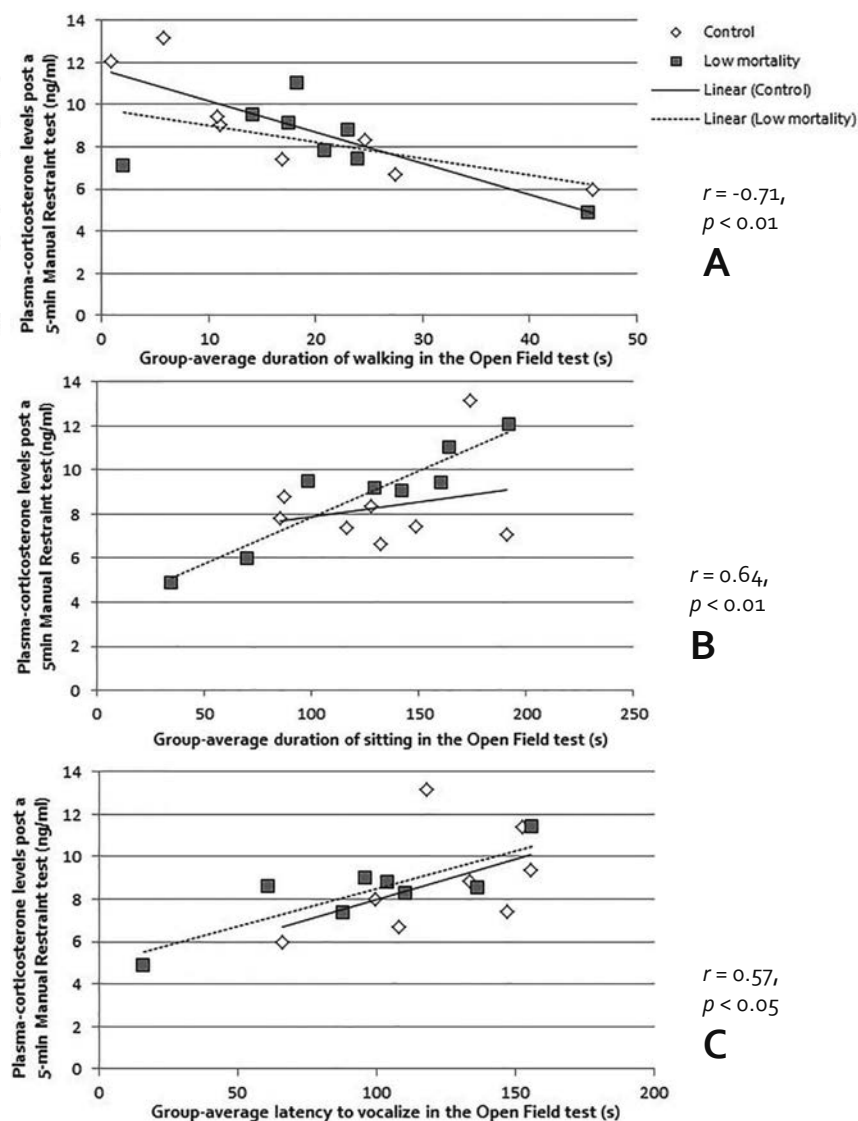
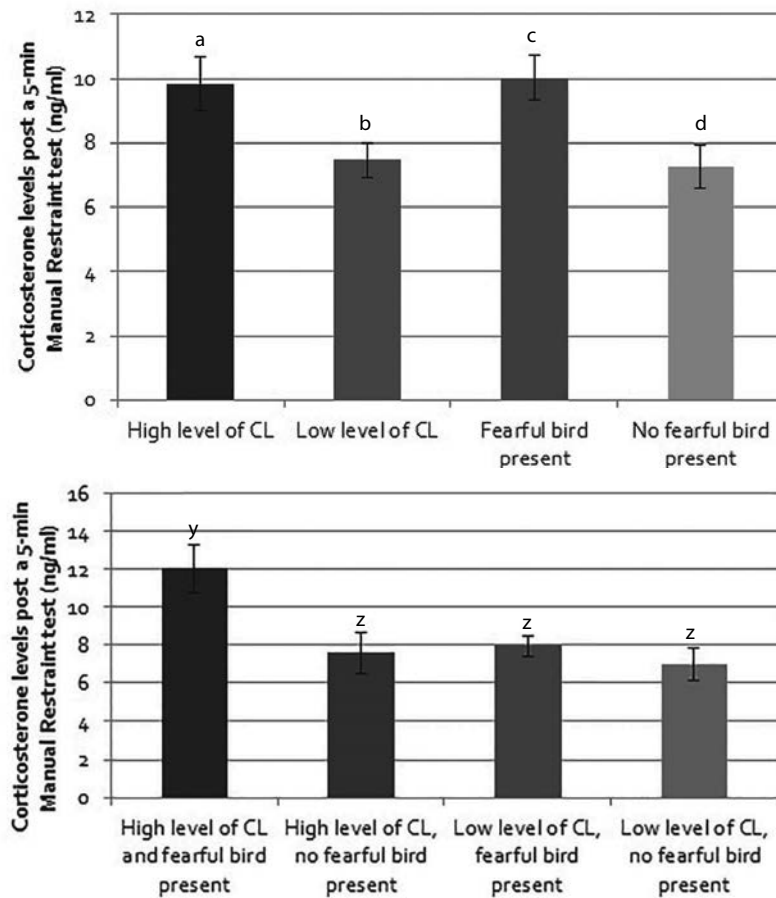


Figure 2.4. Plasma-corticosterone (ng/ml) post a 5-min Manual Restraint test at 33 weeks of age of birds that walked and/or vocalized in the Open-Field (OF) at six weeks of age in: (A) groups with high/low level of comb lesions (CL) and in groups with birds that froze (F) in the OF test present or absent; and the interaction between fearful birds present/absence and level of comb lesions (B)



Means with different superscript abc: $P < 0.05$, yz: $P < 0.1$

2.5.1. LINE EFFECTS

Chicks from the low mortality line expressed more vocalizations and had a shorter latency to stand in the OF than chicks from the control line. Inhibition of birds to ambulate and vocalize serves to avoid predation and therefore most likely represents high fear for predation (Gallup and Suarez, 1980; Jones, 1996a; Forkman et al., 2007). Thus, conversely, ambulation measurements such as number of steps and duration of walking in the OF, as measures of exploration/activity, likely represent low fear for predation (Forkman et al., 2007). Standing up, however, can be a precursor for locomotion (Rodenburg et al., 2003b). A short latency to stand – from a low posture to an upright position – may indicate less fear for predation in the chicks from the low mortality line (also found by (Rodenburg et al., 2009b)), as movement can increase the risk of detection by a predator. A high level of vocalizations (i.e. distress calls: (Collias and Joos, 1953)) in a social isolation test as the OF, can indicate strong motivation for social reinstatement (Gallup and Suarez, 1980). Thus when the number of vocalizations is high, it shows that social reinstatement motivation overcomes fear for predation. Chicks of the second generation of the low mortality line also expressed more vocalizations in the OF test than chicks of the control line (Rodenburg et al., 2009b). In a recent study with the same generation selection lines, chicks of the low mortality line were faster to return to their con-specifics compared to chicks of the control line (Nordquist et al., 2011). Group-selection on low mortality appeared to have influenced both level of fear and sociality. Our results fit with previous studies showing a lower fear level in the low mortality line compared to the control line by: higher activity in an OF (Rodenburg et al., 2009b; Nordquist et al., 2011), earlier reinstatement of behaviour after a sudden human approach, higher activity during MR, higher whole blood 5-HT (serotonin),

lower platelet 5-HT uptake (Bolhuis et al., 2009) and lower post-MR CORT (Rodenburg et al., 2009a). Unexpectedly, lines did not differ in post-MR CORT levels in our study. In the second generation, hens of the low mortality line had lower CORT after a MR test (Rodenburg et al., 2009a), but this was not found in the study of Bolhuis et al. (2009). We and Bolhuis et al. (2009) obtained a blood sample immediately after the MR test, while Rodenburg et al. (2009a) took a sample ten min after the MR test. Our measurement and that of Bolhuis et al. (2009), possibly resemble the rising CORT, while Rodenburg's measurement possibly resembles a peak measure (Cockrem, 2007) and may therefore differ from the other two studies.

2.5.2. RELATION BETWEEN OPEN FIELD BEHAVIOUR AND POST-MANUAL RESTRAINT CORTICOSTERONE

Birds that did not walk or vocalize in the OF (F birds) had higher post-MR CORT levels compared to birds that walked and/or vocalized in the OF. We did not assess basal CORT levels, but due to the type of stressor (sudden restraining); being an unpredictable and uncontrollable event (Koolhaas et al., 2011), and the average height of the CORT response (>8 ng/ml versus 1.5 ng/ml for baseline Cockrem, 2007), we do believe that differences in CORT levels cannot be regarded as baseline but as differences in rising CORT. As we alternated pens and birds within pens during both test days, the higher levels of CORT in F birds cannot be a co-effect of higher basal CORT due to the circadian rhythm. Additionally in mice, housed either in groups or in social isolation, basal CORT levels were unrelated to peak CORT levels in response to a stressful procedure (Nichols and Chevins, 1981). Struggles during the MR test affected CORT levels. This supports the finding that behavioural activity *as such* can increase CORT, and that combining behaviour and physiological measurements is essential to

evaluate stress responses (Koolhaas et al., 2011). The higher CORT levels after MR at 33 weeks of age in F birds show that fearfulness, as assessed early in life, can have a long-term effect on stress sensitivity in laying hens, as shown in rodents (Pawlak et al., 2008; Sandi and Richter-Levin, 2009) and Japanese quail (Calandreau et al., 2011a). In the OF no distinct threat is placed upon the birds, thus animals which respond fearful in the OF test perceive the OF as a potential threat i.e. anxiety (Davis et al., 2010) which can be physical or psychological (Clement and Chapouthier, 1998; Clément et al., 2002). An animal's anxiety state can affect vulnerability to stress (Korte, 2001; Wood et al., 2008; Rosenkranz et al., 2010) and high anxiousness may misbalance the hypothalamic–pituitary–adrenal pathway which responds to stressful events (Eiland and McEwen, 2012). We did, however, not find a relation between behaviour in the OF (potential threat: anxiety (Clement and Chapouthier, 1998) and behaviour during the MR test (actual threat: fear (Beuving and Vonder, 1977)) in the present study. This illustrates that fear tests vary in the degree of threat potential (Lang et al., 2000) and that further research is needed to elucidate the differences between fearful/non-fearful birds in consistency of behavioural and physiological responses over time and contexts.

2.5.3. GROUP EFFECTS

Positive correlations were found between group-average inactive behaviour in the OF test and group-average CORT. Groups with a low average walking duration in the OF had high average CORT after MR. Average CORT was, moreover, affected by the presence of fearful birds (i.e. F birds) in the group. More precisely, CORT levels of WV (walk and vocalize), V (vocalize) and W (walk) birds in groups with at least one fearful, freezing bird present, were higher than

CORT levels of WV, V and W birds in groups where no fearful birds were present. The social environment, i.e. the presence or absence of fearful birds in a group, thus affected the response to a stressful event of all birds. In highly synchronized animals, such as laying hens, social transmission of (fear) behaviour may be the mechanism affecting individuals within a pen (Nicol, 1995; 2004; Hoppitt et al., 2007). Young chicks are mostly guided by the behaviour of their conspecifics (Nicol, 2004). Humans can also be affected by fearfulness of others, which then activates the observers' amygdala (Olsson et al., 2007); the most important brain region involved in emotions and anxiety (Graeff et al., 1993). Fear-responses by single birds (F birds) can thus spread through a group, whereby responses are transmitted upon group mates. In rodents (Cavigelli and McClintock, 2003; Clinton et al., 2008), but also in avian species (Schoech et al., 2011) social transmission of fear and early exposure to stress can have life-long effects on behaviour, physiology and brain development. The contagion of mood of certain individuals can, in humans, affect group behaviour and mood of others (Barsade, 2002; Sy et al., 2005). Certain birds may thus directly or indirectly affect the behaviour and stress sensitivity of others within the same group. A direct effect can be aggressive behaviour from one bird to another. An indirect effect would then be the increase in activity caused by aggressive interactions (as seen by a trend for higher activity at an adult age in the home pen of F birds and birds with no comb lesions) which affects all animals within the group. As we showed by groups where the majority of birds had severe comb lesions, CORT levels were higher. Comb lesions are indicative of dominance-aggression, due to pecking at the comb and neck region (Savory, 1995; Tauson et al., 2005). When many birds in a group have comb lesions, this points to social instability in a group. This in turn can make the whole group more susceptible to stress (Sachser et al., 1998),

as seen by a higher level of CORT in groups with a high level of comb lesions. Additionally, high levels of comb lesions and presence of fearful birds combined tended to result in higher CORT compared to having only comb lesions or fearful birds present in the group. Based on the present data we cannot say whether having fearful birds present in a group causes higher levels of aggression, or vice versa as we only have measurements of aggression at one time point (i.e. at adult age). But this study does give some indications which factors influence stress sensitivity on a group level.

2.6. CONCLUSIONS

High fearfulness of laying hen chicks can have a long-term effect on individual stress physiology. At the group-level, fearfulness at six weeks – as assessed by Open Field behaviour – was correlated with corticosterone levels after a Manual Restraint test at 33 weeks of age. The presence of fearful birds in a group, as well as signs of social instability in a group, affected birds from the same group in coping with stress. The mechanism by which this occurs can lie in social transmission of (fear related) behaviour, but needs further investigation.

2.7. ACKNOWLEDGEMENTS

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Chapter 2

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Chapter 3

Selection for low mortality in laying hens affects catecholamine levels in the arcopallium, a brain area involved in fear and motor regulation

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ABSTRACT

Feather pecking (FP) in laying hens may cause mortality due to cannibalism. Novel breeding methods using survival days of group-housed siblings allow for the genetic selection of laying hens with low mortality (LML: low mortality line) due to cannibalism. Previous studies have demonstrated less fear-related behaviour and also less FP in LML hens compared to CL. Selection also caused changes in locomotor behaviour in an Open Field. It is unknown, however, whether selection for low mortality affects central neurotransmitter levels. In this study, brain monoamine levels were measured in the dorsal thalamus, medial striatum, hippocampus and arcopallium of adult laying hens of both LML and CL using HPLC. Brain samples were collected after 5-min of Manual Restraint. The most prominent line differences were found in the arcopallium. Compared to CL, LML had lower levels of noradrenaline (NA) and 3, 4-dihydroxyphenylacetic acid (DOPAC) and tended to have lower levels of dopamine (DA), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA). Levels of serotonin (5-HT), 5-HT- and DA-turnover in this brain area were not affected by line. LML showed less fear-related behaviour during the restraint than CL. These findings show that selection for low mortality in hens leads to changes of predominantly the dopaminergic system in the chicken's arcopallium, a forebrain somatomotor area also related to fear. This suggests a relationship between catecholamine functioning in this brain area and FP and cannibalistic behaviour in chickens and underpins previously found relationships between FP, fear and high activity.

KEYWORDS: Laying hens; genetic selection; feather pecking; cannibalism; dopamine; arcopallium

3.1. INTRODUCTION

Welfare concerns have led to a European ban on conventional battery cages for laying hens. Since 2012, only alternative housing systems ranging from “furnished” or “enriched” cages to non-caged aviaries or free-range systems are allowed (Rodenburg et al., 2012). Although these systems allow hens more freedom of movement, recent studies report increased mortality rates within alternative housing systems and many casualties are due to cannibalism (Fossum et al., 2009). Cannibalism in laying hens is the act of a bird pecking at the skin and devouring the flesh of other birds (Savory, 1995), which may ultimately lead to the death of the victims. Cannibalistic pecking is often preceded by severe feather pecking (FP) which is pecking at and removing of feathers of conspecifics causing denuded areas in the plumage which subsequently is very attractive for others to peck at (Bilčík and Keeling, 1999). To reduce problems related to severe feather pecking and cannibalism, many hens are exposed to beak-trimming, i.e. removal of the sharp tip of the upper beak. There is a growing societal resistance against animal mutilations, as it in itself beak-trimming induces stress, pain and fear in hens (Gentle and Hunter, 1990; Gentle, 2011). Thus, there is an urgent need for alternatives to reduce severe FP and cannibalism in laying hens.

Multiple factors, such as rearing and housing conditions (Johnsen et al., 1998a; Rodenburg et al., 2008a), and diet (Van Krimpen et al., 2005; 2011) may contribute to the development of FP and cannibalism in laying hens. There are, however, also large individual differences in the vulnerability to develop severe FP and cannibalism and a genetic background for these differences has been found (Cheng et al., 2001; Kjær et al., 2001). Traditionally, laying hens are selected for individual performance, such as egg production (Olsen et al., 1940). Selecting on individual performance can have potentially negative side-effects on

group members (Bijma et al., 2007a; Bijma et al., 2007b). By focusing on group performance and survival, Craig and Muir successfully decreased cannibalism-induced mortality in non-beak trimmed hens (Craig and Muir, 1996; Muir, 1996). Recently, a novel selection method has been developed in which selection of individually housed candidates is partly based on the survival of their group-housed female siblings (Ellen et al., 2007). The advantage of this selection method is that candidates for breeding remain unaffected by group interactions, as they are housed individually, and vital information on individual performance is combined with the information on group performance. Already in the first generation a markedly decreased mortality rate was established in the low mortality line (LML) compared to the unselected control line (CL) (Rodenburg et al., 2010a; Ellen et al., 2010). In the third generation, LML showed less cannibalistic toe and comb pecking than CL (Rodenburg et al., 2009b). Behavioural tests further show that LML hens displayed less fear-related and more active behaviour compared to the unselected CL hens in several behavioural tests, both at young and at adult age (Bolhuis et al., 2009; Rodenburg et al., 2009a; Nordquist et al., 2011; de Haas et al., 2012). Interestingly, LML and CL hens do not only differ in damaging and emotional behaviour, but also in possible underlying physiological mechanisms. For example, differences in the peripheral serotonergic system have been found, with LML hens having higher whole blood serotonin (5-hydroxytryptamine; 5-HT) levels (Bolhuis et al., 2009; Rodenburg et al., 2009a) and a lower platelet 5-HT uptake (Bolhuis et al., 2009) than CL. Also, lower plasma corticosterone levels were measured in LML after a Manual Restraint (Rodenburg et al., 2009a), possibly reflecting decreased fearfulness in the low mortality line. In animals and humans, anxiety (or fearfulness) has been related to brain 5-HT (Lesch et al., 1996). Several genetic

and pharmacological studies further established the involvement of both central 5-HT and DA in FP (Kjær et al., 2004; van Hierden et al., 2004ab ;2005; Flisikowski et al., 2009; Biscarini et al., 2010; Bordnick et al., 1994) and cannibalism (Cheng et al., 2001; Cheng et al., 2003; Dennis et al., 2006; Flisikowski et al., 2009; Rodenburg et al., 2009a; Rodenburg et al., 2009b). More recently, a lower concentration of tyrosine hydroxylase, the rate-limiting enzyme in DA production, was reported in the nidopallium, a “prefrontal” area (Morgensen and Divac, 1982; Kroner and Güntürkün, 1999; Güntürkün, 2005) of LML hens compared with CL hens (Nordquist et al., 2013). Unknown, however, is whether and how selection for low mortality in laying hens affects central neurotransmitter levels in the brain.

The aim of the present study was to compare brain monoamine levels and DA and 5-HT turnover levels between the fourth generation of laying hens selected for low mortality (LML) and the control line (CL). In total, four target regions related to the modulation of fear and motor control were selected ((Durstewitz et al., 1999a; Atoji et al., 2006): a combination of the dorsal (AD) and central (AI) region of the intermediate arcopallium (referred to as arcopallium), the medial striatum, the hippocampus, and the dorsal thalamus. The arcopallium receives input from various associative and sensory forebrain areas and is the source of a major down-sweeping pathway to brainstem motor structures; it thus is a somatomotor forebrain area (Reiner et al., 2004). The medial striatum is the limbic component of the avian striatal complex (Reiner et al., 2004). Given the behavioural and physiological differences between LML and CL, we expect lower levels for both DA and 5-HT in CL hens compared to LML hens, as the former are considered more fearful and display more FP and cannibalistic behaviour leading with higher mortality rates.

3.2. MATERIAL AND METHODS

3.2.1. ETHICAL STATEMENT

The experiment was approved by the Animal Care and Use Committee of Wageningen University, and in accordance with Dutch legislation on the treatment of experimental animals the ETS₁₂₃ (Council of Europe 1985) and the 86/609/EEC Directive.

3.2.2. BIRDS AND HOUSING

In total, 40 adult female White Leghorns (*Gallus gallus domesticus*) of 33 weeks of age were selected for brain analyses. Half of these hens originated from CL (n=20) and the other half from the fourth generation of LML (n=20) aimed at breeding with selection candidates of which siblings showed low group mortality (Ellen et al., 2007) (de Haas et al., 2012). All non-beak trimmed hens came from the same population of 160 hens as described previously (de Haas et al., 2012) and were obtained from ISA, the layer breeder division of Hendrix Genetics, the Netherlands. Hens were housed per line in groups of 10 birds per pen, 8 pens per line thus 16 pens in total. From each pen, two to three hens were randomly selected for brain analyses such that twenty birds were selected per line. Water and a commercial mash diet were provided ad libitum. Pen floors (1.9 by 1.2 m) were covered with sand (1/3) and wood shavings (2/3). For more details on housing conditions, see (de Haas et al., 2012).

3.2.3. MANUAL RESTRAINT

At 33 weeks, each hen was subjected to a Manual Restraint test, using a method previously described (Bolhuis et al., 2009). Briefly, each hen was removed from her home pen and put in a cardboard box to be tested in an adjacent room. The

experimenter used the right hand to place a hen on her right side on a table covered with cardboard and then covered the trunk and the left hand gently stretched the hen's legs. For 5 min, the frequency of consecutive struggles and the number of vocalizations was recorded as well as the latency to struggle and vocalize. After each struggle, hens were placed back into the original test position until the time of the test passed. The Manual Restraint took place on two consecutive days by one researcher and the order of testing was balanced for line. After the Manual Restraint, the hens selected for brain analyses were sacrificed by cervical dislocation.

3.2.4. BRAIN TISSUE PREPARATION

Brains were removed and immediately deep frozen in n-heptane, put on dry ice and stored at -80°C (protocol by Van Hierden et al., 2002ab). Slicing of brains was executed in a cryostat (Frigocut Jung Mod_700) under cold conditions (-10°C). Slice thickness was $400\text{ }\mu\text{m}$. The four regions of interest were located using the brain atlas for 2-week-old chickens (Puelles et al., 2007) and with considering literature on the avian brain (Atoji and Wild, 2009; Metzger et al., 2002; 1998), thereby also taking into account the increased brain size in our hens at 33-weeks of age. Figure 3.1 is a schematic drawing depicting the location of, respectively, the MSt (A) and the dorsal thalamus, hippocampus and the arcopallium (B). The grey dotted shapes illustrate the cutting lines per brain area (carefully cut with a scalpel). Tissue samples were taken from multiple slices, with corresponding figure numbers in the atlas: Medial striatum (MSt; 7.56 - 5.68 mm anterior to the interaural line) including the accumbens (Acb; 8.08 - 7.56 mm anterior to the interaural line), hippocampus (HIP; Hi1, Hi2, PHiM, PHiL, PHiL1, PHiL2, and PHiA; 6.16 - 0.40 mm anterior to the interaural line), and the dorsal thalamus (DPe,

DMA, DIA, DLA; 3.04 - 1.36 mm anterior to the interaural line). For the arcopallium, the area referred to as amygdala core by (Puelles et al., 2007) was sampled (4.24 - 2.08 mm anterior to the interaural line). Brain samples of the left and right hemisphere were taken together and analyzed as one.

CENTRAL MONOAMINE ANALYSIS WITH HPLC

Brain samples were analyzed using a High Performance Liquid Chromatography (HPLC) method. For that, the tissue samples were homogenized in an ice-cold solution containing 5 μ M clorgyline, 5 μ g/ml glutathione and 1.2 μ M N-methylserotonin (NMET, internal standard) using sonication. To 80 μ l homogenate, 20 μ l 2 M HClO₄ was added and mixed. After 15 min in ice water, the homogenates were centrifuged during 15 min at 15000g (4 °C). The supernatants were diluted 10 times with water before HPLC analysis. The concentration of serotonin [5-HT] and its metabolite 5-hydroxyindoleacetic acid [5-HIAA], and dopamine [DA] with corresponding metabolites 3-methoxytyramine [3-MT], 3, 4-dihydroxyphenylacetic acid [DOPAC], and homovanillic acid [HVA], and also noradrenaline [NA] in the tissue extracts were measured by HPLC with electro chemical detection (ECD). The HPLC system consisted of a pump model P100, a model AS300 auto sampler (both from Thermo Separation Products, Waltham, MA, USA), a ERC-3113 degasser (Erma CR. Inc. Tokyo, Japan), an ESA Coulochem II detector with 5011 analytical cell set at potential +550mV (ESA Inc. Bedford MA, USA), a data acquisition program (Atlas 2003, Thermo Separation Products) and a column (150mm x 4.6mm i.d.) packed with Hypersil BDS C18, 5 μ m particle size (Alltech Associates, USA). The mobile phase solution consisted of 50 mM citric acid, 50 mM phosphoric acid, 0.1 mM EDTA, 45 μ l/L dibutylamine, 77 mg/L 1-octanesulfonic acid sodium salt, 10 %

methanol; the pH of the buffer was adjusted to 3.4 with NaOH. Separation was performed at 45 °C using a flow rate of 0.8 ml/min. The concentration of each compound was calculated by comparison with both the internal and the external standards. The protein content of each homogenate sample was determined using the DC protein Assay (Bio-Rad). Monoamine concentrations are expressed as nmol/g protein. Turnover levels of serotonin (5-HIAA/5-HT) and dopamine ((DOPAC+HVA+3-MT)/DA) were calculated as an index for the activity of the serotonergic and dopaminergic system (van Hierden et al., 2002a) high levels indicate a quicker metabolic pathway due to higher biosynthetic enzyme activity.

3.3. STATISTICAL ANALYSIS

SAS version 9.2 was used for statistical calculations [SAS, 1989]. Monoamine and metabolite levels per brain area were tested with a mixed model that included the fixed effects of line (LML vs. CL) and day (test day 1 and 2). Pen nested within line was added as a random effect to the model, thus, effectively, pen (n=16) was used as experimental unit for testing line effects. Post-hoc least square means were used to detect pair-wise differences. A log transformation for DA turnover in the arcopallium and HVA in the dorsal thalamus was executed to obtain normality of residuals. Data are presented as mean \pm SEM. Effects of line on the behavioural responses of hens during the Manual Restraint test were analyzed using Kruskal Wallis tests as data were not normally distributed. If significantly different, values are presented as median (M) with the interquartile range, i.e. lower (Q1) and upper (Q3) quartiles. Many birds did not struggle or vocalize at all during Manual Restraint. Therefore, struggling and vocalizing during the test was also analyzed as a binary (yes/no) variable using a generalized mixed model with

logit link function. Line and day were fixed effects in this model, and pen nested within line was added as random effect. Data are presented as mean \pm SEM.

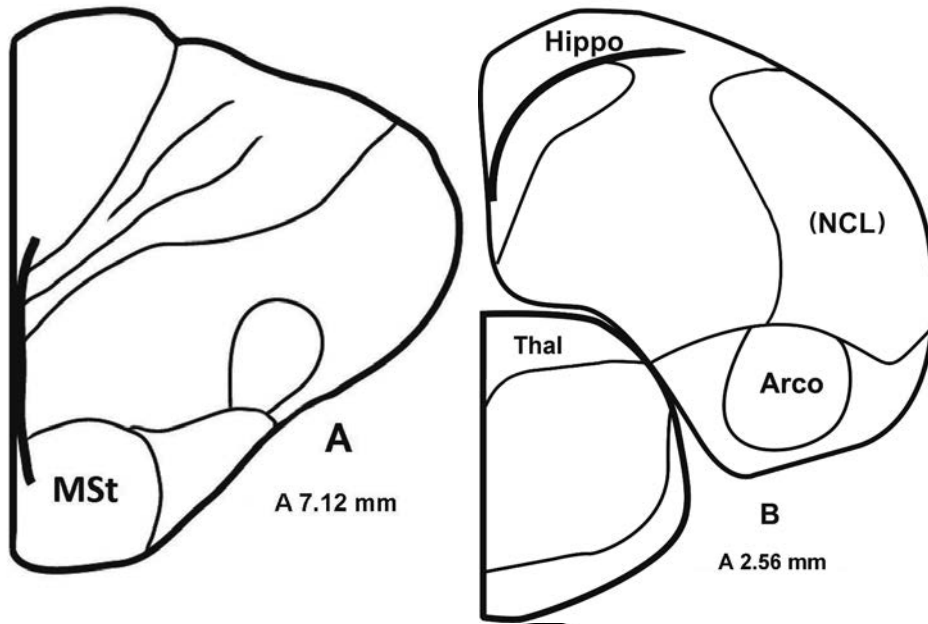


Figure 3.1. Coronal schematic views of the chicken brain illustrating the medial striatum, thalamus, hippocampus, and arcopallium. The schematic views of the left hemisphere of a chicken's brain are drawn after [Atoji and Wild, 2009; Metzger et al., 1998; 2002] with brain coordinates based on the chicken brain atlas [Puelles et al., 2007]. Depicted are the medial striatum (MSt) at 7.12 mm anterior to the interaural line (A), and the thalamus, arcopallium and hippocampus at 2.56 mm anterior to the interaural line (B). The location of the NCL is indicated between brackets (B), because this area was analyzed by [Nordquist et al., 2013], and here compared with our results and discussed. At 33 weeks of age, chicken brains were sampled from both the left (shown here) and right hemisphere (not shown); the gray dotted shapes illustrate the cutting lines per brain area. Abbreviations: A = anterior to the interaural line; MSt = medial striatum; Thal = Thalamus; Arco = Arcopallium; Hippo = hippocampus; NCL = nidopallium caudolaterale

3.4. RESULTS

3.4.1. EFFECTS ON DA

In Figure 3.2, the levels of DA, its three metabolites DOPAC, HVA, and 3-MT, NA and the calculated DA turnover in the arcopallium are shown. DA levels ($F_{1,14} = 4.1$, $P = 0.06$) and levels of its metabolite HVA ($F_{1,14} = 4.1$, $P = 0.06$) tended to be lower for LML birds than for CL birds. LML birds also showed significant lower NA levels ($F_{1,14} = 5.6$, $P = 0.03$) and DOPAC levels ($F_{1,14} = 7.1$, $P = 0.02$) in this brain area compared to CL birds. Levels of 3-MT ($F_{1,14} = 0.0$, $P = 0.95$) and the DA turnover ($F_{1,14} = 1.0$, $P = 0.24$) in the arcopallium were unaffected by line. Table 3.1 shows the levels of DA, DOPAC, HVA, 3-MT, DA turnover, and NA in the dorsal thalamus, medial striatum, and hippocampus per line. DOPAC levels in the thalamus were lower for LML birds compared to CL hens ($F_{1,14} = 6.1$, $P = 0.03$). LML hens showed a higher DA turnover in the hippocampus ($F_{1,14} = 5.2$, $P = 0.04$) than CL hens. No other line effects were found for DA and its metabolites in the dorsal thalamus, medial striatum, and hippocampus.

3.4.2. EFFECTS ON 5-HT

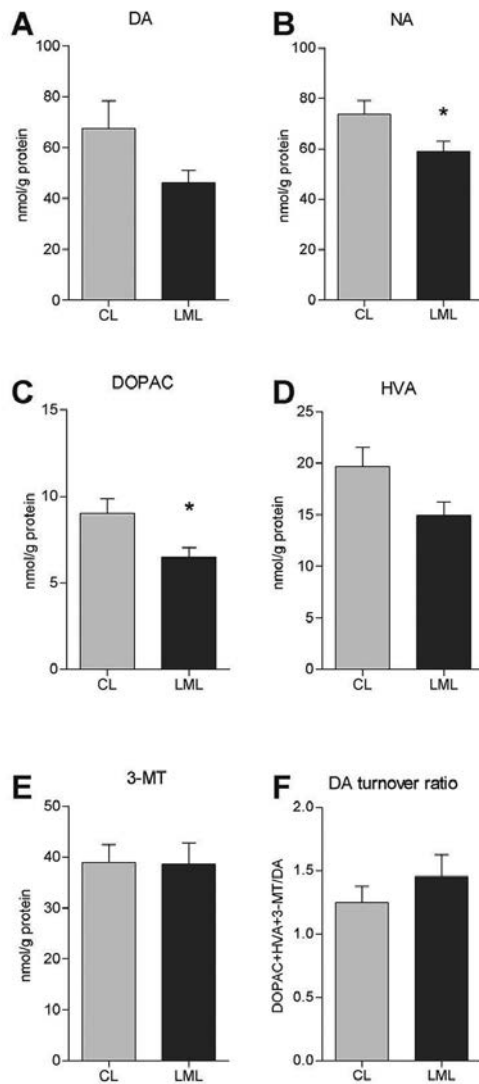
Figure 3.3 shows levels of 5-HT, 5-HIAA, and 5-HT turnover in the arcopallium. There, 5-HIAA levels tended to be lower for LML hens compared to CL hens ($F_{1,14} = 3.6$, $P = 0.08$). Levels of 5-HT ($F_{1,14} = 2.3$, $P = 0.15$) and the 5-HT turnover ($F_{1,14} = 0.6$, $P = 0.45$) in this area were unaffected by line. In the three other brain areas, i.e. the dorsal thalamus, medial striatum, and hippocampus, no effects of line on 5-HT, 5-HIAA, or 5-HT turnover were found (Table 3.2).

Table 3.1. Catecholamine, metabolites, and DA turnover levels in the dorsal thalamus, medial striatum, and hippocampus of hens from the control line (CL) and low mortality line (LML).

		Control (CL)		Low mortality (LML)		P value
		Mean	SEM	Mean	SEM	
Dorsal thalamus						
	DA	12.33	1.64	8.72	0.98	0.105
	NA	94.90	6.71	81.77	4.23	0.145
	DOPAC	0.94	0.13	0.55	0.07	0.027 *
	HVA	3.91	0.65	2.89	0.25	0.200
	3-MT	6.79	0.65	6.73	0.56	0.944
	DA turnover	1.05	0.09	1.18	0.08	0.219
Medial striatum						
	DA	748.75	34.11	701.90	59.08	0.536
	NA	38.25	4.28	31.20	3.17	0.211
	DOPAC	33.70	2.20	30.34	2.89	0.421
	HVA	45.60	3.03	43.00	4.01	0.726
	3-MT	86.10	5.93	95.55	8.28	0.369
	DA turnover	0.23	0.01	0.24	0.01	0.291
Hippocampus						
	DA	4.40	1.09	2.95	0.67	0.320
	NA	66.95	3.70	65.20	3.01	0.723
	DOPAC	0.95	0.26	1.15	0.26	0.600
	HVA	1.35	0.40	2.45	0.59	0.145
	3-MT	18.05	1.91	19.25	2.45	0.774
	DA turnover	0.65	0.15	1.52	0.39	0.043 *

*P < 0.05; Mean (\pm SEM) in nmol /g protein; n = 20/group

Figure 3.2. Catecholamine, metabolite, and turnover levels in the arcopallium of adult laying hens. Mean (\pm SEM) values for catecholamines dopamine (A) and noradrenaline (B), the DA-metabolites DOPAC (C), HVA (D), and 3-MT (E), and the DA turnover ratio ((DOPAC + HVA + 3-MT)/DA) (F) in the arcopallium of hens from the control line (CL) and low mortality line (LML). $n = 20/\text{group}$



* $P < 0.05$

3

Table 3.2. Serotonin, metabolite, and 5-HT turnover levels HT and 5-HIAA, and 5-HT turnover in the dorsal thalamus, medial striatum, and hippocampus of hens from the control line (CL) and low mortality line (LML)

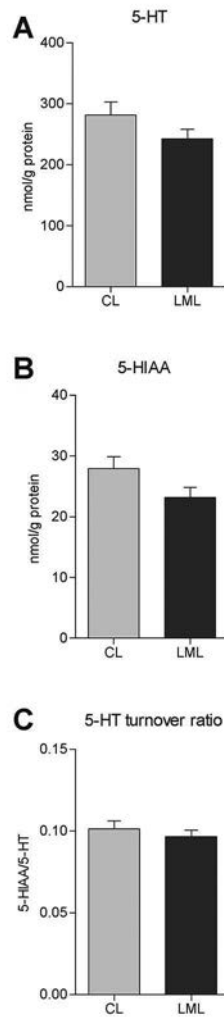
		Control (CL)		Low mortality (LML)		P value
		Mean	SEM	Mean	SEM	
Dorsal thalamus						
	5-HT	92.84	5.56	98.96	5.33	0.533
	5-HIAA	10.16	0.65	10.01	0.51	0.919
	5-HT turnover	0.11	0.01	0.10	0.00	0.246
Medial striatum						
	5-HT	159.60	10.23	162.30	10.88	0.850
	5-HIAA	15.45	0.97	14.75	1.26	0.733
	5-HT turnover	0.1	0.00	0.09	0.00	0.161
Hippocampus						
	5-HT	177.25	11.00	174.00	10.13	0.853
	5-HIAA	23.65	1.15	23.85	1.29	0.904
	5-HT turnover	0.14	0.01	0.14	0.00	0.982

Mean (\pm SEM) in nmol / g protein; n = 20/ group

3.4.3. MANUAL RESTRAINT

During the 5-min Manual Restraint, birds from the two lines differed in their latency to vocalize ($\chi^2 = 5.9$, df = 1, $P = 0.02$) and frequency ($\chi^2 = 6.1$, df = 1, $P = 0.01$), with LML birds vocalizing sooner ($M_{LML} = 296$ sec, $Q_1 = 161$, $Q_3 = 300$) and more ($M_{LML} = 0.5$, $Q_1 = 0.0$, $Q_3 = 16.3$) compared to CL birds ($M_{CL} = 300$ sec, $Q_1 = 300$, $Q_3 = 300$, and, $M_{CL} = 0.0$, $Q_1 = 0.0$, $Q_3 = 0.0$, respectively). When vocalizing was expressed as a binary variable, 50% of all LML hens vocalized, whereas only 15 % of CL hens did ($F_{1,14} = 3.60$, $P = 0.08$). Lines did not differ significantly in their latency to struggle ($\chi^2 = 1.9$, df = 1, $P = 0.167$; $M_{LML} = 300$, $Q_1 = 241$, $Q_3 = 300$ vs. $M_{CL} = 300$, $Q_1 = 300$, $Q_3 = 300$) or in frequency of struggling ($\chi^2 = 2.6$; df = 1; $P = 0.11$; $M_{LML} = 0$, $Q_1 = 0$, $Q_3 = 1$ vs. $M_{CL} = 0$, $Q_1 = 0$, $Q_3 = 0$). Also, the percentage of birds that struggled during the test did not differ between LML (45%) and CL birds (20%) ($F_{1,14} = 2.31$, $P = 0.15$).

Figure 3.3. Serotonin, metabolite, and turnover levels in the arcopallium of adult laying hens. Mean (\pm SEM) values for serotonin (5-HT) (A), its metabolite 5-HIAA (B), and the 5-HT turnover ratio (5-HIAA/5-HT) (C) in the arcopallium of hens from the control line (CL) and low mortality line (LML). $n = 20/\text{group}$



3.5. DISCUSSION

The current study compared brain monoamine levels in four different brain regions of laying hens selected for low mortality using group selection of siblings (LML) with a control line (CL). Selection for low mortality resulted in changes in dopaminergic measures, most prominently present in the arcopallium, but did not significantly affect serotonergic measures.

3.5.1. EFFECTS OF SELECTION FOR LOW MORTALITY ON DOPAMINERGIC MEASURES

Selection for low mortality resulted in lower levels of NA and DOPAC and a tendency for lower levels of DA and HVA, with no line differences for 3-MT levels and DA turnover levels in the arcopallium. In agreement with this observed difference in dopaminergic action, a recent immunohistochemistry study in the same selection lines showed lowered tyrosine hydroxylase concentrations in the nidopallium caudolaterale (NCL) in hens of the LML (second generation) as compared to the CL (Nordquist et al., 2013). Tyrosine hydroxylase catalyzes the production of DA from tyrosine (Doubner et al., 2001) and fewer enzymes might lead to a reduced synthesis of DA and diminished production of metabolites, as shown here. It is speculated that other mechanisms may be involved too, such as an altered activity of dopamine β hydroxylase and monoamine oxidase (MAO) that might affect monoaminergic neurotransmission (Eisenhofer et al., 2004). While Nordquist and collaborators (2013) focused on the NCL we focused on the arcopallium, medial striatum, hippocampus, and the thalamus. The telencephalic areas arcopallium and medial striatum contain a higher distribution of dopaminergic fibers and D₁ receptors than the NCL (Durstewitz et al., 1999b; Steward et al., 1996). A micro dialysis study in pigeons showed a high

release of DA and high production of DA-metabolites in the striatal area; with a relatively low HVA/DOPAC ratio reflecting a fast reuptake by the dopamine transporter (DAT) (Bast et al., 2002). In the present study, lines did not differ in their dopaminergic levels in the MSt. As the hippocampus has very little expression of the DAT (Borgkvist et al., 2012) reuptake of released DA into the presynaptic terminal is hampered, resulting in low levels of DOPAC and HVA. This effect might be stronger in CL hens, which had a lower DA turnover ratio in this area than LML hens. Similar to the arcopallium, DOPAC levels in the thalamus were lower for CL hens than for LML hens.

As LML are selected for low mortality due to FP and cannibalism, these results confirm previously found relationships between FP and the dopaminergic system (Kjær et al., 2004; Uitdehaag et al., 2011). It is shown that LML hens had a lowered DA neurotransmission compared to CL hens, most prominently seen in the arcopallium. It remains unknown, however, how much DA is released by the presynaptic cell thereby contributing to the levels of DOPAC (via reuptake of DA), 3-MT (via released DA) and HVA (via forming of DOPAC and 3-MT) (Eisenhofer et al., 2004). A micro dialysis study could provide more details on the functional aspects of monoamines as this technique enables measuring presynaptic release of DA (and 5-HT) and its metabolites (Bast et al., 2002).

3.5.2.EFFECTS OF SELECTION FOR LOW MORTALITY ON SEROTONERGIC MEASURES

Selection for low mortality tended to lower 5-HIAA levels in the arcopallium of LML hens, but 5-HT levels, albeit numerically lower in LML hens, were unaffected by line. No significant serotonergic effects were found in any of the three other brain areas either. Previously, it has been shown that LML hens had higher

peripheral whole blood 5-HT levels (Bolhuis et al., 2009; Rodenburg et al., 2009a) and a lower platelet 5-HT uptake (Bolhuis et al., 2009; Rodenburg et al., 2009a) than CL hens. In addition, it was shown that hens displaying severe FP had increased 5-HT turnover in the dorsal thalamus and higher levels of the 5-HT metabolite in the medial striatum compared to non-peckers and victims of FP (Kops et al., 2013a). Similar results were found when comparing 5-HT turnover between a flighty, FP-prone line and a more docile, low FP line (Uitdehaag et al., 2011). This suggests that FP may be influenced by brain 5-HT, whereas selection for low mortality is probably affected by more or different traits, obscuring the link with central 5-HT.

3.5.3. EFFECTS OF SELECTION FOR LOW MORTALITY ON DOPAMINERGIC MEASURES

The catecholamines, such as DA and NA, are known to play a role in motivational and reward-related motor and higher cognitive functions like impulsivity (Kalenscher et al., 2006). Pharmacological studies in both birds and mammals support the involvement of the central dopaminergic system in dysfunctional behaviours. For instance, increasing DA activity by administering a DA₂ receptor agonist induced stereotypies in pigeons (Cheng and Long, 1974), increased aggressive pecking in normally low aggressive chickens (Dennis and Cheng, 2011), and increased impulsivity in rats (Winstanley et al., 2010). Administering a DA₂ receptor antagonist, thus decreasing DA activity, reduced FP ratios in laying hens (Kjær et al., 2004), while a DA₁ receptor antagonist decreased the behaviour of already high aggressive chickens (Dennis and Cheng, 2011), and high impulsive rats (Winstanley et al., 2010), but not in low aggressive chickens or low impulsive rats. Thus, high dopaminergic levels in CL might (at least partly) lie at the basis of

FP or impulsivity. Also, a large number of studies have demonstrated that fearfulness is related to FP behaviour (Hughes and Duncan, 1972; Bolhuis et al., 2009; Vestergaard et al., 1993; Rodenburg et al., 2004a; Jensen et al., 2005; Rodenburg et al., 2004a; Uitdehaag et al., 2008a; 2008b). It is assumed that more fearful animals are more prone to display defensive aggression and are more likely to perform severe FP (Rodenburg et al., 2004a; Rodenburg et al., 2008a) and cannibalism (Blokhuis and Beuving, 1993; Keeling and Jensen, 1995; Lesch, 2005). In addition, within a group both fearfulness and FP may be transmitted among pen-mates (Zeltner et al., 2000; Uitdehaag et al., 2008c; de Haas et al., 2012), and also victims of FP show increased fearfulness (Hughes and Duncan, 1972; Vestergaard et al., 1993) with possible activation of the ascending DA system, as seen in rodents suffering from repeated aggressive attacks (Barik et al., 2013). Here, the more active vocal behaviour of LML hens during the manual restraint indicates lower fearfulness compared to CL. That is, a more active behaviour during fear tests represents lower fear levels or a higher social reinstatement motivation in comparison to non-vocalizing and non-struggling behaviour (Forkman et al., 2007) as seen in CL hens. As described before LML showed reduced cannibalistic toe pecking (Rodenburg et al., 2009b) and are characterized as less fearful in numerous behavioural tests compared to the CL (Bolhuis et al., 2009; Rodenburg et al., 2009a; Nordquist et al., 2011; de Haas et al., 2012) including the one in this study. The differences in the dopaminergic activity between LML (low DA activity) and CL (high DA activity) might thus underlie the intergroup differences in damaging behaviours.

Importantly, the strongest dopaminergic effects were found in the arcopallium. Before the avian neuroanatomical nomenclature was changed, the area in the most ventrolateral and posterior part of the bird telencephalon was

called archistriatum. Based on a large amount of neurobiological evidence, the archistriatum is now subdivided into the somatomotor arcopallium and a cluster of sub nuclei that constitutes the amygdala (Reiner et al., 2004; Cheng et al., 1999; Yamamoto et al., 2005). Lesions in the amygdala of Japanese quail increase fear behaviour in an Open Field test, while arcopallium lesions decrease anxiety (Saint-Dizier et al., 2009), which fits with our results. It is also possible, though, that the quails' fearful state was mediated by motor output deficits as fearful quails remained longer immobile in the Open Field (Saint-Dizier et al., 2009). In addition, humans suffering from either obsessive-compulsive disorder or trichotillomania, a hair-pulling disease with similarities to FP (van Zeeland et al., 2009), both have impaired inhibition of motor response (Chamberlain et al., 2006). L-DOPA (L-3,4-dihydroxyphenylalanine) is known to improve motor learning and motor memory by increasing DA levels (Flöel et al., 2005; Pearson-Fuhrhop et al., 2012). Increased DA levels can, however, also induce stereotypes (Deviche, 1983) and deficits in the control of posture and motor activity (Nistico and Stephenson, 1979). Dopaminergic terminals are abundant in the arcopallium (Wynne and Güntürkün, 1995) and activate primarily D1-receptors (Durstewitz et al., 1999b), thereby possibly increasing spike densities of pre-activated neurons (Durstewitz et al., 1999a; Durstewitz et al., 1999b). Consequently, the higher DA activity might lead to a more active motor output system in CL hens, with impulsivity or even hyperactivity (Kjær, 2009) as underlying problems associated with FP and cannibalistic behaviour. Vice versa, it is hypothesized that lower dopaminergic neurotransmission in LML hens decreases the probability that motor-processes related to FP and cannibalism are activated and are executed.

3.6. CONCLUSIONS

The present study shows that selection for low mortality using a sibling group selection scheme affects the dopaminergic neurotransmission of laying hens with possible implications for the motor output of FP and cannibalistic behaviour, as suggested by the strong results found in the arcopallium, a somatomotor area. The stronger effect of line in the arcopallium compared to the absence of significant effects for dopamine or serotonin in the limbic MSt suggests that deficits in motor functioning might be at the base of these behaviours, although fear and even impulsivity might also affect FP and cannibalism. Future studies are needed (e.g. in vivo micro dialysis) on the role of dopamine in FP and cannibalism to further investigate the underlying neural mechanisms.

3.7. ACKNOWLEDGEMENTS

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The Practice



Chapter 4

Fear, stress, and feather pecking in commercial white and brown laying hen parent-stock flocks and their relationships with production parameters

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ABSTRACT

Little is known about the relationship between welfare traits and production in laying hen parent-stock (PS). In commercial laying hens and pure lines, it is known that aspects associated with reduced welfare such as high fear, stress and feather pecking (FP) can have negative effects on production. As PS hens are housed under different conditions than commercial laying hens, the relationship between welfare traits and production may differ. We therefore studied the fear response to a stationary person (SP) and novel object (NO), basal plasma-corticosterone (CORT) and whole-blood serotonin levels (5-HT), and feather damage as proxy for FP, in 10 Dekalb White (DW) and 10 ISA Brown (ISA) commercial PS flocks and related these to production data. As the relationship between welfare traits and production may differ by genetic origin and group size we also assessed genotype and group size effects. Dekalb White birds were more fearful of a stationary person, had more feather damage and lower 5-HT levels than ISA birds. Genotypes did not differ in CORT. A large group size ($n > 5000$) was associated with low feed intake and better feed conversion for ISA flocks. For DW flocks, high fear of the NO was associated with low body weight (BW), low egg weight and low feed intake. For ISA flocks, high fear of the SP was associated with high mortality. For both lines, high CORT was related to low egg-weight. This is the first study to associate levels of fear and CORT to production in commercial PS flocks. Management of PS flocks should take into account breed differences, group size effects and effects of human-animal interactions. Further research is needed to determine the effects of fear, CORT, 5-HT and feather damage in commercial PS flocks on the development of their offspring.

KEYWORDS: parent-stock, laying hens, welfare, stress, productivity

4.1. INTRODUCTION

Parent-stock (PS) flocks produce eggs for production of commercial laying hens. To date, very little is known about the relationship between welfare traits and production in PS flocks. Coping with fear and stress and the development of feather pecking (FP) are aspects affecting the birds' welfare. In commercial laying hens - the offspring of PS flocks - numerous studies have shown that behavioural and physiological measurements related to welfare are associated with productivity (Shini et al., 2009; Sossidou and Elson, 2009; Sosnowka-Czajka et al., 2010; O'Connor et al., 2011; Nasr et al., 2012). For instance in laying hens, negative relationships have been found between fearfulness and egg production (Barnett et al., 1994; Uitdehaag et al., 2008b), between FP and egg weight (Buitenhuis et al., 2004), FP and feed efficiency (Su et al., 2006), between induced high basal plasma-corticosterone and oviposition time (Moudgal et al., 1991), and also between CORT and hen-day egg production in Japanese Quail (Marin et al., 2002).

Parent stock hens are hybrids of a two-way cross of pure lines, housed in different conditions (floor housing) compared to pure lines (frequently cage-housed) and commercial laying hens (frequently housed in aviaries with or without outdoor range, at least in the Netherlands). Further, PS hens are housed together with roosters, under a strict hygienic regime, which limits contact with humans. These factors may cause variation in how birds cope with fear and stress and the relationship between fear and stress with production may thus be different from what is known in pure lines and commercial laying hens. Moreover, it has been suggested that welfare levels are lower in PS birds in comparison to commercial hybrids due to higher levels of aggression and mortality (Sosnowka-Czajka et al., 2011). Parent stock hens produce fertilized eggs containing

commercial laying hen embryos. Fear and stress in PS hens can affect the deposition of hormones in the egg (Henriksen et al., 2011b), and these can, in turn, affect the developing offspring: the laying hen (Janczak et al., 2007b; Guibert et al., 2011).

The level of fear and stress displayed by hens, and their predisposition to develop FP, can be related to their genetic origin. For example, commercial laying hens from a white genotype showed a longer duration of tonic immobility (anti predator response (Jones, 1996b)), indicating higher fearfulness, compared to various brown genotypes (ISA Brown, Colombian Blacktail, Ixworth (Albentosa et al., 2003); brown Hyline hens (Fraisse and Cockrem, 2006)). Also, white commercial laying hens displayed greater CORT response to human handling than brown Hyline hens (Fraisse and Cockrem, 2006). In pure lines, hens from a White Leghorn origin (WL: white) were more fearful in various fear tests than hens from a Rhode Island Red origin (RIR: brown) and developed more feather damage due to FP than RIR hens when tested in conventional cages (Uitdehaag et al., 2008b). Purebred WL hens also had lower whole-blood serotonin (5-HT) levels than hens from a Rhode Island Red origin (RIR; brown) (Uitdehaag et al., 2011). Lower 5-HT levels have been associated with high fearfulness and predisposition for FP (Bolhuis et al., 2009).

The relationship between behavioural and physiological measurements related to welfare and productivity has not yet been studied in PS flocks. Our aim was to assess the relationship between fear responses, physiological measurements of basal plasma-corticosterone and whole-blood serotonin, feather damage and productivity in commercial PS flocks from a WL or RIR origin. Based on the literature mentioned above our hypothesis was that flocks from a WL origin would be more fearful, have higher levels of basal plasma-

corticosterone, lower levels of 5-HT, and have higher levels of feather damage than flocks from a RIR origin. Additionally, we expected that high levels of fear, basal plasma-corticosterone, and feather damage would be associated with reduced production. As group size varied between commercial flocks, we also assessed group size effects. Group size, under commercial conditions, is known to affect behaviour and FP (Zimmerman et al., 2006).

4.2. MATERIALS AND METHODS

4.2.1. ANIMALS AND HOUSING

This study was approved by the Institutional Animal Care and Use Committee of Wageningen University. Two commercial PS genotypes were used: ISA Brown (ISA) and Dekalb White (DW). The ISA birds originate from a RIR origin, while the DW birds originate from a WL origin. The ISA flocks contained Rhode Island White hens with RIR (brown) roosters, while DW stock flocks contained WL (white) hens and WL (white) roosters. Rooster/hen ratio was 1:10 for all flocks. Ten ISA PS flocks and 10 DW PS flocks from Hatchery Ter Heerdt BV, Babberich, The Netherlands, were visited at 40 weeks of age between August 2010 and August 2011. Flocks were housed on commercial propagator-farms, using floor housing with partly slatted floors. Houses provided a litter area and nest-boxes but no perches. Number of birds per m² was similar for all flocks (8 birds/m²), but group size varied between 2,235 and 9,262 birds (DW: min=3,941, max=8,937; ISA: min=2,235, max=9,262). Light was provided for 15-16.5 hours per day. We measured light intensities by means of a Voltcraft MS-1300 light meter (Conrad Electric Benelux, Oldenzaal, The Netherlands), measuring LUX under a light source and not under a light source at three locations in the chicken house (front,

middle and back). Light intensities ranged from 1.3 to 42.1 LUX (average 25.6 LUX), with minimal daylight entering the house.

4.2.2. PRODUCTION PARAMETERS

During lay, production data of the flocks were recorded by the farmers from 20 until 65 weeks of age. We used data which was consistently present for all flocks containing the average data from week 25 until 40 (start), week 40 only (top) and from week 41 until 65 (end). For each production parameter per flock the following parameters were recorded:

- Laying percentage (expressed as average number of eggs laid per day in relation to number of hens per flock),
- Average egg weight (g) per flock based on weight of minimum 180 eggs per week,
- Average feed-intake per bird/day (g) based on feed intake per day divided by total number of birds per flock present,
- Average feed-conversion expressed as g feed/egg,
- Average hen-body weight based on weight of 50 hens per week (only recorded during the start and the top period),
- Cumulative mortality levels at the start and end (expressed as percentage of birds/flock that died),
- Occurrences of smothering events which led to mortality of large number of birds at specific time points (Bright and Johnson, 2011). We used farmer's reports to determine whether, at least once, a smothering event occurred in a particular flock.

4.2.3. BEHAVIOURAL PARAMETERS AT WEEK 40 OF AGE

All behavioural observations were conducted at 40 weeks of age, by one of three observers. Observation methods were brought into conformity with each other prior to the farm visits, by comparing observations between observers. Number of samples and sample size per measurement were based on previous on-farm methodologies (e.g. (Rodenburg et al., 2008b)) and based on guidelines of Welfare Quality® (2009) in order to make reliable predictions for the whole flock.

STATIONARY PERSON TEST

On six places in each chicken house a Stationary Person (SP) test was performed, derived from the Welfare Quality® protocol for on-farm assessment of welfare of poultry (2009). The observer, dressed in clothes similar to that of the farmer, walked through the chicken house at a slow pace (1 m per sec) and ceased walking on six predetermined locations: on the slats or litter area, always under a light source. The test-locations were equally distributed over the chicken house (front, middle and back). When the observer stopped walking, every 10 sec the number of birds within 25 cm distance of the observer was recorded for a total duration of 2 min. Proximity of 25cm was based on the whole bird being in 25 cm of the SP. The latency until the first bird approached the SP and the average number of birds that approached the SP within the test duration were recorded. The average number of birds that approached was calculated by taking the average of all 12 time points. If no bird approached within the 2 min of the test, the maximum test duration was set at 130 sec. The data from all six locations were averaged per flock. Whether any bird approached or did not approach within the 2 min of all the six tests was added to the data as a binary variable

(yes/no). In two ISA flocks no SP test was performed due to practical circumstances.

NOVEL OBJECT TEST

The procedure for the Novel Object (NO) test was comparable to the SP test, derived from the Welfare Quality® protocol for on-farm assessment of poultry welfare (2009). On six different locations in the chicken house the NO test was performed after the SP test had taken place (i.e. each SP test was followed by a NO test, but on a different location in the chicken house). A plastic stick (*length*: 50 cm, *diameter*: 3 cm) covered with coloured tape markings (red, white, green, black and yellow) was used as a NO. For all farms the same NO was used. The NO was placed under a light source either on the slats or in the litter area with an equal distribution of places throughout the chicken house (front, middle and back). After placing the NO, the observer withdrew to a distance of 2 m from the NO and recorded every 10 sec for a maximum duration of 2 min, the number of birds within 25 cm of the NO. We ruled out that hens approached the NO to escape the human observer, by placing the NO on the floor and then retracing our steps until we were 2 m distant from the NO. Hereby, we observed birds which came towards the NO and not away from the observer, but rather coming closer to the observer. The latency until at least three birds approached the NO and the average number of birds that approached were recorded. If no bird approached within the 2 min of the test, the maximum test duration was set at 130 sec. The data from all six locations were averaged per flock. Whether any bird approached or did not approach the NO within the 2 min of all the six tests, was added to the data as binary variable (yes/no).

4.2.4. QUALITATIVE BEHAVIOURAL ASSESSMENT

On two locations in the chicken house a Qualitative Behavioural Assessment (QBA) was performed on flock level, based on the Welfare Quality® protocol for on-farm assessment of welfare of poultry (2009). Scoring was done on a six-point-scale of 20 behavioural expressions (such as, for instance distressed, fearful, relaxed, comfort, positively occupied, see Welfare Quality®, 2009). Low values indicate low levels of behavioural expression, while high values indicate high levels of behavioural expression. The output from both locations was averaged per flock, as no effect of location was found on QBA scores. Two ISA flocks were missing in the QBA assessment due to practical circumstances.

4.2.5. FEATHER DAMAGE SCORE

Feather damage was assessed for twenty hens per flock at 40 weeks of age. Hens were taken individually from the chicken house to an adjacent room. We randomized the location in the chicken house from where hens were taken (left, right and front, middle and floor, slats and nest-boxes). Choice of hen was based on the principle: "choose one hen and take the 2nd closest to that hen". As choice of location where hens were taken from was alternated, and thus randomization of chosen hens was obtained. Feather damage to neck, back and belly were assessed on a three point scale: intact/slight wear (a), moderate wear (b) and featherless areas (c), and summed to give a whole body index. The total score was either 0 (all regions had "a"), 1 (only one "b" led to a total score of 1) or 2 (only one "c" led to a total score of 2) (Welfare Quality®, 2009). We calculated the average of 20 hens' feather damage scores per flock and the proportion of hens per flock with feather damage b or c per region (neck, back and belly) to

discriminate between regions for assessing different types of feather pecking (Savory, 1995).

4.2.6. BLOOD PARAMETERS AT 40 WEEKS OF AGE

Before feather damage scoring, 2.5 mL blood was drawn from the wing vein of the hens (n=20/flock). Flocks were sampled during the late morning (i.e. between 10 and 12 a.m.), approximate 20 minutes after behavioural observations had taken place. Time of catching and post-sampling was recorded to ensure samples were taken within 3 min. Blood was collected in 4 mL EDTA-tubes and stored on ice immediately after blood collection.

BASAL PLASMA-CORTICOSTERONE

For basal plasma-corticosterone (CORT) analysis, 1.4 mL of blood was centrifuged at 5251 RCF for six min to obtain plasma. Plasma was stored at -4 F (-20°C) before corticosterone was analysed at the Department of Biomedical Sciences/Biochemistry at the University of Veterinary Medicine in Vienna (Austria). Plasma (0.5 mL) was extracted with 5 mL diethylether. After evaporation the ether and re-dissolving steroids in assay buffer (0.5 mL), an aliquot (50 µL) was measured in a corticosterone enzyme immunoassay (described in detail by Palme and Mostl, 1997).

WHOLE-BLOOD SEROTONIN

For whole-blood serotonin (5-HT) analysis 1.1 mL of blood was stored at -112 F (-80°C). Blood samples were thawed and serotonin concentration (nmol/mL) was assessed by fluorescence assay. Fluorescence was determined in a Perkin-Elmer 2000 Fluorescence spectrophotometer at 283 and 540 nm after blood samples were prepared; see description by Bolhuis et al. (2009).

4.3. STATISTICAL ANALYSIS

Data were analysed with SAS 9.2. The experimental unit was flock. The general linear model consisted of fixed effect of genotype (DW or ISA), and the linear effect of group size (to test for group size effects) and the interaction between genotype and group size for production parameters, CORT, 5-HT and feather scoring. A backwards regression procedure was applied in case group size or the interaction genotype*group size effects were below a P level of < 0.05 . Effects of genotype and group size (group size bigger or smaller than 5,000) on the occurrences of smothering were tested with a GenMod procedure with a binary distribution and a logit link function. The latency to approach in the SP and NO was not distributed normally, due to a positive exponential distribution (i.e. a large number of data points at the far-extreme). Therefore, we assessed effects of genotype and group size (bigger or smaller than 5,000) on the binary variable (approaching yes/no) by means of a GenMod procedure with binary distribution and logit link function. An exploratory factor analysis with orthogonal rotation was used to determine similarity of (normally distributed) variables in the QBA. Factors with an eigenvalue of > 1 were retained in the analysis. For the effects of physiology and feather damage on production, we used a general linear model with the fixed effect of genotype, and the linear effect of group size, to which – independently – average CORT, average 5-HT or average feather damage were added as co-variables, including their interaction with genotype. Due to lack of variation in behavioural response to the NO and SP in ISA and DW flocks respectively, only within-genotype relationships with production could be assessed for either the NO (DW) or SP (ISA). For the effects of behaviour in the NO and SP on production variables we used a general linear model with the linear effect of group size to which – independently – latency to approach and number

of birds that approached were added. The relationship between production and NO was, thus, assessed within the DW genotype only, and the relationship with production and SP was assessed within the ISA genotype only. Only main effects and interactions with P -value < 0.05 are presented.

4.4. RESULTS

4.4.1. PRODUCTION PARAMETERS

Table 4.1 shows the production parameters per genotype during the start, top and end of the production period. In the top period, DW hens had a lower bodyweight than ISA hens ($F_{1,17} = 13.1$, $P = 0.003$). Egg weight was lower for DW hens in the end period ($F_{1,19} = 4.24$, $P = 0.05$). During the start of the production period DW birds had a lower feed conversion than ISA birds ($F_{1,19} = 8.3$, $P = 0.01$). Feed conversion at the top and end of production was lower in large ISA flocks compared to small ISA flocks while no group size effects were found for DW flocks (top β_{ISA} -39 gr: $F_{1,19} = 8.22$, $P = 0.01$, end β_{ISA} -17 gr: $F_{1,19} = 4.85$, $P = 0.04$). In small ISA flocks mortality levels until week 40 of age were higher than in large flocks, while in DW flocks mortality levels were unaffected by group size (genotype * group size: $F_{1,18} = 5.04$, $P = 0.04$, small flocks with less than 5,000 birds: 5.1% vs. 3.9% for large flocks with more than 5,000 birds). The number of farms in which smothering occurred was higher for ISA flocks than for DW flocks ($X^2_1 = 13.1$, $P = 0.003$, Table 4.1), and higher for small flocks than for large flocks ($X^2_1 = 5.3$, $P = 0.02$, probability of a smothering event occurring: 70% for large flocks vs. 0% for small flocks). Feed intake per animal per day was higher in small flocks compared to large flocks at the start of the production period ($F_{1,19} = 13.8$, $P = 0.002$; small flocks: 129.4 g/animal/day vs. 124.0 g/animal/day). No other

effects of genotype, group size or their interaction on production parameters were found.

Table 4.1. Average production parameters of Dekalb White (DW) and ISA Brown (ISA) parent stock flocks on production parameters at the start (25-40 weeks), the top (week 40) and the end (41-65 weeks) of the laying hen-production period

Age	Production parameters	DW (n=10)	ISA (n=10)	P-value
<i>Start: week 25-40</i>	Hens in lay (%)	93.9	91.9	0.06
	Egg weight (g)	58.2±0.4	58.7± 0.4	0.12
	Hen-bodyweight (g)	1,662.0±33	1,872.0±26	0.24
	Cumulative mortality (%)	4.2	4.9	0.26
	Feed-intake (g/animal/day)	116.0±0.8	120.0±0.8	0.33
	Feed conversion (g feed/ egg)	124.0±1.0^a	131.0±1.7^b	0.01
<i>Top: week 40</i>	Hens in lay (%)	93.3	92	0.12
	Egg weight (g)	60.4±0.8	60.8± 0.4	0.14
	Hen-bodyweight (g)	1,686.0±22^a	1,920.0±18.3^b	0.003
	Feed-intake(g/animal/day)	122.0±1.7	130.0±3.0	0.12
	Feed conversion (g feed/ egg)	136.0±2.3	149.0±3.6	0.002
<i>End: week 41-65</i>	Hens in lay (%)	88.9	88.7	0.19
	Egg weight (g)	60.3±0.8^a	61.6± 0.2^b	0.05
	Cumulative mortality (%)	5.9^a	7.8^b	0.03
	Feed-intake (g/animal/day)	118.6±1.3	119.8±1.2	0.39
	Feed conversion (g feed/ egg)	133.5±2.1	135.1±1.6	0.42
<i>Overall: week 25-65</i>	Occurrences of smothering events (% of farms)	10^a	80^b	0.01

Means in bold with different superscript between columns indicate difference (P<0.05)

4.4.2. BEHAVIOURAL OBSERVATIONS

In only 10% of the DW flocks, birds approached the stationary person (SP) before the test ended, while in 75% of the ISA flocks birds approached before ending of the test ($X^2_1 = 8.4$, $P = 0.001$) (see Table 4.2). No effect of group size was found on response to the SP. Of all the flocks, irrespective of genotype, in which birds

approached, the average latency for the first bird to approach was 78 ± 12 seconds, with on average 2.4 ± 0.5 birds approaching within the duration of the test. In only 30% of the ISA flocks, birds approached the novel object (NO), while in 90% of the DW flock birds approached the NO ($X^2_1 = 10.66$, $P = 0.007$). In small flocks the likelihood that birds approached the NO was smaller than in large flocks ($X^2_1 = 7.49$, $P = 0.006$, likelihood to approach 60% in small flocks vs. 80% in large flocks). Of all the flocks, irrespective of genotype, in which birds approached the NO, the latency for the first three birds to approach was 60.3 ± 10.2 seconds with on average 4.6 ± 1.14 birds approaching within the duration of the test.

Table 4.2. Fear response, feather damage and physiological data of Dekalb White (DW) and ISA Brown (ISA) parent-stock flocks at 40 weeks of age

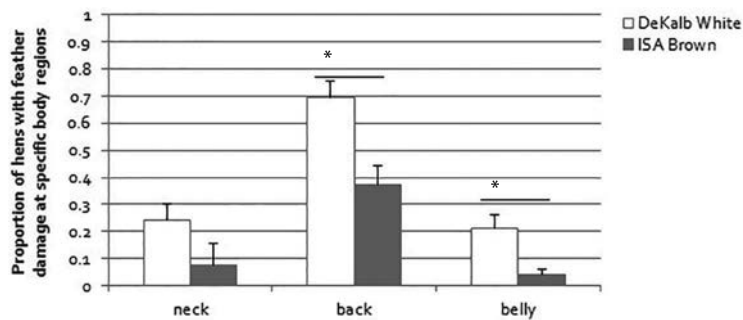
Test variables	DW	ISA	P-value
<i>Stationary person test</i>			
Number of flocks in which hens approached expressed as % of the total flocks (%)	10^a	75^b	0.007
<i>Novel object test</i>			
Number of flocks in which hens approached expressed as % of the total flocks (%)	90^a	30^b	0.001
<i>Qualitative behavioural assessment</i>			
Factor 1 "distressed"	0.6±0.3^a	-0.8±0.2^b	0.002
Factor 2 "comfort"	-0.08±0.2	0.1±0.5	0.69
Factor 3 "active"	0.1±0.4	-0.2±0.3	0.55
<i>Feather damage score</i>			
Average feather damage score (0-2)	1.0±0.1^a	0.5±0.1^b	0.006
<i>Physiological measurements</i>			
Plasma-corticosterone (ng/ml)	1.05±0.1	0.99±0.1	0.76
Whole-blood serotonin (nmol/ml)	53.0±5.3^a	79.1±6.4^b	0.005

Means in bold and with different superscript between columns indicate difference ($P < 0.05$)

4.4.3. QUALITATIVE BEHAVIOURAL ASSESSMENT

Exploratory factor analysis revealed three factors for the QBA (see Table 4.3). Factors were labelled based on the expression that loaded most strongly on the factor, leading to; factor 1 “distressed”, factor 2 “comfort”, factor 3 “active”, explaining respectively 53%, 25% and 7% of the variance. DW flocks had higher scores for the factor “distressed” than ISA flocks ($F_{1,16} = 16.5$, $P = 0.002$) but no differences were found between the two genotypes for the factor “comfort” ($F_{1,16} = 0.16$, $P = 0.69$) or the factor “active” ($F_{1,16} = 0.40$, $P = 0.55$), see Table 4.2. Group size or its interaction with genotype did not affect factor scores.

Figure 4.1. Proportion of 20 hens per flock with feather damage on neck, back and belly region in Dekalb White and ISA Brown Parent Stock laying hen flocks at 40 weeks of age



* indicates $P=0.02$

4.4.5. FEATHER DAMAGE SCORE

Dekalb White hens had a higher average feather damage score than ISA hens ($F_{1,19}=9.83$, $P=0.006$, Table 4.2). This was caused by a higher proportion of DW hens/flock with belly damage ($F_{1,19}=8.1$, $P=0.02$) and back damage ($F_{1,19}=7.0$, $P=0.02$) in comparison to ISA flocks [see Figure 4.1]. Group size or its interaction with genotype did not affect feather damage score.

Table 4.3. Factors based on an exploratory factor analysis of behavioural expressions of the Qualitative Behavioural Assessment

	Factor 1	Factor 2	Factor 3
	Distressed	Comfort	Active
distressed	0.9	-0.2	-0.02
fearful	0.9	-0.4	0.2
scared	0.9	-0.3	-0.2
tense	0.9	-0.3	-0.2
unsure	0.9	-0.3	-0.2
nervous	0.9	-0.3	-0.2
frustrated	0.9	0.1	-0.3
bored	0.9	0.1	-0.4
depressed	0.8	0.2	-0.4
agitated	0.7	-0.4	-0.2
comfort	-0.4	0.9	-0.1
calm	0.2	0.9	-0.2
positively occupied	0.04	0.9	0.2
content	-0.2	0.9	0.1
happy	-0.3	0.8	-0.2
relaxed	-0.3	0.8	-0.14
friendly	-0.4	0.7	-0.3
confident	-0.6	0.6	-0.2
active	-0.3	-0.1	0.9
energetic	-0.4	-0.1	0.8

4.4.6. BLOOD-PARAMETERS

Basal plasma-corticosterone (CORT) did not differ between genotypes ($F_{1, 19} = 0.09$, $P = 0.76$, Table 4.2). Whole-blood serotonin (5-HT) was higher in ISA hens than in DW hens ($F_{1, 19} = 10.0$, $P = 0.005$). Neither group size nor the interaction with genotype affected the blood-parameters.

4.4.7. RELATIONSHIP BETWEEN PRODUCTION PARAMETERS AND FEAR RESPONSE, QBA, FEATHER DAMAGE AND PHYSIOLOGICAL MEASUREMENTS.

The relationships between fear response, QBA, feather damage, physiological measurements and production parameters are given in Table 4.4.

FEAR BEHAVIOUR AND PRODUCTION

Due to lack of variation in the behavioural response to the NO and SP in ISA and DW flocks respectively, only within-genotype relationships with production could be assessed for the NO (DW only) or SP (ISA only). Only when including the ISA flocks that approached, a long latency to approach the SP and a low number of birds that approached the SP was related to high mortality levels at the end of production (latency: $\beta+0.05\%$, $F_{1,5} = 7.70$, $P = 0.05$, number of birds that approached: $\beta-1.12\%$, $F_{1,5} = 7.78$, $P = 0.05$). Only when including the DW flocks that approached, a long latency to approach the NO was related to low egg weight at the top ($\beta-0.03\text{gram}$, $F_{1,8} = 9.53$, $P = 0.02$) and at the end of production ($\beta-0.06\text{gram}$, $F_{1,8} = 15.19$, $P = 0.01$). A long latency to approach the NO was also related to a low bodyweight of DW hens at the top of production ($\beta-1.49\text{gram}$, $F_{1,8} = 9.11$, $P = 0.02$). If more DW hens approached the NO the feed intake per animal was higher at the end of production ($\beta+0.70\text{gram/day}$, $F_{1,8} = 2.40$, $P = 0.05$).

QUALITATIVE BEHAVIOURAL ASSESSMENT AND PRODUCTION

Over genotypes, flocks which had high scores for the QBA factor "comfort" had hens with a higher BW at the top of production than flocks with low scores for this factor ($\beta+2.6\text{gram}$: $F_{1,15} = 4.95$, $P = 0.05$). Of the DW flocks which had high

Table 4.4. Linear effects (positive (+) or negative (-) β -values) of fear-response, Qualitative Behavioural Assessment (QBA) factors, feather damage and physiological measurements on production parameters of Dekalb White and ISA Brown parent stock flocks

Production parameters Behaviour, QBA, Feather damage and physiology	Hen-body weight (g)	Egg weight (g)	Cumulative mortality (%)	Feed intake (g/animal/ day)	Feed conversion (g feed/ egg)
<u>Stationary person test</u> (ISA only)					
Latency to approach (s)	P>0.05	P>0.05	+0.05 ^{end}	P>0.05	P>0.05
Number of hens that approached	P>0.05	P>0.05	-1.12 ^{end}	P>0.05	P>0.05
<u>Novel object test</u> (DW only)					
Latency to approach (s)	-1.49 ^{top}	-0.03 ^{top} -0.06 ^{end}	P>0.05	P>0.05	P>0.05
Number of hens that approached	P>0.05	P>0.05	P>0.05	+0.7 ^{end}	P>0.05
<u>QBA</u>					
Factor 1: Distressed	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Factor 2: Comfort	+2.6 ^{top}	+2.51 ^{top}	P>0.05	P>0.05	P>0.05
Factor 3: Active	P>0.05	P>0.05	P>0.05	-5.7 ^{top} ; -2.3 ^{end}	P>0.05
<u>Feather damage</u>					
Average feather damage/flock	P>0.05	P>0.05	-2.7 ^{start}	P>0.05	P>0.05
<u>Physiological measurements</u>					
Plasma-corticosterone (ng/ml)	P>0.05	-1.55 ^{top} -0.47 ^{end}	P>0.05	P>0.05	P>0.05
Whole-blood serotonin(nmol/ml)	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

QBA=Qualitative Behavioural Assessment with 20 behavioural expressions (Welfare Quality [®], 2009). Factor 1, 2 and 3 were respective factors based on an explanatory factor analysis with the 20 QBA behavioural expressions; P>0.05=P-value >0.05; ^{start} = start of production between 25 and 40 weeks of age; ^{top}= top of production at 40 weeks of age; ^{end}= end of production: between 40-65 weeks of age

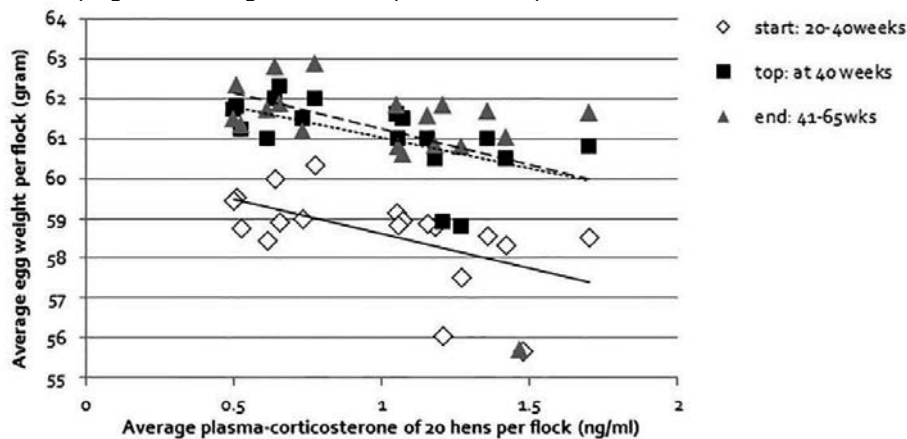
scores for the QBA factor “comfort” also had higher egg weight at the top period ($\beta_{\text{Dekalb White}} + 2.51\text{gram}$: genotype*group size interaction: $F_{1,16} = 7.52$, $P = 0.02$). Flocks with a high score for the QBA factor “active” had lower feed intake per animal at the top and end of production than flocks with a low score (β_{top} -

5.7gram/day: $F_{1,16} = 6.4$, $P = 0.03$ and $\beta_{\text{end}} - 2.3\text{gram/day}$: $F_{1,16} = 6.8$, $P = 0.03$). The QBA factor “distressed” was not related to any of the production parameters.

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High basal plasma-corticosterone was related to low egg weight at the top ($\beta_{\text{top}} - 1.55\text{gram}$: $F_{1,19} = 11.4$, $P = 0.01$) and at the end of production ($\beta_{\text{end}} - 0.47\text{gram}$: $F_{1,19} = 8.8$, $P = 0.01$, Figure 4.2 and Table 4.4). High levels of feather damage in week 40 were related to lower mortality levels during the start of production ($\beta - 2.7\%$: $F_{1,18} = 7.89$, $P = 0.02$).

Figure 4.2. Relation between average plasma-corticosterone and egg weight in parent stock laying hens during the start, top and end of production



4.5. DISCUSSION

Although much research has been done on laying hens, not much is known about their parents. Such knowledge might help to understand their behaviour, stress sensitivity and variation in production parameters. We analysed genotype

differences and group size effects on production, behaviour, feather damage and physiology as well as the relationship among these. For production performance standards for parent stock hens of DW, see: <http://www.isapoultry.com/en/Products/Dekalb/Dekalb%20White.aspx> , and for parent stock hens of ISA, see <http://www.isapoultry.com/en/Products/Isa/Isa%20Brown.aspx>.

4.5.1. PRODUCTION

Dekalb White (DW) parent stock (PS) hens had a lower bodyweight (BW), a lower feed conversion and produced lighter eggs compared to ISA brown PS hens. This is in line with other studies in pure-bred layer lines and commercial lines (Benyi et al., 2006; Singh et al., 2009; Bonekamp et al., 2010; Silversides, 2010). The differences in BW and feed efficiency between DW and ISA hens are most probably due to genetic differences in body constitution and activity patterns between hens derived from a white line and brown line (Luiting, 1990). In small flocks, mortality was higher in ISA flocks than in DW flocks. Hens from a brown line often have higher mortality than hens from a white line, when reared in conventional cages (Singh et al., 2009), housed at point-of-lay in furnished cages (Wall et al., 2008) or in floor systems (Tauson and Abrahamsson, 1996). We also found higher occurrences of smothering in ISA flocks than in DW flocks. It is known by the industry (see management guide for PS: www.isapoultry.com), that hens from brown lines have a stronger tendency to crowd than hens from white lines, which can cause mortality due to smothering. Smothering has been associated with panic and hysteria (Mills and Faure, 1990). In ISA hens social adherence seem to also play a role (www.isapoultry.com). Smothering in or near the nests mostly occurs during onset and peak of lay (Bright and Johnson, 2011), which we also noticed (data not shown). Other forms of smothering can be

observed in the litter area or even in the outdoor run (Sparks et al., 2008; Rodenburg et al., 2012). Smothering occurred most frequently in small flocks. Since density was the same for all flocks, small flocks were housed in barns with a smaller surface than large flocks, providing less space for escaping smothering events. As DW birds have 13% lower BW than ISA birds, the effective stocking density of ISA birds under the same number of hens per m² of DW birds may constrain the actual space per hen, and thereby increase the risk of smothering in ISA flocks. ISA birds also had higher feed conversion and higher mortality in small flocks, which was not the case for DW birds. These results indicate that DW birds were able to cope with varying group sizes while ISA birds appeared to perform better in large flocks. This is in line with findings that hens from a brown line showed more behavioural problems in smaller flocks than in larger flocks (Zimmerman et al., 2006). For both genotypes, feed intake per animal per day was lower in large flocks than in small flocks. Since there was no effect of group size on body weight, this could be related to the increase in activity in large flocks. This is supported by the negative correlation between the QBA factor activity and feed intake. In large flocks there may be more competition for food which leads to increased activity. This in turn may lead to birds eating more often and, probably in smaller portions.

4

4.5.2. BEHAVIOUR, PHYSIOLOGY AND FEATHER DAMAGE

Partially confirming our hypothesis, we found that DW birds in our study showed higher fear of a stationary person (SP), had higher scores for QBA factor “distressed” and lower whole-blood serotonin levels than ISA birds. Hens from a white line are known to be more fearful in various fear tests than hens from a brown line (Mahboub et al., 2004; Fraisse and Cockrem, 2006; Uitdehaag et al.,

2009). Higher fear of humans in DW birds fits with previous findings that cage housed pure White Leghorn hens were more fearful than Rhode Island Red hens when approached by a human holding a novel object (NO) (Fraisie and Cockrem, 2006; Uitdehaag et al., 2008ab). In contrast, DW birds were less fearful of the novel object than ISA birds. In the previously mentioned tests (Mahboub et al., 2004; Fraisie and Cockrem, 2006; Uitdehaag et al., 2009), hens from a white line showed higher fear in tests with human involvement than hens from a brown line. It appears that hens from a white line do not necessarily have higher general fear levels than hens from a brown line, but show higher fear of humans specifically. The finding that the loadings for the QBA factor "distressed" were higher for DW than for ISA flocks also indicate that DW flocks appeared more distressed to the observer than ISA flocks. In summary, DW flocks appear to be more fearful towards a human, which opens the route for improvement of the human-animal relationship.

Unexpectedly, DW and ISA hens did not differ in basal plasma-corticosterone (CORT) levels. For both genotypes, CORT represented basal levels (<1.5 ng/ml) (Cockrem, 2007). White commercial layers differ from brown layers in CORT response but not in basal levels which could be similar for the PS hens in our study (Fraisie and Cockrem, 2006). Whole-blood serotonin (5-HT) was lower in DW hens than in ISA hens. Pure cage-housed WL hens also had lower whole-blood 5-HT than cage-housed RIR hens (Uitdehaag et al., 2011). Activity of the brain serotonergic system shows an inverse relationship with feather pecking (FP) (van Hierden et al., 2002a; 2004a)). Hens from a low FP line (Buitenhuis et al., 2006) and a line selected for low mortality (mainly due to reduced FP and cannibalism) (Bolhuis et al., 2009) also had higher blood 5-HT levels compared to their counterparts. Hens from a white line frequently have more feather damage

than hens from a brown line (Uitdehaag et al., 2006; Biscarini et al., 2010). Our results fit with previous studies both for 5-HT and for feather damage as the DW hens had more feather damage than the ISA hens. Feather damage in PS hens is partly caused by mating, especially damage to the back. However, the higher proportion of hens with feather damage to the belly region in DW flocks points to the presence of vent pecking (Savory, 1995). The prevention of vent pecking may thus require extra attention in white flocks, which is addressed in the management guide for PS management of ISA (www.isapoultry.com).

4.5.3. RELATIONSHIPS BETWEEN PRODUCTION, BEHAVIOUR, PHYSIOLOGY AND FEATHER DAMAGE.

Our expectations were that high fear, corticosterone and feather damage would negatively affect production. Indeed, in DW flocks, a long latency to approach the novel object (NO) was associated with a low egg weight and a low BW. Also, in flocks where many hens approached the NO, the birds feed intake per day was high. Taken together, these results indicate that high levels of fear in a flock may have caused a poorer production performance. As high levels of fear can affect the stress response (de Haas et al., 2012) this may negatively affected production performance (Barnett et al., 1992). In a genetic association study, associations between fear (measured by duration of tonic immobility) and egg-weight were found as well as associations between the response to a NO and growth in male offspring of a White Leghorn and red jungle-fowl cross (Schutz et al., 2004). A freezing response to a NO has also been associated with reduced hen-day egg-production and tendencies were found for a reduced BW (Uitdehaag et al., 2008b). High fear of a NO could affect egg weight and BW by coinciding selection for the same loci. Conversely, if low fear of the NO (high number of birds

approaching) relates to high feed intake, this can positively affect BW and egg weight. Also, flocks which scored high on the QBA factor "Comfort" had a higher hen BW, and in the DW flocks also higher egg-weight. Within and between lines, flocks which were less fearful (relationship between response to NO and BW in DW flocks) and appeared more comfortable (both lines), generally had higher BW than more fearful and less flocks which scored low on the QBA factor "Comfort". In ISA flocks, a long latency to approach the SP and a low number of hens approaching the SP was associated with high mortality levels at the end of production. Fear of humans has been associated with production traits in laying hens (Barnett et al., 1992), but not with mortality. However, in non-beak trimmed hens selected for low mortality, escape attempts waned sooner after a human suddenly appeared in front of their cage than in hens that were not selected for low mortality (Bolhuis et al., 2009). A cause of high mortality within ISA flocks was due to smothering events. Although the cause of these events was unclear, a combination of avoidance of the farmer out of high general fear levels (i.e. seen by high fear of the NO) and social adherence with various underlying causes could have caused smothering. Also for the ISA flocks, the human-animal relationship should be taken into account to reduce the risk of high mortality by reducing fearfulness.

High levels of feather damage were related to low mortality at the start of production. Feather damage can originate from feather pecking (FP) which can lead to mortality due to cannibalism (Savory, 1995). A possible explanation for the unexpected negative relationship between feather damage and mortality is that birds with severe damage or cannibalized birds have been taken out of the flocks before we measured feather damage at 40 weeks. What remains in the flocks are hens with low levels of feather damage in flocks with initial high

mortality due to FP but with a lower density. In PS flocks however, feather damage and associated effects should be further investigated, as the damaging effect roosters have on hens' feather damage cannot be excluded.

We also found that high basal plasma-corticosterone (CORT) was related to low egg weight at the top and end of production. Elevated CORT is associated with enhanced energy expenditure, due to increased protein and lipid metabolism in avian species (Pilo et al., 1985; Shini et al., 2009). In relationship to protein metabolism, high CORT leads to increased net breakdown of protein especially in muscle tissue in broilers (Lin et al., 2006; Mumma et al., 2006; Dong et al., 2007). There are two possible explanations for the relationship between CORT levels and egg weight. First, albumen and yolk weight are reduced by lack of available protein due to enhanced proteolysis caused by high CORT. Albumen comprises 60% of the egg weight and consists mainly of proteins (Moran, 1987). As high CORT intensifies proteolysis, a trade-off for albumen synthesis may take place, causing a reduction in egg weight. For yolk (33% of egg weight), treatment with CORT in zebra finches showed to inhibit yolk precursor production (Salvante and Williams, 2003), which consequently affects yolk synthesis (Moran, 1987). Second, retention time of the egg in the oviduct may be shortened due to high CORT. Hens injected with CORT after ovulation had a shorter interval from injection to oviposition than hens injected with progesterone (Etches and Cunningham, 1976). Time in the oviduct affects egg-shell weight, which comprises 12% of the egg weight (Melek et al., 1973). A shorter time in the oviduct may thus restrict egg-shell development, but also formation of albumen and yolk which are dependent on protein synthesis in the oviduct (Moran, 1987). Thus egg formation may be penalized due to high basal CORT levels. High basal CORT may be high due to high fear levels, and thus possibly indicative of chronic

stress, which should be reduced in order to minimize negative effects on production and welfare of PS flocks. Additionally, since egg weight and maternal CORT levels can also affect offspring quality, including stress sensitivity (Henriksen et al., 2011ab), further research is needed to determine the effect of fear, stress and feather damage in commercial PS flocks on the development of their offspring.

In summary, this is the first study to show that fear, stress and feather damage is associated with production in PS laying hens. DW flocks were generally more fearful of humans than ISA birds. Low levels of fear of a novel object were associated with higher BW and egg weight in DW birds. In ISA birds increased fear of humans was associated with higher mortality. Our study also showed that ISA birds were more at risk to smothering events than DW birds, especially in small flocks, possibly due to social adherence either due to fearful events or other causes. Management of PS flocks should therefore take the human-animal relationship into account and realize that measures can have differential effects for ISA and DW hens in large and small flocks.

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Chapter 5

Parents and early life environment affect behavioural development of laying hen chickens

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ABSTRACT

Severe feather pecking (SFP) in commercial laying hens is a maladaptive behaviour which is associated with anxiety traits. Many experimental studies have shown that stress in the parents can affect anxiety in the offspring, but until now these effects have been neglected in addressing the problem of SFP in commercially kept laying hens. We therefore studied whether parental stock (PS) affected the development of SFP and anxiety in their offspring. We used flocks from a brown and white genetic hybrid because genetic background can affect SFP and anxiety. As SFP can also be influenced by housing conditions on the rearing farm, we included effects of housing system and litter availability in the analysis. Forty-seven rearing flocks, originating from ten PS flocks were followed. Behavioural and physiological parameters related to anxiety and SFP were studied in the PS at 40 weeks of age and in the rearing flocks at one, five, ten and fifteen weeks of age. We found that PS had an effect on SFP at one week of age and on anxiety at one and five weeks of age. In the white hybrid, but not in the brown hybrid, high levels of maternal corticosterone, maternal feather damage and maternal whole-blood serotonin levels showed positive relations with offsprings' SFP at one week and offsprings' anxiety at one and five weeks of age. Disruption and limitation of litter supply at an early age on the rearing farms increased SFP, feather damage and fearfulness. These effects were most prominent in the brown hybrid. It appeared that hens from a brown hybrid are more affected by environmental conditions, while hens from a white hybrid were more strongly affected by parental effects. These results are important for designing measures to prevent the development of SFP, which may require a different approach in brown and white flocks.

KEYWORDS: chickens, parental effects, anxiety, feather pecking, behaviour development

5.1. INTRODUCTION

In mammals, but also in avian and fish species, mothers can affect the behavioural development of their offspring both before and after birth or hatch (e.g. humans (Lumey et al., 2007; Viltart and Vanbesien-Mailliot, 2007), rodents (Weaver et al., 2004; Champagne and Rissman, 2011), fish (Eriksen et al., 2011), wild birds (Groothuis et al., 2005) and domesticated birds (Janczak et al., 2007b); for reviews see: (Brunton and Russell, 2010; Charil et al., 2010; Gudsruk and Champagne, 2011), farm animals (Rutherford et al., 2012), birds (Richard-Yris et al., 2005; Henriksen et al., 2011b)). Mechanisms by which birds may pass information to their offspring are through hormone transfer to the egg (Groothuis and Schwabl, 2008; Henriksen et al., 2011b) and/or via epigenetic pathways (Lindqvist et al., 2007a; Nätt et al., 2009; Goerlich et al., 2012). By these mechanisms the developing embryo may be better prepared for its future environment; this is also referred to as a “predictive adaptive response” (Gluckman et al., 2005; Bateson, 2007). In poultry, yolk-hormone levels can vary according to stressful environmental conditions (Janczak et al., 2009). Exposure to repeated, unpredictable events (Japanese quail (Guibert et al., 2011), domestic chicken (Goerlich et al., 2012)) and daily exposure to humans (Japanese quail (Bertin et al., 2008)) can alter egg-hormone levels. Stress experienced by the hen can also reduce her own body weight (Janczak et al., 2007b) and egg weight (Henriksen et al., 2011a; de Haas et al., 2013a), and in this way influence offspring development too. Such maternal effects may underlie the repeated finding that offspring of stressed birds have higher anxiety levels compared with offspring from non-stressed birds (Janczak et al., 2007a; Janczak et al., 2007b; Davis et al., 2008; Guibert et al., 2010; 2011).

These maternal effects may have important implications for the poultry industry, but have so far been overlooked. In commercial laying hens, feather pecking (FP), the plucking of- and pecking at feathers of conspecifics (Savory, 1995), is a maladaptive behaviour. The severe form of FP (severe feather pecking: SFP) has serious consequences for animal welfare as it causes pain and stress in the recipient and can lead to mortality due to cannibalism. Counter measures against FP, such as beak trimming, adjustments of light intensity or supply of foraging materials (Nicol et al., 2013), are only partially successful and we studied the possibility that maternal effects play a role. The tendency to develop SFP appears to be related to anxiety-related behavioural and physiological traits (Jones et al., 1995; Rodenburg et al., 2004a; Bolhuis et al., 2009; Rodenburg et al., 2009a). For example, chicks which show high anxiety in an Open Field test (social isolation in a novel environment) have stronger tendencies to perform SFP (Jones et al., 1995; Rodenburg et al., 2004a; 2009b; 2013; Uitdehaag et al., 2009). Also, birds with high anxiety levels show high post-stress plasma corticosterone levels whilst having low whole-blood serotonin levels, which were linked to feather pecking tendencies (Bolhuis et al., 2009; Rodenburg et al., 2009a). The predisposition to be more anxious and develop FP has a genetic component, as birds of a white ancestor origin are generally more anxious than birds of a brown origin (Fraisse and Cockrem, 2006; Uitdehaag et al., 2008ac; 2009; 2011; de Haas et al., 2013a). The predisposition for anxiety can be affected by level of stress of the parents (Janczak et al., 2006; Janczak et al., 2007b). Therefore, it is important to assess this relationship under commercial conditions where it can affect millions of laying hens. In the poultry industry, parental flocks (parent stock: PS) are flocks which contain thousands of breeder hens and roosters housed together. They produce a multitude of offspring flocks (rearing flocks) which themselves contain

thousands per flock. Additionally, the housing conditions during the offspring's early life can affect development of behaviour (Rogers, 1995; Rodenburg et al., 2008a) including FP (Gilani et al., 2012; Rodenburg et al., 2013). Factors such as a large group size (Bilčík and Keeling, 2000; Kjær, 2004), a high stocking density (Zimmerman et al., 2006; Rodenburg and Koene, 2007) and a lack of litter or unsuitable litter (Huber-Eicher and Wechsler, 1997; 1998; de Jong et al., 2013) have been shown to increase the development of FP. In this study, we examined in two crosses of laying hens (Dekalb White: DW and ISA Brown: ISA) whether parent stock had an effect on the development of FP and anxiety in their offspring. To understand the relation between parents and offspring, we studied which behavioural and physiological parameters (feather damage, plasma corticosterone levels and serotonin levels) of the parent stock coincided with high levels of SFP and anxiety in their offspring. In addition, we studied how litter supply and housing conditions during rearing affected the development of FP. Commercial PS flocks had an impact on the development of anxiety and SFP in their offspring, especially for the DW hybrid. Litter conditions and housing system also showed to have a substantial effect on SFP and anxiety, especially for the ISA hybrid.

5.2. MATERIAL AND METHODS

As one-on-one relations between parents and offspring cannot be determined under commercial conditions - due to the impossibility of individual recognition within large flocks of birds - data were assessed on flock level for both PS and rearing flocks.

5.2.1. ETHICAL STATEMENT

This study comprises an on-farm longitudinal follow-up study on commercial laying hens, conducted between August 2010 and March 2012, which was approved by the Institutional Animal Care and Use Committee of Wageningen University, The Netherlands (permit number for parental flocks: DEC 2010042, permit number for rearing flocks: DEC 2010083).

5.2.2. PARENT STOCK

EXPERIMENTAL ANIMALS AND HOUSING.

Ten commercial flocks of parent stock (PS) of the rearing company Ter Heerdt BV, Babberich, The Netherlands were studied. Five of these were ISA Brown (ISA) parent stock (white hens, brown roosters) and five were Dekalb White (DW) parent stock (white hens and roosters). ISA Brown PS chickens originate from a Rhode Island Red and a Rhode Island White pure line. Dekalb White chickens originate from two White Leghorn pure lines. The ten PS flocks were situated at 7 different breeding farms, meaning that 3 farms had both hybrids while the remaining had either DW or ISA only. Flocks of different hybrids from the same breeding farm were taken as separate flocks. Rooster/hen ratio was approximate 1:10 for all flocks. Flocks were kept on commercial propagator farms with floor housing, partly slatted floors, and litter. For details on housing see (de Haas et al., 2013a).

MEASUREMENTS.

At 40 weeks of age, levels of feather damage, basal plasma-corticosterone and whole-blood serotonin levels of parental hens were assessed. For a detailed description of the measurements, see (de Haas et al., 2013a). For 20 hens per flock, blood samples were drawn from the wing vein within two min after

capturing the hen. Blood samples were analysed for plasma-corticosterone (CORT) and whole-blood serotonin (5-HT) levels (for details, see de Haas et al., 2013a). Each hen was individually taken from a random location in the chicken house (left or right; front or middle; floor or slats or nest boxes) to an adjacent room. After blood sampling, feather damage on neck, back and belly was assessed, and scored on a 3-point scale: no damage (a), slight damage (b), severe damage (c). Scores per area were summed to give a total body score (Welfare, 2009) between 0 (no damage) and 2 (most severe damage). Fertilized eggs were collected daily and were incubated in a commercial incubator of the hatchery of Ter Heerdt BV, Zevenaar, The Netherlands. Fertilized eggs were collected per farm and hybrid. The pooled data per farm and hybrid are referred to as parent stock (PS).

5.2.3. REARING FLOCKS

EXPERIMENTAL ANIMALS AND HOUSING.

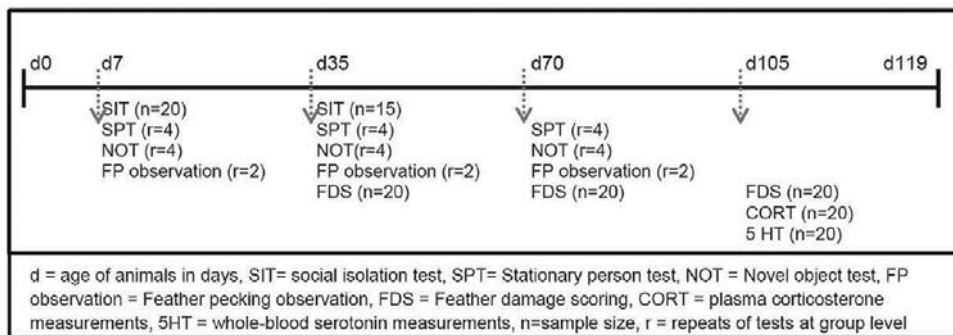
Per PS flock (n=10) between three to seven rearing flocks were studied, of which 23 were DW and 24 were ISA (n=47 rearing flocks in total). The 47 rearing flocks were situated at 25 different rearing farms. Age of the parents at time of incubation varied from 30 to 60 weeks of age, with a majority around 40 weeks. The rearing flocks contained only hen-chicks. At one day after hatch chicks arrived at the rearing farm on which they stayed until approximate 17 weeks of age. All rearing flocks were housed in a tier-system of which 39 flocks were housed in an aviary system and 8 flocks in a floor system to which levels were gradually added (level system). All systems provided tiers, a litter area, slatted area, perches, multiple nipple drinkers and feeding troughs at different levels but no nest boxes or outdoor area. During the first five weeks of life, in the aviary

system adjacent cages were either closed, restricting the number of chicks within the same enclosure (between 30-60), or partly-open (between 30-100). Chicks in the level system were placed in one large flock which varied between 10.000 and 30.000 chicks. Upon arrival, chicks were housed under temperatures ranging between 30 and 33°C with humidity levels between 50 and 65%. Temperature was gradually decreased to approximately 19°C at 10 weeks of age, which was maintained from 10 to 17 weeks of age. Chicks were kept under artificial light either with or without additional LED light with intensities ranging from 1 – 25 LUX measured with a Voltcraft MS-1300 light meter (Conrad Electric Benelux, Oldenzaal, The Netherlands) on bird level. Light regime was a 4-h light/2-h dark cycle for the first seven days of life. After seven days, light regime was adjusted to a 16-h light/8-h dark cycle and light was subsequently decreased gradually from 16 to 9 consecutive hours per day. Each week, one hour of light was removed from the schedule, until 9 hours per day was reached (at 10 weeks of age). Chicks received a commercial diet: mashed starter 1 from one until four weeks of age; semi mashed starter 2 from four until ten weeks of age; and crumbled pre-lay diet from 10 until 17 weeks of age. Chicks were placed within the aviary system on cardboard paper (also called chick paper: (de Jong et al., 2013)) varying from 50 to 90 grams per square meter. This cardboard paper prevented the chicks getting stuck or falling through the mesh wire of the system due to their small body size. It also enabled the accumulation of spilled food, excretions and/or litter and thus provided a foraging substrate. Around five weeks of age, exposure to the litter area within the system was enabled for all flocks. In the aviary system, all walls of the cage tiers were opened and the corridor between tiers became litter area. In the open level system the side walls of the system were opened, and the outside corridor became litter area. Litter supply

could, however, be disrupted from seven to 10 days prior to opening the system by the removal of cardboard paper without additional litter being supplied (hereafter named litter disruption). Farmers use this approach to accustom chicks to their new flooring condition (i.e. wire or plastic surface without cardboard paper). Also, litter supply could be limited by supplying the cardboard paper remnants without additional flooring substrate such as wood-shavings or alfalfa (hereafter named litter limitation). The code of practice of maximum stocking densities was applied, enabling sufficient space per bird in the chicken house. Birds were vaccinated according to the standard vaccination protocol used by the rearing company. Extra specific vaccinations could be requested by the laying hen farm for which the birds were reared.

MEASUREMENTS. At four age points during the rearing period behavioural observations were conducted: week one, five, ten and fifteen weeks of age [see Figure 5.1].

Figure 5.1. Time line of age of birds in days (d) with tests executed at specific ages



ANXIETY RELATED TESTS. Tests related to fear and anxiety were conducted at one, five and 10 weeks of age. Fear of humans was assessed by exposure to either a human arm in their home cage (at one and five weeks of age) or a human standing in the litter area (at 10 weeks of age). In the level system, fear of humans was assessed only by a human standing in the litter area at all ages. Fear of novelty was assessed by exposure to a novel wooden box (5*5*2 cm) with coloured tape (red, yellow, white and green) at one and five weeks of age, and a novel stick with coloured tape (a 50 cm PVC tube with coloured tape) at 10 weeks of age (Welfare Quality, 2009). In both tests, birds were exposed for two min to the human observer and the novel object separately. Every ten seconds, we counted the number of birds within close proximity (i.e. 25cm). For the novel object test, we calculated at which time point at least three birds approached. As birds often did not approach within 25 cm during the human observer test, we estimated the minimal distance in cm of hens that approached over the total test duration. For each flock, tests were repeated four times at different locations in the chicken house (front, middle-front, middle-back, back) always under a light source to limit lack of visibility. A preliminary analysis was performed to assess the effect of location and as location did not affect the latency to approach the novel object or the minimal distance to the human observer, we averaged all values over our four tests. Separation anxiety was measured by a social isolation/novel environment test. Individual chicks, selected from random locations in the chicken house (n=20 in week one, n=15 in week five), were tested. Chicks were positioned inside a round orange bucket (30 cm Ø, with 30 cm height) at one week of age and a round white bucket (40 cm Ø, with 50 cm height) at five weeks of age for a

duration of one min. At five weeks of age a larger bucket was needed to prevent chicks from jumping out the smaller bucket. The observer was out of sight of the chick while testing, but was able to record high pitched vocalizations; i.e. latency to vocalize and number of vocalizations. High pitched vocalizations are referred to as alarm or distress calls (Collias, 1987; Sufka and Hughes, 1991). They are interpreted as an attempt to reinstate contact with conspecifics and as indicating separation anxiety (Warnick et al., 2009).

FEATHER PECKING AND FEATHER DAMAGE.

At one, five and 10 weeks of age feather pecking (FP) behaviour was recorded during two 20-min observations in each flock. For each observation, FP was recorded by means of behaviour sampling at a predetermined location of approximately 1 m² within the chicken house, covering all resources (feeding through, drinking nipples, litter area, tiers and perches). FP was recorded as the frequency of pecks/20 min observation time. Gentle FP (GFP) was recorded as nibbling and gentle feather pecks without a reaction in the receiver, while severe FP (SFP) was recorded as forceful pecks with attempts to pull feathers out to back of the recipient body generally leading to a withdrawal response of the receiver (Savory, 1995; Rodenburg et al., 2013). Aggressive pecks to neck and head were also recorded but due to limited observation numbers, these data were not further analysed. Prior to observations, the observer waited until birds were habituated to her presence by the criterion that 80% of chicks present were not directing their attention to the observer. The number of chicks within the observation area could vary between 15 and 50 chicks due to unrestricted physical boundaries. Feather damage was assessed at five, 10 and 15 weeks of age. At each age point, 20 chicks per flock, chosen selectively from random locations within the chicken house, were assessed for feather damage to the



Picture 5.1 Pictures of measurements and tests conducted of parent stock hens (A and B) and rearing flock chicks (C-F). A: blood sampling for basal plasma-corticosterone and whole-blood serotonin analysis, B: feather damage assessment, C: social isolation/novel environment test conducted at one week of age, D: feather pecking observations conducted at one week of age, E: human arm in home cage conducted at one week of age, F: novel object test conducted at five week of age

neck, back and belly region, similar to feather damage scoring in PS hens (Welfare, 2009). However, the wing and tail area were included as extra areas of measurement using a 0/1 scale, as slight damage to the tips of the feathers in these regions early in life possibly indicates the presence of SFP before severe damage is perceived. Total body score (FS) was the sum of values for all body

regions, similar to the scoring system for PS hens, but damage to the tips of wings was added to the total body score as a value of 0.5.

BLOOD PARAMETERS.

At 15 weeks of age, prior to assessment of feather damage, 20 hens per flock were blood sampled. Samples were always collected around 11-12 a.m. before feeding. An identical procedure was applied for blood sampling and analysis as with the PS hens (for details, see de Haas et al., 2013a). In short, individual hens were chosen selectively from random locations (floor, tier, perch, front and middle) in the chicken house and sampled within two minutes after capture. Blood (2.5-mL) was stored in 4-mL EDTA tubes and immediately put on ice. For whole-blood serotonin (5-HT) analysis, 1.1 mL of blood was pipetted out of the total amount and stored at -80°C . 1 mL of blood was used for analysis (see (Bolhuis et al., 2009) for detailed description). 5-HT concentrations (nmol/mL) were assessed by fluorescence assay and compared with a standard curve of 5-HT stock of increasing dilutions. A Perkin-Elmer 2000 Fluorescence spectrophotometer was used to determine fluorescence at 283 and 540 nm. For basal plasma corticosterone (CORT) analysis, 1.4 mL of blood was centrifuged at $2,095 \times g$ at 21°C for 6 min to obtain plasma. Plasma was stored at -20°C before CORT was analysed at the Faculty of Bio Engineer Science, University of Leuven (Belgium). For the determination of corticosterone concentrations, a competitive radio-immunoassay was performed with the ImmuChem Double Antibody Corticosterone 125I RIA Kit for Rats and Mice of MP Biomedicals LLC (Bio-Connect Diagnostics BV, The Netherlands) with appropriately diluted plasma specimens (for details see (Rodenburg et al., 2009a)).

5.3. STATISTICAL ANALYSIS

Data were analysed with SAS 9.2. For each flock, flock averages were calculated. A general linear model (GLM) included the fixed effects of PS, hybrid (DW vs. ISA) and housing system (open, partly open, closed). For the variables which showed an effect of PS, an additional analysis was conducted to investigate the underlying factors. The average level of CORT, 5-HT and feather damage of the PS hens and age of the PS were added separately as a covariate in the model, which substituted the factor PS, and were tested with its interaction with factor hybrid. For the variables measured from five weeks of age onwards, the effects of limitation of litter (yes/no), disruption of litter supply (yes/no) and the interaction between limitation and disruption of litter supply, and their single interaction with hybrid were added to the model. Post-hoc least square means were used to assess pair-wise differences. Correlations between the residuals of the variables (based on a GLM with PS) were assessed, by hybrid, to determine relations between variables related to anxiety and FP. Plots were examined for outliers to confirm the calculated *R*-values. The normality of the distribution of the residuals was checked, and no transformations were needed. All data is expressed as means \pm SEM.

5.4. RESULTS

5.4.1. PARENTAL EFFECTS

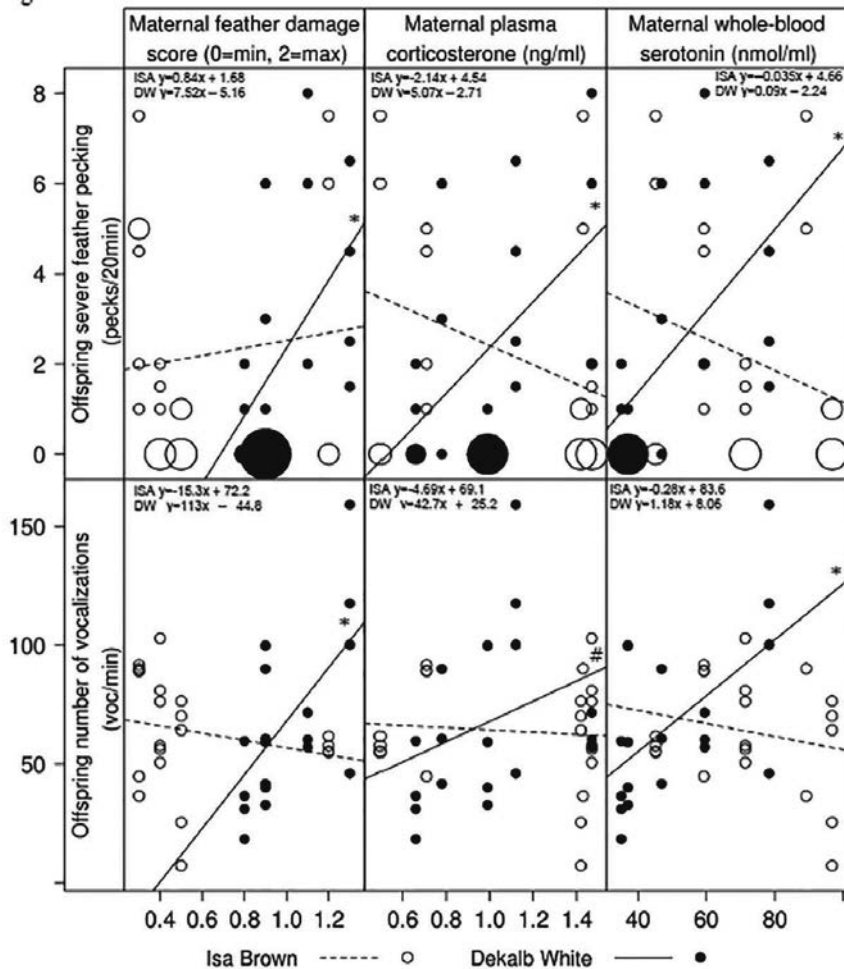
SFP at one week of age was affected by parent stock (PS) ($F_{8, 39} = 4.09$, $P = 0.002$). Additional analysis revealed that for the DW hybrid, but not for the ISA hybrid, offspring' SFP at one week of age was related to high maternal plasma-CORT (CORT*hybrid: $F_{1,39} = 6.25$, $P = 0.02$), high maternal whole-blood 5-HT (5-HT*hybrid: $F_{1,39} = 7.72$, $P = 0.01$) and high maternal feather damage score

(FS*hybrid: $F_{1,39} = 5.02$, $P = 0.03$), see Figure 5.2 [top panel]. For the ISA hybrid, no effects of maternal CORT, 5-HT or feather damage was found on offsprings' SFP at one week of age. PS affected the number of vocalizations in the social isolation test at five weeks of age ($F_{8, 43} = 2.56$, $P = 0.03$) and tended to affect the number of vocalizations at one week of age ($F_{8, 39} = 2.21$, $P = 0.06$). PS did not affect the latency to vocalize at one week ($F_{8, 39} = 0.22$, $P = 0.98$) or five weeks of age ($F_{8, 43} = 1.48$, $P = 0.20$). Additional analysis revealed that for the DW hybrid but not for the ISA hybrid, a high level of vocalizations in the social isolation test at one week of age were related to high levels of maternal whole-blood 5-HT (5-HT*hybrid: $F_{1,39} = 9.18$, $P = 0.005$) and high maternal feather damage (FS*hybrid: $F_{1,39} = 9.16$, $P = 0.005$) and tended to relate to high levels of maternal plasma-CORT (CORT*hybrid: $F_{1,39} = 3.48$, $P = 0.07$) see Figure 5.2 [bottom panel]. High number of vocalizations at five weeks of age were related to high maternal feather damage in the DW hybrid (FS*hybrid: $F_{1, 43} = 5.98$, $P = 0.02$: DW $y = 38.4x - 26.98$). For the ISA hybrid, no effects of maternal CORT, 5-HT or feather damage was found on number of vocalizations of the offspring at one or five week of age. Neither PS age nor its interaction with hybrid affected SFP, or vocalizations in the social isolation test at one week of age (SFPweek1: PS age: $F_{1, 39} = 0.75$, $P = 0.39$, PS age * hybrid $F_{1, 39} = 2.19$, $P = 0.15$; vocalizationsweek1: PS age: $F_{1, 39} = 0.26$, $P = 0.61$; PS age * hybrid $F_{1, 39} = 0.09$, $P = 0.76$). PS did not affect SFP and GFP at five or ten weeks of age, feather damage, fearfulness at any other age.

5.4.2. HOUSING EFFECTS

See table 5.1 for differences and pair-wise comparisons of housing system for FP, fear and feather damage. SFP at ten weeks and GFP at one and ten weeks was

Figure 5.2. Average level of maternal feather damage [left panel], average level of maternal plasma-corticosterone [middle panel] and average level of whole-blood serotonin levels [right panel] with their offsprings' average level of severe feather pecking at week one of age [upper panels] and the number of vocalizations in a social isolation at one week of age [lower panels]



* near line indicates slope $P < 0.05$, # near line indicates slope $P = 0.07$, size of dots represents sample size of a given x, y combination. Equation represents regression line, with y = either level of severe feather pecking (based on average of 2 * 20 min observation/flock) or average number of vocalizations in a one minute social isolation test of 20 chicks/flock, both assessed at one week of age, with x = either average level of maternal feather damage (based on feather damage of 20 hens/flock, sum of damage to neck, back and belly (min=0, max=2) based on Welfare Quality, 2009), average level of plasma corticosterone (based on plasma corticosterone levels of 20 hens/flock), and average level of serotonin (based on whole-blood serotonin levels of 20 hens/flock), expressed by genetic laying hen hybrid (Isa Brown or Dekalb White).

highest, and SFP at five weeks tended to be highest, in the open level system compared to the closed and partly-open aviary system (housing-system effect: SFPweek1: $F_{2,39} = 1.93$, $P = 0.16$, SFPweek5: $F_{2,43} = 2.62$, $P = 0.10$; SFPweek10: $F_{2,45} = 11.55$, $P = 0.002$; GFPweek1: $F_{2,38} = 4.09$, $P = 0.03$, GFPweek5: $F_{2,44} = 0.38$, $P = 0.69$, GFPweek10: $F_{2,45} = 4.48$, $P = 0.02$, see Table 5.1). Feather damage score at ten weeks, but not at five or fifteen weeks, was highest for flocks that were housed in an open level system compared to an aviary system (housing-system effect: FSweek5: $F_{2,45} = 1.81$, $P = 0.18$, FSweek10: $F_{2,45} = 3.14$, $P = 0.05$, FSweek15: $F_{2,42} = 1.26$, $P = 0.30$). At one and five weeks of age, the latency of at least three birds to approach a novel object (NOT) was shortest in the open level system compared to the open and partly-open aviary system (housing-system effect: NOTweek1: $F_{2,39} = 17.02$, $P < 0.0001$, NOTweek5: $F_{2,45} = 4.81$, $P = 0.01$, NOTweek10: $F_{2,43} = 0.65$, $P = 0.53$). In the fear for humans test at one week of age, the effect of housing-system was significant ($F_{2, 39} = 16.7$, $P < 0.0001$: open: $96.1 \pm 26\text{cm}$, closed: $29 \pm 3.2\text{cm}$, partly-open: $23.6 \pm 1.8\text{ cm}$). This effect is, however, an artefact caused by the different spatial dimensions of the systems on the test variable (minimal distance, i.e. the minimal distance can be larger in an open system vs. the other systems purely due to the systems' spatial dimension) and the setting of the test (i.e. in the aviary systems response to a human arm, while in the level system response to a standing person is measured). Therefore, this result is not reported in Table 5.1. Housing system did not affect minimal distance to the human observer at five or ten weeks of age (housing-system effect: SPTweek5: $F_{2, 44} = 0.13$, $P = 0.87$; SPTweek10: $F_{2, 44} = 0.51$, $P = 0.60$).

Table 5.1. Means \pm SEM of response variables of the behavioural tests, feather pecking observations and feather damage scoring of rearing flocks housed in an open, closed or partly-open system

Variables			System		
Tests	Age	Response variables	Open (n=8)	Closed (n=25)	Partly open (n=14)
Stationary person test					
	Week 1	Minimal distance (cm)	-	-	-
	Week 5	Minimal distance (cm)	71.7 \pm 19.2	78.2 \pm 13.7	74.7 \pm 22.9
	Week 10	Minimal distance (cm)	45.9 \pm 17.4	113.7 \pm 17.8	117.5 \pm 22.6
Novel object test					
	Week 1	Latency of 3 birds to approach (s)	33.2 \pm 6.5 ^a	87.6 \pm 6.0 ^b	94.2 \pm 8.0 ^b
	Week 5	Latency of 3 birds to approach (s)	17.2 \pm 2.6 ^a	69.5 \pm 8.5 ^b	68.1 \pm 11.5 ^b
	Week 10	Latency of 3 birds to approach (s)	14.3 \pm 1.7	30.0 \pm 5.6	24.8 \pm 4.6
Social isolation test					
	Week 1	Number of vocalizations/min	55.3 \pm 7.0	70.6 \pm 6.8	61.2 \pm 8.8
	Week 1	Latency to vocalise (s)	9.8 \pm 1.5	11.6 \pm 1.4	10.4 \pm 2.0
	Week 5	Number of vocalizations/min	24.5 \pm 8.6	21.3 \pm 2.7	15.1 \pm 1.5
	Week 5	Latency to vocalise (s)	23.6 \pm 5.1	24.0 \pm 2.8	27.8 \pm 4.0
Feather pecking behaviour (pecks/20 min)					
	Week 1	Gentle feather pecking	24.4 \pm 1.9 ^a	9.7 \pm 1.7 ^b	16.3 \pm 4.1 ^c
	Week 5	Gentle feather pecking	70.6 \pm 27.4	74.8 \pm 17.1	41.9 \pm 9.7
	Week 10	Gentle feather pecking	71.1 \pm 14.6 ^a	23.8 \pm 5.2 ^b	42.9 \pm 11.8 ^c
	Week 1	Severe feather pecking	4.0 \pm 1.0	1.7 \pm 0.5	2.1 \pm 0.9
	Week 5	Severe feather pecking	15.4 \pm 5.8 ^x	9.6 \pm 1.6 ^x	4.0 \pm 1.6 ^y
	Week 10	Severe feather pecking	7.3 \pm 1.9 ^a	1.6 \pm 0.5 ^b	2.2 \pm 0.8 ^c
Feather damage scoring (min=0, max=2)					
	Week 5	Average feather score	0.24 \pm 0.07	0.28 \pm 0.05	0.31 \pm 0.05
	Week 10	Average feather score	0.29 \pm 0.08 ^a	0.23 \pm 0.03 ^b	0.22 \pm 0.05 ^b
	Week 15	Average feather score	0.23 \pm 0.04	0.14 \pm 0.02	0.13 \pm 0.02

Bold number with superscripts a,b,c indicate P-value of <0.05 ; bold numbers with superscripts x,y,z indicate P-value $<0.1>0.05$ (different superscript letters indicate pair-wise differences), "—" indicate non determined effect due to effects of an artefact of the system on the response variable

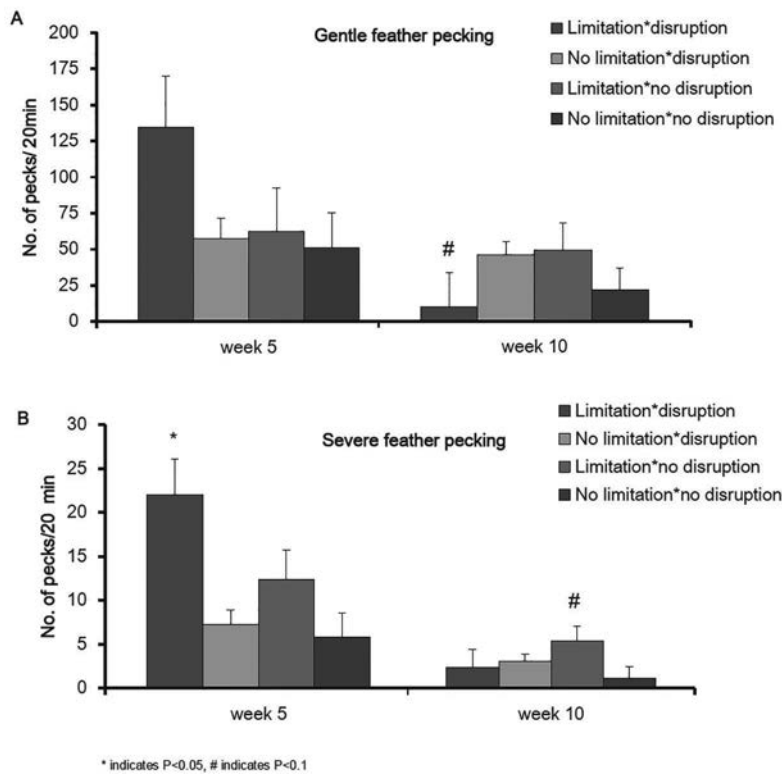
5.4.3. GENETIC EFFECTS

GFP tended to be higher for DW than for ISA birds at one week of age (GFPweek1: $F_{1, 38} = 3.69$, $P = 0.06$: DW: 16.8 ± 3.2 pecks/20 min vs. ISA: 11.4 ± 1.7 pecks/20 min). At five and ten weeks of age GFP did not differ between hybrids (GFPweek5: $F_{1, 44} = 0.11$, $P = 0.73$, GFPweek10: $F_{1, 45} = 0.01$, $P = 0.94$). SFP was not affected by hybrid at one or ten weeks of age (SFPweek1: $F_{1, 39} = 0.00$, $P = 0.97$; SFPweek10: $F_{1, 45} = 1.16$, $P = 0.29$). SFP at week 5 of age was affected by the interaction of hybrid with litter limitation, which will be explained further-on under litter effects. At ten weeks of age, but not at one or five weeks of age, DW birds kept a greater distance to the human observer than ISA birds (SPTweek1: $F_{1, 28} = 0.77$, $P = 0.39$; SPTweek5: $F_{1, 28} = 0.09$, $P = 0.76$; SPTweek10 $F_{1, 28} = 12.15$, $P = 0.002$: DW: 152.9 ± 17.8 cm vs. ISA: 57.9 ± 11.0 cm). Whole-blood serotonin (5-HT) was higher for ISA birds than for DW birds ($F_{1, 44} = 64.03$, $P < 0.001$: DW: 60.8 ± 1.26 nmol/ml vs. ISA: 88.6 ± 2.54 nmol/ml). Plasma CORT was not affected by hybrid ($F_{1, 44} = 0.00$, $P = 0.96$: DW: 1.85 ± 0.06 ng/ml vs. ISA: 2.05 ± 0.15 ng/ml).

5.4.4. LITTER EFFECTS

The combination of both litter disruption and litter limitation resulted in the highest levels of SFP at five weeks of age (litter disruption * litter limitation: $F_{1, 43} = 4.12$, $P = 0.05$, Figure 5.3A) and a similar but non-significant trend for GFP at five weeks (litter disruption * litter limitation: $F_{1, 44} = 1.13$, $P = 0.30$, Figure 5.3B). GFP and SFP at week 10 of age also tended to be affected by the interaction between limitation and disruption (litter limitation * litter disruption: GFPweek10: $F_{1, 45} = 3.12$, $P = 0.08$; SFPweek10: $F_{1, 45} = 3.32$, $P = 0.08$, Figure 5.3 A, B). Limitation of litter alone increased SFP at five weeks in the ISA hybrid but not in the DW hybrid (hybrid * limitation: $F_{1, 43} = 7.36$, $P = 0.01$, see Figure 5.4A)

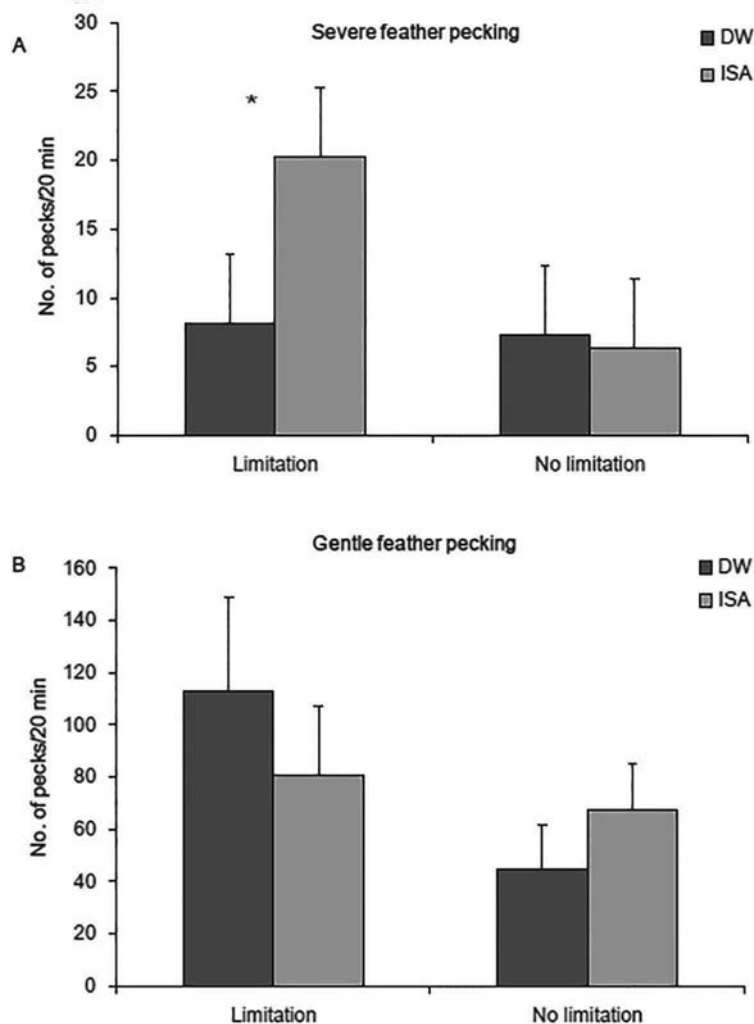
Figure 5.3. (A) Gentle feather pecking at 5 and 10 weeks of age in relation to litter disruption and litter limitation and (B) severe feather pecking at 5 and 10 weeks of age in relation to litter disruption and litter limitation



while GFP did not differ between hybrids (hybrid * limitation: $F_{1,44} = 0.04$, $P = 0.84$, Figure 4B). Disruption of litter alone increased feather damage score at week 5 and 10 but not at 15 weeks of age (disruption: FSweek5: $F_{1, 45} = 18.55$, $P = 0.002$, FSweek10: $F_{1, 45} = 6.55$, $P = 0.02$, FSweek15: $F_{1, 45} = 0.48$, $P = 0.51$, Table 5.2). These effects were most strong for the DW hybrid at five weeks of age

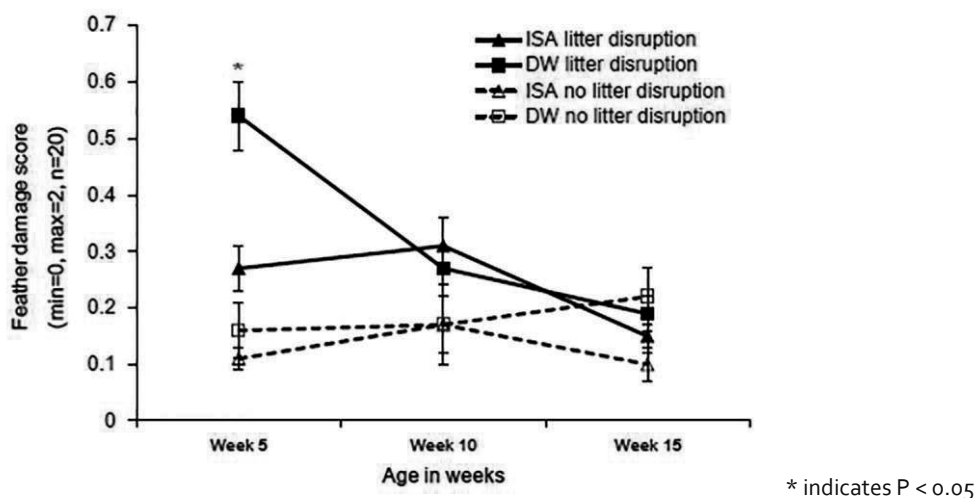
(hybrid * disruption: FSweek5: $F_{1, 45} = 4.21$, $P = 0.05$, FSweek10: $F_{1, 45} = 0.34$, $P = 0.56$, FSweek15: $F_{1, 45} = 0.79$, $P = 0.35$, Figure 5.5).

Figure 5.4. (A) Severe feather pecking at 5 weeks of age in Dekalb White (DW) and ISA brown (ISA) birds in relation to litter limitation and (B) gentle feather pecking at 5 weeks of age in Dekalb White (DW) and ISA brown (ISA) birds in relation to litter limitation.



* indicates $P < 0.05$

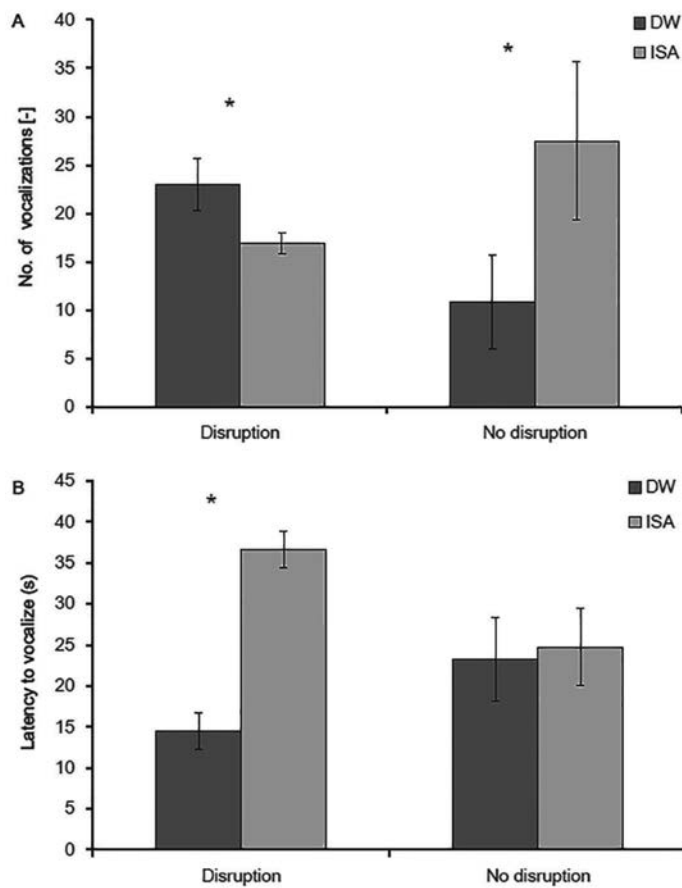
Figure 5.5. Feather damage score of Dekalb White (DW) and ISA brown (ISA) at 5, 10 and 15 weeks of age in relation to litter disruption



Independent of hybrid, in flocks which experienced a litter disruption, birds tended to keep a greater distance to the human observer (litter disruption: $F_{1,44} = 3.00$, $P = 0.09$; disruption: $126.7 \pm 15.7\text{cm}$ vs. no disruption: $63.9 \pm 17.0\text{cm}$) and tended to approach a novel object later (litter disruption: $F_{1,43} = 3.78$, $P = 0.06$, disruption: $31.1 \pm 5.0\text{ s.}$ vs. no disruption: $17.0 \pm 2.4\text{ s.}$) in comparison to flocks that did not experience litter disruption. Whole-blood 5-HT was higher when litter was disrupted then when litter was not disrupted (litter disruption: $F_{1, 44} = 4.24$, $P = 0.05$; disruption: $64.2 \pm 3.6\text{ nmol/ml}$ vs. no disruption: $57.0 \pm 3.5\text{ nmol/ml}$). Plasma-corticosterone was not affected by litter supply (litter disruption: $F_{1, 44} = 0.49$, $P = 0.48$, litter limitation: $F_{1, 44} = 0.18$, $P = 0.67$). Disruption in access to litter affected the response to social isolation at five weeks differently between the hybrids; ISA birds that had a disruption in litter supply vocalized less than ISA birds that did not have a disruption in litter supply (hybrid * disruption: $F_{1, 43} =$

4.08, $P = 0.05$) and had a longer latency to vocalize (hybrid * disruption: $F_{1, 43} = 3.63$, $P = 0.04$) while the opposite was the case for the DW birds [Figure 5.6].

Figure 5.6. (A) Number of vocalizations upon social isolation at 5 weeks of age in Dekalb White (DW) and ISA Brown (ISA) chicks in relation to litter disruption and (B) latency to vocalize upon social isolation at 5 weeks of age in Dekalb White (DW) and ISA Brown (ISA) chicks in relation to litter disruption.



* indicates $P < 0.05$

Table 5.2. Feather damage score at week five, ten and fifteen of age in relation to litter disruption

Feather damage score	Disruption	
	Yes	No
week 5	0.45(0.06) ^a	0.18(0.05) ^b
week 10	0.29(0.06) ^a	0.15(0.06) ^b
week 15	0.16(0.03)	0.15(0.03)

Numbers with subscripts a,b indicate P-value of <0.05

5.4.5. RELATIONS BETWEEN ANXIETY AND FEATHER PECKING

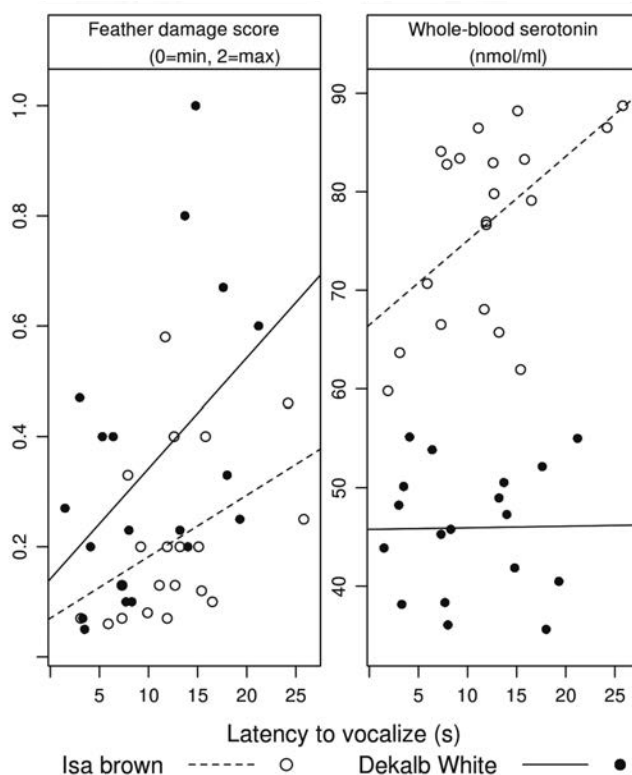
For both hybrids, average feather damage score at five weeks was higher when the latency to vocalize in the social isolation test at one week of age was higher ($r = 0.46$, $P < 0.003$, Figure 5.7). In the ISA birds, whole-blood serotonin levels were higher if the latency to vocalize in the social isolation test at one week was higher ($r_{ISA} = 0.67$, $P < 0.001$, Figure 5.7), but this was not significant in the DW birds ($r_{DW} = 0.22$, $P = 0.37$). As 5-HT was higher for birds which experienced a litter disruption, we assessed the correlation within litter disruption groups within the ISA hybrid. For litter disruption the correlation between 5-HT and vocalizations at one week was positive ($r_{\text{limitation}} = 0.72$, $P = 0.02$), while without litter disruption the correlation was not significant ($r_{\text{no limitation}} = 0.17$, $P = 0.70$).

5.5. DISCUSSION

This is the first on-farm study in which maternal effects on the behavioural development of offspring are described for laying hens. We explored and examined which maternal and environmental effects act on the development of feather pecking (FP) from one until fifteen weeks of age in two hybrids: Dekalb White (DW) and ISA Brown (ISA). As FP is related to anxiety (Jones et al., 1995;

Rodenburg et al., 2004a), we also assessed this relationship under commercial conditions.

Figure 5.7. Average level of feather damage score at five weeks of age [left panel] and average level of whole-blood serotonin at 15 weeks of age [right panel] related to the average latency to vocalize in a social isolation at one week of age in flocks of Dekalb White (DW) and ISA Brown (ISA) laying hens.



* near line indicates slope $P < 0.05$. Equation represents regression line, with y = average latency per flock to vocalize in social isolation test at one week of age based on 20 chicks/flock, with x = either average level of feather damage per flock at five weeks of age on 20 hens/flock, sum of damage to neck, back and belly (min=0, max=2) based on Welfare Quality, 2009, and average level of whole-blood serotonin (based on 20 hens/flock), expressed by genetic laying hen hybrid (Isa Brown or Dekalb White).

5.5.1. MATERNAL EFFECTS

In the DW hybrid, high maternal plasma-corticosterone (CORT), whole-blood serotonin (5-HT) and feather damage were positively related to offsprings' severe FP (SFP) at one week of age and offsprings' vocalizations upon social isolation at one and five weeks of age. The latter are indicative of fearfulness and anxiety (Gallup and Suarez, 1980; Faure et al., 1983; Suarez and Gallup, 1983). These results suggest that within the DW hybrid, maternal state can affect behavioural development of the offspring and thereby cause high fearfulness (Gallup and Suarez, 1980; Suarez and Gallup, 1983) and SFP. These maternal effects may derive from high levels of stress (affecting CORT) and feather pecking in the maternal birds (affecting 5-HT and feather damage, for details see (de Haas et al., 2013a). Offspring of mothers with high CORT have repeatedly shown to have high levels of fearfulness (hens (Janczak et al., 2007b), quail (Bertin et al., 2008; Bertin et al., 2009; Houdelier et al., 2011) and emotional reactivity (hens (Nätt et al., 2009; Goerlich et al., 2012), quail (Guibert et al., 2010; 2011; 2013). Altered deposition of nutrients and hormones in the egg may underlie the maternal effects we found (for review see (Henriksen et al., 2011b)). High CORT of the mother, due to living in a stressful environment, can affect yolk-hormones such as testosterone (Schwabl, 1997; Janczak et al., 2007b; Guibert et al., 2010; 2011; 2013), progesterone (Bertin et al., 2008; Guibert et al., 2013) and oestrogens (Janczak et al., 2009; Nätt et al., 2009) which can influence offspring behaviour (Groothuis and Schwabl, 2008; Henriksen et al., 2011b). Additionally, high maternal CORT has been related to low egg weight (Henriksen et al., 2011a; de Haas et al., 2013a) and chick weight post hatch (Janczak et al., 2007b; Bertin et al., 2008; Goerlich et al., 2012). It is known that ISA and DW birds with high maternal CORT induced by CORT implants differ in yolk-steroid levels and yolk-

mass (Henriksen et al., 2013). Offsprings' fearfulness and SFP in our study may thus be influenced by egg hormone and nutrient content as affected by maternal physiology. In the present study these maternal effects depended on genotype. Breed-dependent differences in epigenetic programming (similar gene-expression patterns over generations and other non-genetic inheritable traits, see reviews on epigenetic studies in mice and chickens (Champagne, 2010; Curley et al., 2011; Champagne, 2012; Berghof et al., 2013)) have also been identified as a putative mechanism of maternal effects (Lindqvist et al., 2007a; Nätt et al., 2009; Goerlich et al., 2012). These epigenetic changes may even be induced by altered egg-hormone content (Nätt et al., 2009; Ho and Burggren, 2010; Guibert et al., 2013). Differences in genetic and epigenetic inheritance between laying hen lines may be the reason why we only recorded maternal effects in the DW hybrid and not in the ISA hybrid.

5.5.2. ENVIRONMENTAL EFFECTS

Housing effects. In the open level system, chicks had a shorter latency to approach a novel object, but also had the highest gentle FP (GFP) at one and ten weeks of age, highest SFP at five (a tendency $P < 0.1$) and ten weeks of age, and highest feather damage at ten weeks of age compared to chicks housed in a closed or partly-open aviary system. Although GFP and SFP originate from different behavioural needs (Savory, 1995; Rodenburg et al., 2013) and involvement of different genes (Buitenhuis et al., 2004) and gene-expression patterns (Hughes and Buitenhuis, 2010), one does not necessarily lead to the other (Rodenburg et al., 2004a; 2013 Newberry et al., 2007), but the co-existence of both may result in feather damage. In the open level system chicks are placed together with thousands of other individuals inside a large area from day one. In

both aviary systems group size is substantially smaller than in the level system as the (partially) closed walls of the aviary system limit the space nor group size to extent to over hundreds. Effects of system are therefore likely to partly be group size related. Social transmission of behaviour (Tolman, 1964; Nicol, 1995), such as the approach of novel objects and FP, may have occurred more readily in a large group (Croney and Newberry, 2007) as there are more birds from which to copy and synchronize behaviour. Previous studies suggest that FP is socially transmitted within a group (SFP (Zeltner et al., 2000; McAdie and Keeling, 2002; Uitdehaag et al., 2009), GFP (McAdie and Keeling, 2000; 2002; Riedstra and Groothuis, 2002). In the closed aviary system we recorded a peak in GFP at five weeks of age. GFP appears to stem from social exploration (Savory, 1995; Riedstra and Groothuis, 2002) and presumably underlies this result. Birds are mixed at around four to five weeks, and this may elicit social exploration, which presumably would have already occurred in the other systems. These results indicate that housing system (possibly related to differences in group size which affect social exploration and social transmission) influences the development of FP and feather damage on-farm.

Litter effects. Litter disruption (taking away foraging substrate for a period of 7-10 days) and litter limitation (limited supplementation in the form of remnant of chicken paper) had a substantial effect on FP and fear responses (Table 5.3). Especially at five weeks of age, disruption of litter led to high SFP, GFP and feather damage. During litter disruption, three factors are at play: 1) disturbances by the farmer, who takes out cardboard paper, handles and mixes birds, 2) removal of cardboard paper and thus removal of foraging material, and 3) disrupted uptake of fibres or excretions from cardboard paper.

Table 5.3. Effects of litter supply (litter disruption and litter limitation) on feather pecking, fearfulness and whole-blood serotonin in laying hens

Effects of litter supply	Severe feather pecking	Gentle feather pecking	Feather damage	Stationary person test	Novel object test	Social isolation test	Whole-blood 5-HT	Correlations
Disruption			↑wk. 5** ↑wk. 10 [#]	↑ week 10 [#]	↑ week 10 [#]	↑ wk. 5 (ISA)*	↑ week 15*	5-HT at 15 weeks & social isolation test at 5 weeks $r=0.67_{**}$
Limitation	↑ week 5 (ISA)*	↑ week 5*						
Disruption* limitation	↑ week 5* ~ week 10 [#]	↑ week 5 ^{NS}						
Effects displayed in:	Figure 5.3	Figure 5.3	Figure 5.5	text	text	Figure 5.6	text	Figure 5.7

↑ Increase due to described effect, ~ effects dependent on interaction, NS: not significant, ** indicates effects $P < 0.01$, * indicates effects $P < 0.05$, # indicates effects $P < 0.1$, (ISA) effects only valid for ISA hybrid, r = correlation coefficient, week = age of hens in weeks

The first factor, disturbance by the farmer, may elicit stress related to fear of humans, as indicated by the greater distance to the human observer in flocks in which access to litter was disrupted. Additionally, absence of litter may induce frustration which can result in SFP (Rodenburg et al., 2004b). The act of SFP itself (pecking and pulling feathers) causes distress in the victims i.e. withdrawal, escape attempts, vocalizations (Rodenburg et al., 2013) and can lead to disturbances in the flock (Koene et al., 2001; Bright, 2008). Taken together, litter disruption can, either directly or indirectly via SFP, increase a flock's fear level.

The second factor, removal of foraging substrate, has most probably the largest influence on the occurrence of FP. FP is considered redirected foraging pecking (Blokhuis, 1986), and increases when foraging material is limited (Nicol et al., 2001b; Gilani et al., 2012), especially at an early age (Huber-Eicher and Wechsler, 1998; de Jong et al., 2013). As said before, lack of foraging material can

induce frustration when the need to forage is thwarted (Rodenburg et al., 2004b) and results in SFP which in turn can lead to feather damage, as shown in this study. On top of litter disruption, a subsequent limitation of litter brought an additive effect in the development of SFP. At any given time, foraging material is important in prevention of SFP (Nicol et al., 2001b). Feather damage seemed to reduce when birds age, irrespective of litter supply. This may be influenced by the molting periods around 10 weeks of age (Savory and Mann, 1997a), making loose feathers available for ingestion from the floor (Harlander-Matauschek et al., 2007a; 2009). Feather pecking during early rearing, as affected by litter supply, may however still yield a risk of later outbreaks of feather damage during lay (Bright, 2009; Rodenburg et al., 2013).

The third factor, lack of uptake of fibres or excretions, probably affected the level of 5-HT, as our study shows increased whole blood 5-HT levels in flocks with litter disruption. Litter (often wood shavings, alfalfa or remaining cardboard paper) contains fibres, excretions and feather particles. Uptake of these large particles can stimulate gut motility (Amerah et al., 2007), alter gut micro biota (Meyer et al., 2012; Meyer et al., 2013) and activate immunity in various ways (Mossner and Lesch, 1998). Particularly feather eating, which is linked with FP (Harlander-Matauschek et al., 2006a; 2007ab) has been associated with increased gut motility (Harlander-Matauschek et al., 2006b). The enterochromaffin cells in the gut contain 5-HT which are released upon stimulation of the intestinal tract (Ebert-Zavos et al., 2013). As a result of a temporary lack of litter birds may have a strong need to forage, possibly enhancing feather and litter uptake afterwards as over-compensation (Harlander-Matauschek et al., 2009) which altogether affects 5-HT release. Our study shows that whole-blood 5-HT can be influenced by litter disruption.

In the ISA hybrid, especially under disruption of litter, a positive correlation between fear-response at one week of age and 5-HT at fifteen weeks of age was detected. In a previous study, in Rhode Island Red birds (RIR), one of the pure lines of ISA, the correlation between fear responses and brain 5-HT was also dependent on the environment. RIR birds mixed with birds of another line showed a negative correlation between fear and 5-HT while RIR birds which we kept in non-mixed groups showed a positive correlation (Uitdehaag et al., 2011). The positive correlation between fear-responses and 5-HT under litter disruption in our study could be influenced by effects of mixing and substrate intake but probably also by the high levels of SFP occurring under litter disruption. 5-HT activity has been suggested to relate to the development of SFP (Rodenburg et al., 2013) (brain 5-HT young (van Hierden et al., 2004a) and adult birds (Kops et al., 2013a), peripheral 5-HT (Bolhuis et al., 2009; Rodenburg et al., 2009a) and both brain and peripheral 5-HT (Uitdehaag et al., 2011). Both brain and peripheral 5-HT have also been associated with fearfulness (Bolhuis et al., 2009; Rodenburg et al., 2009a; Kops et al., 2013b), and, in our study, peripheral 5-HT was influenced by litter disruption. Within the ISA hybrid, disruption of litter also caused higher anxiety in the social isolation test at five weeks of age. ISA birds appear to be more strongly affected by their (social) environment than DW birds (Uitdehaag et al., 2011). In comparison to other hybrids, birds from a brown origin (in the PS (de Haas et al., 2013a) and pure lines (Uitdehaag et al., 2006; 2008a) repeatedly show higher fear in response to social isolation (Hocking et al., 2001; Ghareeb et al., 2008a; Ghareeb et al., 2008b) and novel items in their home environment. ISA birds are also more affected by social factors such as group size (de Haas et al., 2013a) and mixing (Uitdehaag et al., 2008c; 2009) than DW birds. Taken together with other studies, it appears that ISA birds are more strongly

affected by their (social) environment in comparison to DW birds that are more sensitive to maternal effects.

5.5.3. FEAR AND FEATHER PECKING

For both hybrids we found that latency to vocalize during social isolation at one week of age was related to feather damage at five weeks of age, which complements the relationship between anxiety traits in social isolation tests and FP (Jones et al., 1995; Rodenburg et al., 2004a). This may also explain why we still see FP under optimal conditions with regard to litter. In DW birds, fear of humans was higher than in ISA birds, which was similar to the study of the PS (de Haas et al., 2013a). DW birds are more easily frightened by exposure to humans (Uitdehaag et al., 2009) as indicated by higher fear-responses and plasma-CORT after human handling (Fraisse and Cockrem, 2006; Uitdehaag et al., 2006; 2009; 2011). DW birds also have relatively low levels of whole-blood 5-HT compared to ISA birds (shown in this study, in the PS (de Haas et al., 2013a) and pure lines (Uitdehaag et al., 2011), which may represent a risk in the development of FP (Rodenburg et al., 2013). In addition, the maternal effects on fearfulness in early life may predispose DW birds to develop SFP. The predisposition to develop FP may thus stem from different origins depending on genotype.

5.6. CONCLUSIONS

This study shows for the first time that maternal effects in commercial laying hens play an important role in early life behavioural development of their offspring. Our study indicates that maternal stress can create a risk for the development of anxiety and maladaptive behaviour such as feather pecking (FP) in laying hens. These maternal effects depend on genotype, with birds from a

White Leghorn origin being sensitive. Litter availability is of utmost importance for laying hens, and reduced the risk of FP, especially for birds from a Rhode Island Red origin that also become more anxious and fearful as a result of disruption in litter supply. These results provide new knowledge that is important for preventing the development of anxiety and FP in laying hens.

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Chapter 6

Is it the past or is it the present? Effects of the rearing environment and laying environment of feather damage in commercially housed laying hens

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ABSTRACT

Feather damage due to severe feather pecking (SFP) is most severe during the laying period. However, SFP can develop from an early age and is influenced by early rearing conditions. In this study we combined information about the rearing and laying periods to assess risk factors influencing the level of feather damage at 40 weeks of age. As SFP is known to be related to high fearfulness which can differ between crosses, we also assessed the relationship between fearfulness and SFP in two commercial crosses: ISA brown and Dekalb White. Feather damage, FP and feather eating during rearing were identified as risk factors for feather damage during laying, but did not always lead to severe damage indicating that management on the laying farm also plays an important role. High levels of feather damage at laying were associated with high SFP levels at five weeks of age and elevated fear of humans. During laying, feather damage was more severe in floor housing compared to aviary housing, while an adjusted management (radio, pecking blocks, round drinkers and/or roosters) reduced feather damage compared to standard management. A large group size and fear of humans at laying was associated with higher levels of feather damage than a small group size. Approximately, 49% of the laying flocks and 60% of the rearing flocks in this study showed high SFP or feather damage. This high incidence emphasizes the severity of the problem and the importance of finding a solution. The results of this study may aid in providing practical solutions to this serious animal welfare problem.

KEYWORDS Laying hens, Feather pecking, Rearing, Laying, Litter, Genotype

6.1. INTRODUCTION

In laying hens, severe feather pecking (SFP) - the plucking and pecking of feathers of conspecifics resulting in feather damage and mortality (Savory, 1995) - still remains a serious welfare problem in commercial enterprises despite the large number of studies focusing on the prevention of the behaviour (Nicol et al., 2013; Rodenburg et al., 2013). Outbreaks occur mostly during laying (Newberry et al., 2007; Bright, 2009) but SFP and consequent damage can develop already at an early age (Blokhuys and van der Haar, 1992; Johnsen et al., 1998b; Riedstra and Groothuis, 2002; de Haas et al., 2014). The percentage of laying flocks affected by SFP ranges from 65% (Gilani et al., 2013), 69% to 86% in free range systems (Lambton et al., 2010) and organic systems (Bestman and Wagenaar, 2003), respectively. The percentage of rearing flocks affected by SFP ranges from 27% in free range systems (Gilani et al., 2013) to 54% in organic rearing systems (Bestman et al., 2009), whereas the prevalence in other systems is unknown due to the limited number of studies (de Jong et al., 2013). In approximately 90% of the cases in which feather damage is observed during rearing, feather damage also occurs during laying (Bestman et al., 2009; Gilani et al., 2013).

Feather pecking occurs more frequently when a foraging substrate, i.e. litter, is absent (Huber-Eicher and Wechsler, 1997; Huber-Eicher and Sebo, 2001b). As such, litter absence during early life may have a strong influence on the development of SFP during rearing (Blokhuys and van der Haar, 1992; Johnsen et al., 1998b; Nicol et al., 2001b; Gilani et al., 2013; de Haas et al., 2014). Absence of litter during early life does, however, not always lead to SFP or feather damage during the laying period when litter is adequately available to the adult birds (Nicol et al., 2001b; de Jong et al., 2013). Moreover, the availability of litter during lay strongly affects the occurrence of SFP at laying (Pötzsch et al., 2001; Nicol et

al., 2003; Lambton et al., 2010), indicating that the need of hens to forage can lead to SFP at any time when this behaviour is thwarted. Litter absence during early life may, however, result in a preference for pecking at feathers rather than pecking at litter. Under suboptimal conditions such as a high light intensity, this environmentally-induced predisposition may contribute to the development of SFP (Kjær and Vestergaard, 1999; de Jong et al., 2013).

Feather pecking is a multifactorial problem (Rodenburg et al., 2013). Apart from an insufficient substrate availability, high prevalence of SFP has been recorded when hens were housed in large flocks (Zimmerman et al., 2006; Bestman et al., 2009; Lambton et al., 2010), under high stocking densities (Nicol et al., 2006), under high light intensities (Mohammed et al., 2010), when fed pelleted food (van Krimpen et al., 2005; Lambton et al., 2010), or given immunological challenges (Parmentier et al., 2009). These unfavourable conditions may impose stress in the hens. High fearfulness has also been associated with SFP (Jones et al., 1995; Rodenburg et al., 2004a) and an elevated sensitivity to stress (de Haas et al., 2012), which makes animals more likely to have difficulty coping with challenges. For example, hens are adapted to living in small flocks of eight to ten individuals, which is the flock size observed under natural conditions (Collias, 1996), and housing in large flocks is therefore likely to be challenging for the birds. Additionally, genetic selection for egg production may have caused strain-dependent differences in the ability to cope with challenging (social) conditions. Supporting this, there are many studies indicating that commercial lines originating from a White Leghorn (WL) or Rhode Island Red (RIR) genetic background differ in levels of fearfulness, stress sensitivity, and the propensity to develop SFP (Hocking et al., 1999; Hocking et al., 2001; Uitdehaag et al., 2008a; 2009; 2011; de Haas et al., 2013a; 2014). We have recently shown that hens

originating from a RIR line are more strongly affected by social factors such as large group size (de Haas et al., 2013a) and mixing with other genotypes (Uitdehaag et al., 2009; 2011) than hens from a WL line.

In this prospective longitudinal study, our aim was to assess which factors during rearing and laying affect feather damage at laying (i.e. at 40 weeks of age), under commercial conditions in non-cage systems in two commercial crosses originating from a WL and RIR origin. In The Netherlands, non-organic laying hen farms constitute over 90% of all laying hen production (CBS, 2013a; b). Beak treatment is currently still applied in these systems to reduce damage due to SFP, but will be prohibited in The Netherlands from 2018 onwards. This makes it extremely important to find a practical solution to limit the development of SFP in commercial flocks.

6.2. MATERIAL AND METHODS

All hens in this study originated from the Ter Heerdt hatchery and rearing company, Zevenaar, The Netherlands (www.broederijterheerdt.nl) and were reared by the same company on contract rearing farms. Chicks' beaks were infrared treated and chicks were vaccinated at the day of hatching by the hatchery. This study was conducted between August 2010 and August 2012 on farms in The Netherlands and Germany. Approval of the Animal Care and Use Committee of Wageningen University was given for rearing and laying flocks (permit number for rearing flocks: DEC 2010083; permit number for laying flocks: DEC 2010042). For both rearing and laying farms, the codes of practice for maximum stocking densities were applied enabling sufficient space per hen. For an overview of housing factors of both rearing and laying farms, see Table 1.

6.2.1. REARING FARMS

Thirty-five flocks situated at 19 rearing farms were followed until 40 weeks of age. These flocks were a subset of a sample of 45 flocks, which we studied only during the rearing period (de Haas et al., 2014). Thirteen flocks were of an ISA brown cross, originating from a RIR origin, and 22 flocks were of a Dekalb White cross, originating from a WL origin. Chicks/pullets stayed on the rearing farm from one day of age until 17 weeks of age. Three flocks were kept in a level system and 32 flocks were kept in an aviary system (Table 6.1). The aviary system consisted of a tiered system with a litter area between the tiers. The first four to five weeks of life chicks were locked in the tier system. The level system consisted of elevated platforms above a main middle platform and a litter area around the main middle platform, for details see (de Haas et al., 2014). Chicks were housed on cardboard paper until approximately 3.5 weeks of age onwards inside the tiers or on the main platform of the level system. Housing on cardboard paper prevented the chicks falling through the mesh wire of the system due to their small body size. Access to the litter area was enabled from approximately four to five weeks of age onwards. Some farmers removed the cardboard paper and left chicks inside the system without providing additional substrate, we recorded this practice as "litter disruption". Litter provided by the farmers was either wood shavings, alfalfa or cardboard paper remnants. In case of only supplying paper remnants or small amounts of litter, we recorded this practice as "litter limitation". All flocks were housed under artificial light. Light intensities were measured at bird level at one, five and 10 weeks of age with a Voltcraft MS-1300 light meter (Conrad Electric 124 Benelux, Oldenzaal, The Netherlands). We recorded an increase or decrease in light intensity over ages, by comparing minimal light intensities between ages. Commercial feed was provided by one of

four Dutch feed suppliers. From one until four weeks of age, chicks received starter¹ mashed-diet. From week five till 10, pullets received starter² semi-mashed diet. From week 10 till 17, a pre-lay diet in the form of mash, pellets and grains was provided. Chicks received multiple vaccinations in accordance to a pre-set vaccination schedule advised by the rearing company. Thirteen flocks received additional medical treatments (Table 6.1). In eight farms there were sound influences from a radio or children playing inside the chicken house.

6.2.2. LAYING FARMS

At 17 weeks of age, pullets were transported to the laying farms. All laying farms were visited around 40 weeks of age. For details on laying factors see Table 6.1. All rearing flocks were placed at different laying farms, except for three flocks which were placed at the same laying farm. Farms either had an aviary system or a floor housing system. Both systems provided perches, a litter area and nest-boxes. The aviary system consisted of tiers with in between tiers a litter area. The floor system consisted of a middle platform with litter area around the platform. Most farmer supplied litter when flocks arrived at the farm, although eight did not. Litter quality was assessed qualitatively. Litter was assessed as wet, when an ammonia odour and visible wet areas were detected, and dry when litter was in a friable state without a strong ammonia odour or visible wet areas. Flocks were kept in one group or divided into subgroups. Seven farms had an outdoor area either in the form of a Wintergarten or an outdoor range. Multiple flocks received medical treatments. Artificial light was provided for 8-9 hours a day.

Table 6.1. Overview of factors during the rearing period and laying period with corresponding sample size (n)

Rearing factors	n	Laying factors	n
Housing system^a		Housing system^h	
Aviary system	32	Aviary system	28
Level system	3	Ground system	7
Litter type^b		Litter suppliedⁱ	
Wood shavings or alfalfa	32	Yes	27
Paper remnants	3	None	8
Disruption in litter supply^c		Litter quality^j	
Yes	23	Dry	23
No	12	Wet	12
Limitation in litter supply^d		Medical Treatments^f	
Yes	8	Medical treatments given	18
No	27	No medical treatments given	17
Light intensity changes^e		Subgroup^k	26
Adjustments from 1 to 5 weeks	24	No	9
No adjustments from 1 to 5 weeks	11	Outdoor or wintergarden	
Adjustments from 5 to 10 weeks	27	Yes	7
No adjustments from 5 to 10 weeks	7	No	28
Missing data	1	Light intensity^l	
Medical Treatments^f		Dark	8
Medical treatment given	13	Bright	14
No medical treatments given	22	Extra light	13
Sound influences^g		Specific management^m	
Yes	8	Yes	9
No	27	No	26

Footnote: ^a Aviary system: tiers with in between tiers litter area, level system: elevated platforms with litter area around main platform; ^b Litter substrate provided ^c Yes indicates litter supply disruption during first 4 wks of life; ^d Yes indicates only paper remnant or small amount of wood shavings; ^e Light intensity measurements were compared between ages to assess an adjustment; ^f Medical treatments consisted of extra feed-supplements, vitamins, vaccinations or medications; ^g Radio playing or excessive noise from children playing inside the chicken house; ^h Aviary system with tiers and litter area between tiers, ground system with main elevated platform and litter area around the platform; ⁱ Yes indicated litter supplied or no litter supplied; ^j Wet when ammonia odour or visible wet areas, dry: litter friable state no ammonia odour or visible wet areas; ^k Subgroup if flocks were separated in small groups; ^l Dark: visibility less than 20 m or bright visibility more than 20 m; ^m Yes: either a radio playing, aerated beton blocks, round drinkers, or roosters and a combination of the aforementioned.

Light intensity was assessed as dark when observer visibility was less than 20 m, bright when visibility was more than 20 m. In five farms, birds were exposed to daylight, and in eight farms light cables were placed throughout the system. Feed was provided by one of 15 commercial feed producers of the farmer's choice. Specific management was applied by certain farmers which consisted of either providing a radio to diminish disturbances by sounds, aerated concrete blocks for pecking, round bell drinkers, roosters in the flock or combinations of the aforementioned.

6.2.3. ON-FARM MEASUREMENTS

We visited the flocks at week 1, 5, 10, 15 and 40 of age. At each visit we conducted observations related to fearfulness and SFP. One researcher performed the majority of farm visits on the rearing farms, with the exception of three flocks. Another researcher performed the majority of farm visits on the laying farms, with the exception of two flocks. Beforehand, measurements of the same flocks and birds were checked for conformity and similarity between researchers to optimize inter-observer reliability.

BEHAVIOURAL TESTS

Behavioural tests related to fearfulness were: a novel object test, a stationary person test, and a novel environment test. The novel environment test was only conducted during rearing.

NOVEL OBJECT TEST

The novel object test (NOT) was conducted at 1, 5, 10 and 40 weeks of age. At one and five weeks of age, the novel object was a wooden box (5*5*2cm) taped with coloured scotch tape (green, yellow, red and white). At 10 and 40 weeks of

age, a plastic PVC tube (50cm) taped with coloured scotch tape of similar colours was used. The novel object was placed on the floor in the housing area, after which the observer moved away to a distance of 3 meters from the object. The NOT was repeated four times on different locations in the chicken house i.e. at each end of the chicken house, and $\frac{1}{3}$ and $\frac{2}{3}$ of the end of the chicken house. At each location, birds were exposed for two min. Every 10 sec we recorded the number of birds which were within 25cm of the novel object. Afterwards we determined the time point at which at least three birds were in close proximity of the object which we used as the latency of three birds to approach. At 40 weeks of age we estimated the minimal distance in cm of the bird closest to the novel object by approximation.

STATIONARY PERSON TEST

A stationary person test (SPT) was conducted at 1, 5, 10 and 40 weeks of age. At one and five weeks, the observer placed her right arm inside the system. At 10 and 40 weeks, the observer stood still inside the litter area of the system (based on Welfare Quality[®], 2009). Birds were exposed to the human arm or stationary person for two min. The SPT was repeated four times at four different locations in the chicken house (i.e. at each end of the chicken house, and $\frac{1}{3}$ and $\frac{2}{3}$ of the end of the chicken house). We estimated the minimal distance of the bird closest to the stationary person in cm.

NOVEL ENVIRONMENT TEST

A novel environment test (NET) was conducted at 1 and 5 weeks of age. The novel environment was an orange round bucket at one week of age (30 cm diameter with 30 cm height) and a white round bucket at five weeks of age (40 cm diameter with 50 cm height). The larger white bucket was needed to prevent

chicks from jumping out, which occurred in the smaller orange bucket. Chicks were selected from random locations (n=20 at week one, n=15 at week five) and their response was recorded, individually, for one minute in this novel environment. During the NET, the observer was visually out of sight of the test subject. We recorded the latency to vocalize and the number of vocalizations.

FEATHER PECKING OBSERVATIONS

Severe feather pecking (SFP) behaviour was recorded at 1, 5 and 10 weeks of age. We recorded SFP for 20 min by means of behaviour sampling at two locations in the chicken house. The observation area was approximately 1 m² and contained all resources (litter, feeding trough, perches). We recorded the total number of pecks of SFP for all birds in the observation area. Severe pecking was defined as forceful pecks and feathers pulls that resulted in a reaction from the recipient (Savory 1995). We used a variable habituation time before initial behaviour scoring. The criterion was that 80% of chicks within the observation area did not direct their attention to the observer. We used this criterion to ensure that the chicks were not distracted by the observer. During observations we also recorded if feather eating occurred (yes/no).

FEATHER DAMAGE

Feather damage score (FS) was recorded at 5, 10, 15 and 40 weeks of age. During rearing, sample size was 20 birds per flock. During laying, sample size was 50 birds per flock. Birds were selected from random locations in the chicken house. Feather damage was assessed by scoring damage to neck, back and belly region on a three point scale (a=no damage, b=moderate damage, c=severe damage (>5cm), based on Welfare Quality[®], 2009). During rearing, cuts in the wings and tails were included as an indication of early feather damage (ab score). All areas

together gave a total feather score ($a=0$, one $ab=0.5$, one $b=1$, one $c=2$). We calculated the average feather damage per flock, per body area and the proportion of hens with damage per body area and severity of damage (i.e. 0, 0.5, 1 or 2 score). The presence of severe damage in the flock during rearing i.e. hens with wounds was scored as a nominal variable. The presence of severe damage during laying was recorded if more than 10% of the measured hens had severe feather damage (score 2). During laying, it was not always possible to capture hens. When capture was not possible, assessment of feather damage was done by assessing feather damage of individual hens from a small distance (Bright et al., 2006).

6.3. STATISTICAL ANALYSIS

Statistical analysis was performed with SAS version 9.2, and included analysis using 1) PCA analysis, 2) a two-way ANOVA, 3) final model building 4) a mixed model for binomial data, 5) a Chi-Square test and 6) Pearson correlations. The dependent variable feather damage at 40 weeks of age per flock conformed to the assumptions of a general linear model (normal distribution of residuals, equality of variance, and linearity) based on a GLM including cross as a class variable.

First, a PCA with an orthogonal varimax rotation was used to test for similarity between variables recorded in the behavioural tests conducted during the rearing period (see Table 6.2). This was done in order to remove redundancy in the data used for later analysis. Factors were retained if their eigenvalue was more than 1. As loadings of variables rely partly on the sample size (Hair et al., 1998) and our sample size was relatively small ($n=35$) we used a stringent cut-off point of 0.5 to retain variables assigned to the factors. Results of the PCA were

used to generate factor scores related to fearfulness for use as a response variable in GLM as described in the following.

Second, we tested the effect of each independent factor or variable (both during rearing and laying) and its interaction with cross on dependent variable feather damage at 40 weeks of age.

Third, factors were only included in the model if after inclusion of all factors or variables they remained to have an alpha level of less than 0.1, if they were equally balanced, and if there were no interactions with other factors (Table 6.3). Cross was included as it was part of the experimental set-up. The fixed factors of the final GLM were: cross (DW/ISA), laying hen housing system (floor/aviary) and specific management during laying (yes/no). Covariates in the final model were PCA-factor 2 (see Table 6.2), SFP at five weeks of age, and minimal distance to stationary person at 40 weeks of age and group size at laying. The variance explained by the model was interpreted by the R^2 of the model, while the respective variation explained by each factor was calculated by dividing the factor's SUM of SQUARES by the sum of all factors' SUM of SQUARES.

Fourth, proportions which did not fulfil the assumptions of GLM were analysed with a GLIMMIX to test effect of cross, age and their interaction, using a binary distribution with logit link function and a contrast statement to assess specific comparisons.

Fifth, binominal variables of severe damage at laying were related to binominal variables of severe feather damage and feather eating at rearing comparing each age (5, 10 and 15 weeks of age) separately by means of a Chi-square test. For correlations between variables, Pearson correlations within cross were calculated.

6.4. RESULTS

6.4.1. PREVALENCE OF FEATHER PECKING AT REARING AND FEATHER DAMAGE AT LAYING

See Table 6.4 for percentage of flocks with severe feather damage during rearing and laying. During the laying period, 49 % of the flocks visited had signs of severe feather damage, i.e. more than 10% of the sampled hens had moderate or severe feather damage. During rearing, between 37% and 66% of the flocks had severe damage (i.e. recordings of wounds). Average feather damage per flock was at five weeks: 0.31 ± 0.04 , ten weeks: 0.22 ± 0.03 , fifteen weeks: 0.16 ± 0.02 and 40 weeks 0.86 ± 0.07 . At five weeks of age, 36% of the flocks showed high SFP (more than 10 pecks/20min), 24% showed moderate SFP (between 4-10 pecks/20min), 27% showed low SFP (2 pecks or less/20min) and 12% showed no SFP.

Table 6.2. Factor loadings of a Principal Component Analysis with orthogonal varimax rotation, with interpretation of factors and variance explained by each factor of variables of the behavioural tests measured at one, five and ten weeks of age.

Factors of the Principal component analysis					
Nr of factors		Factor1	Factor2	Factor3	Factor4
Naming of factors ¹		Approaching novel object	Distance to stationary person	Fear of novelty	Vocalizations in social isolation/novel environment
Variance explained per factor		38%	24%	16%	11%
Behavioural test	Age at testing (weeks)				
Variable measured					
Novel object test					
Latency to approach	1	-0.86	-0.02	-0.02	-0.16
Latency to approach	5	-0.62	0.36	0.35	0.29
Latency to approach	10	-0.24	-0.02	0.78	0.04
Average number of hens that approached	1	0.91	-0.10	-0.05	-0.09
Average number of hens that approached	5	0.75	0.06	-0.44	-0.14
Average number of hens that approached	10	0.19	0.20	-0.80	-0.09
Stationary person test					
Minimal distance of hens that approached	1	0.27	0.08	-0.29	-0.74
Minimal distance of hens that approached	5	-0.02	0.89	-0.08	0.06
Minimal distance of hens that approached	10	-0.15	0.90	0.13	-0.02
Novel environment test					
Number of vocalizations	1	0.24	0.06	-0.10	0.84
Latency to vocalize	1	0.15	0.13	0.68	0.01
Number of vocalizations	5	0.73	0.42	0.03	0.21
Latency to vocalize	5	-0.46	-0.69	0.18	0.11

Footnote: ¹ Name given to each factor was based on the highest positive loadings (> 0.5) of the variables on these factors; for factor 1: average number of hens approaching a novel object; factor 2: minimal distance to stationary person, factor 3: both latency to approach novel object and latency to vocalize in the novel environment/social isolation test; factor 4: vocalizations in the novel environment social isolation test

Table 6.3. Overview of rearing and laying factors which showed a main effect or an interaction with cross ($P < 0.1$) on average level of feather damage at 40 weeks of age

REARING PERIOD	Main effect		Interaction with cross	
Management	F	P	F	P
Sound influences (radio or children playing) ^{1,4}	15.35	0.005	11.99	0.002
Severe feather pecking (SFP)				
SFP at 5 weeks of age	5.34	0.03	3.32	0.08
Average feather damage score/flock (FS)				
FS at 15 weeks of age ^{2,4}	4.10	0.05	4.21	0.05
Feather eating (yes/no)				
At 15 weeks of age ¹	3.96	0.02	2.11	0.12
PCA factor based on behavioural tests of fearfulness (Table 2)				
Factor 2 "distance to humans" ⁴	13.04	0.001	9.62	0.001
LAYING PERIOD	Main effect		Interaction with cross	
Litter conditions	F	P	F	P
Litter quality ^{1,2,4}	6.77	0.003	9.52	0.004
Outdoor ^{2,3,4}	0.05	0.83	9.45	0.004
Light intensity				
Dark, bright or extra light ¹	3.68	0.01	2.51	0.10
Specific management (radio, beton blocks, round drinkers, roosters)				
Yes/no	11.76	0.00	1.50	0.24
Housing system				
Floor/avairy	4.30	0.05	1.18	0.32
Groupsize	10.31	0.00	0.01	0.93
Medical treatments				
Yes/no ^{1,4}	9.40	0.001	5.80	0.02
Fearfulness at lay ⁴				
Minimal distance to stationary person	4.44	0.05	4.58	0.04

Footnote: no superscript: factors included in final model, 1: factor excluded from final model as effect did not remain $P < 0.1$ with inclusion of all potential factors; 2: unequal distribution to assess interactions; 3: factor excluded from final model due to confounding with other factor (i.e. housing system); 4: interactions were removed when inclusion in the final model did not remain $P < 0.1$, Highlighted factors included in the final analysis

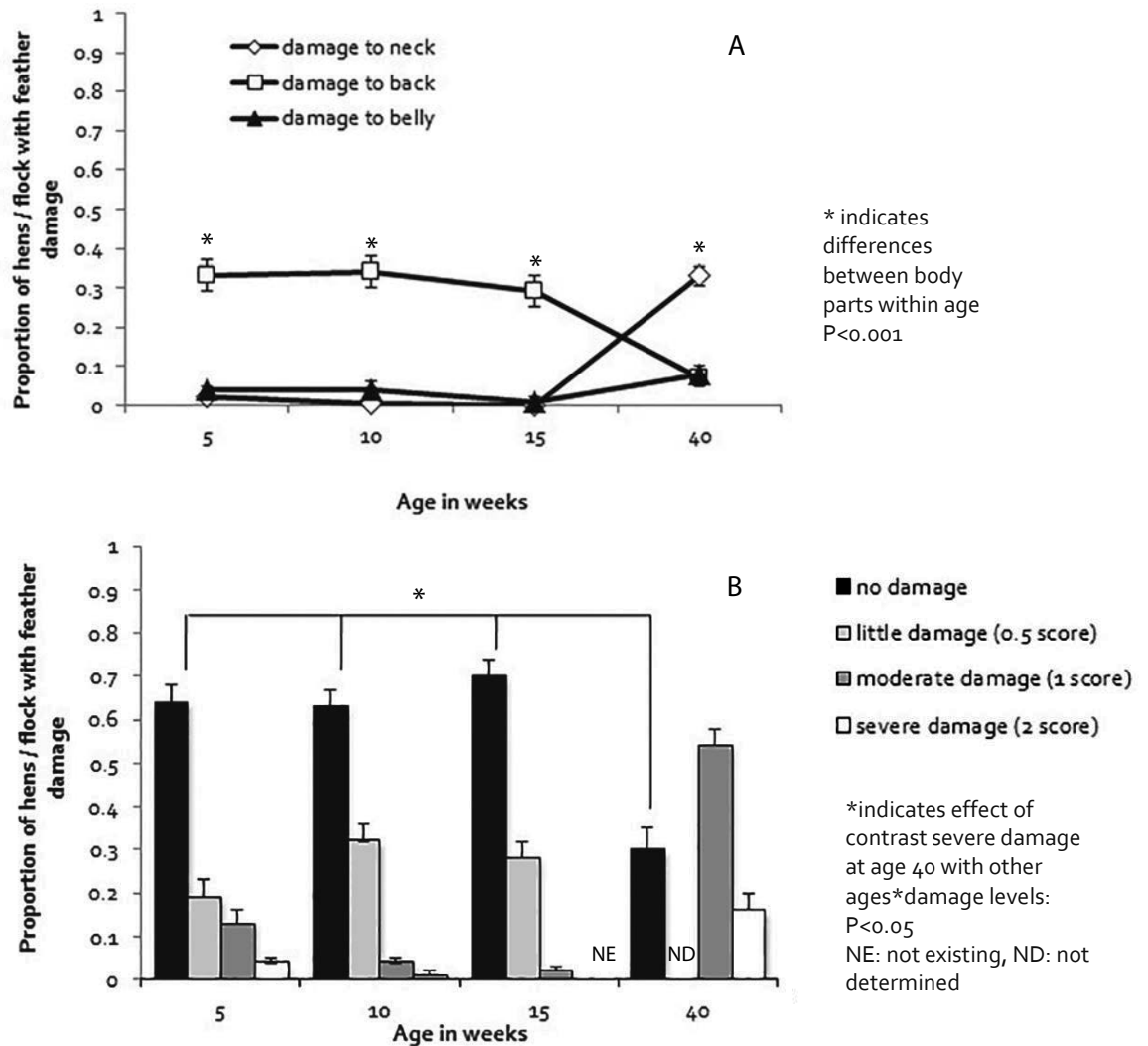
During the laying period, more hens had feather damage to the neck and fewer hens had damage to the back compared to the rearing period (Figure 6.1A). During the laying period, more hens had severe feather damage compared to the rearing period [see Figure 6.1B]. The percentage of flocks with back damage was higher at five weeks of age compared to 15 weeks and tended to be higher at 10 compared to and 40 weeks of age (back₅: 71%, back₁₀: 17%, back₁₅: 71%, back₄₀: 29%, $X^2_1 = 2.8$, $P = 0.09$; back₅ vs. back₁₅ $X^2_1 = 4.32$, $P < 0.05$; back₅ vs. back₄₀ $X^2_1 = 3.2$, $P < 0.07$). Flocks with severe damage in the laying period also had a higher occurrence of feather damage at five weeks of age compared to flocks with no damage at laying ($X^2_2 = 7.8$, $P < 0.05$, Table 6.4), whereas severe feather damage at 10 and 15 weeks were not associated with having severe damage at laying (10 weeks: $X^2_1 = 0.01$, NS, 15 weeks $X^2_2 = 0.45$, NS, Table 6.4). In flocks with severe damage at 40 weeks of age, feather eating during rearing tended to occur more often, especially at 15 weeks of age in comparison to flocks with no severe damage at 40 weeks of age ($X^2_2 = 4.8$, $P = 0.09$; Table 6.4).

Table 6.4. Percentage of severe feather damage at laying (40 weeks of age) in relation to severe feather damage and feather eating at rearing (5, 10 and 15 weeks of age)

	Severe damage ¹ at laying (49%)	No severe damage at laying (51%)
Severe damage²		
5 weeks	53% ^a	22% ^b
10 weeks	65%	67%
15 weeks	71%	61%
Feather eating		
5 weeks	42%	17%
10 weeks	6%	17%
15 weeks	23% ^x	5% ^y

¹severe feather damage at laying was counted as occurring when 10% or more birds with severe feather damage (score 2) were recorded, ²severe feather damage at rearing was counted as occurring when birds with severe damage (wounds and denuded areas) were recorded in the flock, values with superscript ^{ab} between rows differ $P < 0.05$, values with superscript ^{xy} between rows differ $P < 0.1$

Figure 6.1. Proportion of sampled hens with feather damage to neck, back and belly (A) and with no, little, moderate or severe feather damage (B) at five, ten, fifteen and forty weeks of age



The crosses did not differ in average feather damage at 40 weeks of age ($F_{1, 21} = 0.78$, $P = 0.39$, DW: 0.88 ± 0.07 vs. ISA 0.80 ± 0.16). The proportion of hens with damage to the back was higher for ISA than for DW flocks at 40 weeks of age, while at 10 weeks of age the proportion of hens with damage to the back was higher in DW flocks vs. ISA flocks (cross * age: $P < 0.01$: 40 weeks: 0.22 vs. 0.10; 5 weeks: 0.16 vs. 0.40). For DW flocks average damage to the back was not related to average feather damage ($r_{back} = 0.26$, $P = 0.24$) while it was for ISA flocks ($r_{back} = 0.82$, $P = 0.0005$).

6.4.2. PRINCIPAL COMPONENT ANALYSIS

The Principal Component Analysis of the variables from the Novel Object test (NOT), Stationary Person test (SPT) and Novel Environment test (NET) performed during the rearing period resulted in four factors (see Table 2 for variance explained by each factor and contribution of variables to each factor). For factor 1 the highest loading was for the number of hens that approached the novel object and to a lesser extent vocalizations in the NET, hence this factor was named "approaching novel object". Factor 2 consisted mainly of distance to stationary person (SP); hence this was the corresponding name for factor 2. Factor 3 consisted of latency to approach in the NOT and latency to vocalize in the NET, and as both may reflect fear responses to a novel stimulus or environment, thus this factor was named "fear of novelty". Factor 4 consisted of vocalizations in the NET, and was named similarly (see Table 6.2). Only factor 2 (distance to SP) affected feather damage at laying and was therefore retained in the analysis.

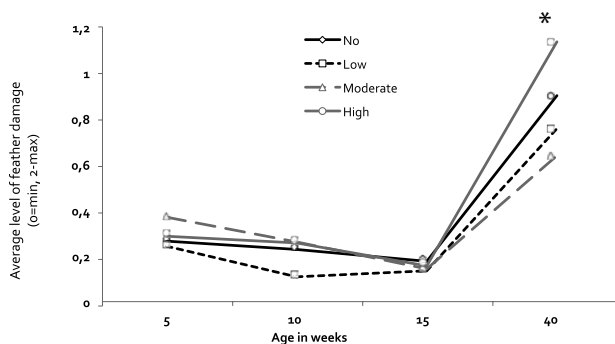
6.4.3. RISK FACTORS FOR FEATHER PECKING

See Table 6.3 for an overview of potential risk factors based on the two-way model testing for main and interaction effects on level of feather damage at laying. Factors at rearing that were associated with feather damage at 40 weeks were: sound influences on the rearing farm, SFP at five weeks of age, feather damage and feather eating at 15 weeks of age and PCA-2 distance to SP. Factors at laying associated with feather damage were: litter quality, outdoor access, light intensities, specific management, housing system, group size, medical treatments and minimal distance to stationary person. The final model, based on $P < 0.1$ for each factor, included cross, SFP at five weeks of age, PCA-2, specific management, system at laying, group size at laying, and minimal distance to stationary person at 40 weeks of age. The model explained 91% of the total variance (R^2). Of that variance, little was explained by cross (0.7%), 34.3 % was explained by factors at rearing and 64 % was explained by factors at laying.

6.4.4. RISK FACTORS DURING REARING

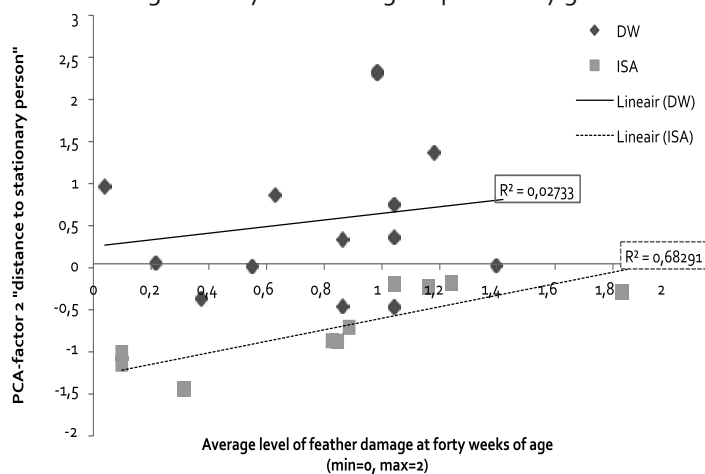
High SFP at five weeks of age resulted in more feather damage at 40 weeks of age (Figure 6.2, $F_{1, 21} = 4.62$, $P = 0.05$; variance explained: 29%). A high score for distance to SP tended to be related to increased feather damage at 40 weeks of age for both crosses (Figure 6.3, $F_{1, 21} = 3.31$, $P = 0.06$; variance explained: 5.3%).

Figure 6.2. Level of severe feather pecking at five weeks of age with feather damage at five, ten, fifteen and forty weeks of age



Severe feather pecking (SFP) at 5 weeks of age was classified as no, low, moderate and high based on the average number of pecks from 2*20 min observations. No: no SFP recorded: 0 pecks/20min; Low: min=1, max=2 pecks/ 20 min; Moderate: min=3, max=10 pecks/20 min; High: >10 pecks/20 min (min=10, max=23 pecks/20 min); * indicates $P < 0.05$ difference between groups

Figure 6.3. Principal component (PCA) factor 2 “distance to stationary person” at rearing and feather damage at forty weeks of age expressed by genetic-cross



PCA-factor 2 distance to stationary person is based on high loadings for minimal distance from stationary person at five and ten weeks of age and to a lesser extent number of vocalizations in the novel environment test at five weeks of age and negative loadings for latency to vocalize at five weeks of age.

6.4.5. RISK FACTORS DURING LAYING

Average feather damage was higher when flocks were housed in a floor system compared to an aviary system (Figure 6.4: $F_{1, 21} = 19.3$, $P < 0.001$; variance explained: 21%). Flocks in which specific management was applied had lower average feather damage than flocks in which no specific management was applied (Figure 6.5, $F_{1, 21} = 11.8$, $P < 0.001$; variance explained: 26%). A smaller group size tended to associate with lower average levels of feather damage ($\beta - 0.0004$; $F_{1, 21} = 3.73$, $P = 0.07$; variance explained: 1%). A larger distance from the SP at laying was associated with more feather damage for both crosses ($\beta + 0.002$; $F_{1, 21} = 5.76$, $P = 0.03$; variance explained: 16%), similar to response to SP during rearing.

Figure 6.4. Average feather damage of 50 hens/flock in flocks which were housed in an

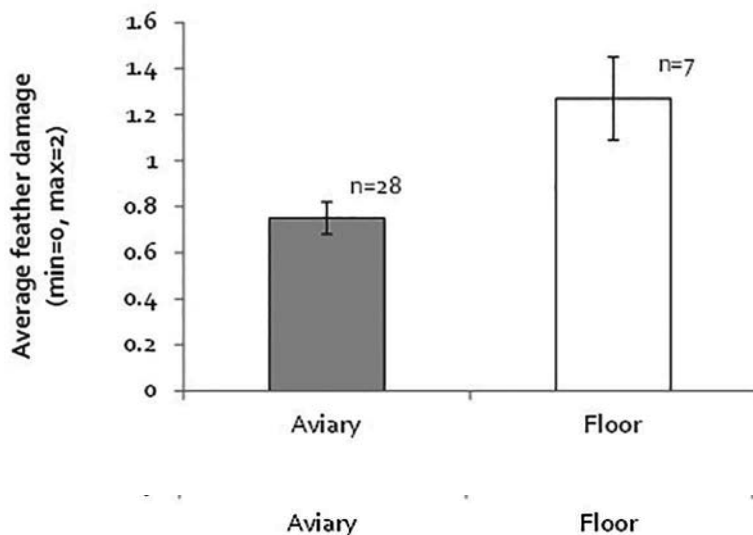
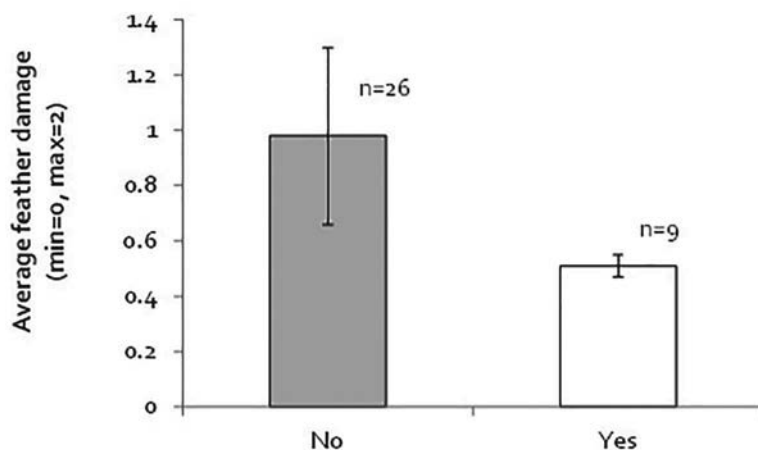


Figure 6.5. Average feather damage of 50 hens/flock in flocks which had (yes) or had not (no) specific management



6.4.6. CROSS EFFECTS

None of the DW flocks approached the SP within 25cm at 40 weeks of age, while 60% of the ISA flocks did not approach the SP within 25cm ($X^2_2 = 14.9$, $P < 0.001$). At 40 weeks of age, DW hens also kept a greater distance to the novel object than ISA hens (96 ± 13 cm vs. 39 ± 8 cm: $F_{1, 22} = 22.14$, $P < 0.01$) and to the stationary person (264 ± 41 cm vs. 38 ± 9 cm: $F_{1, 22} = 41.4$, $P < 0.01$). Similarly, in the fear tests during rearing, distance from SP was higher for DW hens than for ISA hens (PCA-2: $F_{1, 22} = 15.72$, $P < 0.001$; 0.56 ± 0.25 vs. -0.73 ± 0.17) while less hens approached the novel object in DW flocks compared to ISA flocks (PCA-1: $F_{1, 22} = 6.85$, $P = 0.02$; -0.42 ± 0.23 vs. 0.55 ± 0.30). For both crosses, number of hens approaching the novel object during rearing (PCA-1) was related to latency to approach novel object at 40 weeks of age ($r = 0.54$, $P < 0.01$). Distance to SP during rearing (PCA-2) was related to distance to SP at laying ($r = 0.45$, $P = 0.03$).

6.5. DISCUSSION

Is it the past or is it present? This study aimed to assess effects of the rearing environment and laying environment on feather damage at 40 weeks of age in laying hens. We showed that factors at rearing as well as at laying were both related to feather damage at 40 weeks of age. Severe feather pecking (SFP) and elevated fearfulness during rearing were related to higher levels of feather damage at 40 weeks of age. At laying, hens housed in a floor system had more feather damage than hens in an aviary system. Hens housed under specifically adjusted management at laying (i.e. a radio playing in the chicken house, aerated concrete blocks for pecking, round drinkers and/or roosters) had less feather damage than hens housed under standard management. During laying, feather damage mainly consisted of severe damage to the neck area, while during rearing feather damage mainly consisted of moderate damage to the back area. DW hens were more fearful of NO and during SPT than ISA during rearing and laying. ISA flocks had more hens with feather damage to the back than DW flocks at 40 weeks of age, while at 10 weeks of age this was vice versa.

6.5.1. REARING FACTORS

Severe feather pecking, feather damage and feather eating during rearing were associated with feather damage at laying. Especially SFP at five weeks of age was associated with higher levels of feather damage at 40 weeks of age. This is in agreement with other studies which show that SFP and feather damage during rearing increases the risk of feather damage at laying (Nicol et al., 2001a; Bestman et al., 2009; Lambton et al., 2010; Gilani et al., 2013). Feather eating at rearing may also indicate the presence of SFP as it has been repeatedly associated with SFP (Harlander-Mataushek et al., 2006; 2007b; Harlander-

Matauschek and Häusler, 2009). In our previous study of 47 rearing flocks, we found that litter disruption and limitation in access to litter during the first four weeks of age substantially increased SFP at five weeks of age and feather damage during rearing (de Haas et al., 2014). Feather damage at laying may thus be indirectly affected by litter availability during rearing. This finding supports the other aforementioned studies which show that a lack of foraging substrate at an early age yields a risk for the development of feather damage during the production phase. Based on our results, however, we can only conclude that SFP at five weeks of age is a risk factor for more feather damage at laying. PCA-factor 2, which consisted mainly of keeping a large distance from the stationary person during rearing was associated with high feather damage for both crosses. High fearfulness at a young age, as measured in a novel environment/social isolation test, has been shown to be associated with an elevated propensity to develop SFP at adult age (Jones et al., 1995; Rodenburg et al., 2004a). Here, we see similar results at flock level, as factor 2 consisted, partly, of the response to a novel environment combined with social isolation. However, factor 2 consisted more strongly of distance to humans during the rearing period, which in turn was associated with distance to humans during laying and with feather damage during laying. These results indicate that especially fear of humans, both at a young and adult age, may pose a risk for the development of SFP. Furthermore, fearfulness may (further) increase due to SFP (Blokhuys and Beutler, 1992; Vestergaard et al., 1993). The two crosses compared in this study differed in fearfulness both during rearing and laying, with DW hens being generally more fearful than the ISA hens in response both to the novel object and the stationary person. Supporting these findings, hens from a White Leghorn origin (i.e. DW) have been shown repeatedly to have higher fear-levels than hens from a Rhode

Island Red origin (i.e. ISA) when tests measured responses to humans (Uitdehaag et al., 2006; 2008a; de Haas et al., 2013a; 2014). Fear of humans can have a negative effect on production (Hemsworth and Barnett, 1989), while additional exposure to humans can reduce fear of humans through habituation (Barnett et al., 1994). Limited exposure to humans, combined with the highly stressful experiences by being manually vaccinated, transported and handled by humans, may lead to high fear of humans and maybe even chronic stress caused by intermittent exposure to fear- and stress-inducing stimuli. A positive human animal relationship (Hemsworth et al., 1993) from an early age onwards combined with a predictable and controllable environment may reduce stress (Koolhaas et al., 2011), including stress induced by contact with humans, and thereby potentially lessens the risk of developing SFP.

6.5.2. LAYING FACTORS

The system, management and group size on the laying farm affected feather damage. These are similar to the factors at rearing which were found to be related to feather damage at rearing (de Haas et al., 2014). Hens housed in a floor system had more feather damage than hens housed in an aviary system. This has also been reported for organic laying farms (Bestman and Wagenaar, 2003). In an aviary system, hens have more possibilities to escape from feather pecking birds (i.e. more levels and elevated perches), and local density may be lower as hens' living space is spread over multiple levels of the aviary system compared to a floor system with only floor level. Additionally, the floor systems studied here, were of an older type than the aviary systems studied, and had a limited litter area available for foraging compared to the aviary systems where hens were

more spread out over the system which consequently resulted in lower local densities in the litter area.

Flocks in which an adjusted management in the form of supplying aerated blocks for pecking, having a radio playing, round drinkers provided or roosters in the flock had lower levels of feather damage compared to flocks without such management. Some of these measures may reduce fear as suggested by a previous study indicating a fear-reducing effect of intermittent exposure to recorded sounds (Campo et al., 2005). By reducing fear these measures might have had a positive effect on feather pecking. Exposure to varied sound levels have been associated with reduced feather damage (Gilani et al., 2013). Other measures may reduce feather damage, as has for instance been shown for having roosters in a flock (Bestman and Wagenaar, 2003) which potentially also reduce fearfulness in a flock. Round drinkers on the other hand are often associated with higher feather damage (Green et al., 2000; Zimmerman et al., 2006) probably due to reduction of litter quality due to spilling of water over round drinkers as suggested by Gilani et al. (2013). In our case, round drinkers were supplied additionally to nipple drinkers, which reduce competition for water resources and expected less spilling. The success of modified management in reducing feather damage can also mean that these are the farmers that are aware of the problem and are actively working to prevent feather damage. Recently a custom-made management package consisting of varying curative measures to reduce SFP was tested on problem flocks and control flocks in a free-range system (Lambton et al., 2013). Although it was not specified which factors were implemented, use of a large number of different management measures also led to a decrease in SFP and feather damage in problem flocks. We argue, based on our findings and those of Lambton and co-

workers that management practices which facilitate foraging behaviour (such as aerated concrete blocks) and reduce fearfulness (radio playing) may further lessen the risk of SFP development.

A larger group size tended to increase feather damage, which has also been found in previous studies (Zimmerman et al., 2006; Bestman et al., 2009; Lambton et al., 2010). In a larger group, SFP birds can peck more victims. Additionally, transmission of SFP may have occurred because of birds attraction to ruffled plumage or damaged feathers and denuded area's or wounds (McAdie & Keeling, 2002). Consequently this may lead to more birds being involved in SFP and creating more birds with feather damage. Adjusting the management, as previously noted, may thus be especially important in a larger flock.

6.5.3. PREVALENCE OF FEATHER PECKING DURING REARING AND LAYING

The prevalence of feather damage during laying was 49% at flock levels. In other studies prevalence was substantially higher: 57% (Green et al., 2000), 65% (Gilani et al., 2013), 71% (Bestman and Wagenaar, 2003), 69-86% (Lambton et al., 2010), which might be due to a lack of beak treatment, different age at the time of recording, threshold level used, genetic crosses and recording in different housing systems (Nicol et al., 2013). The prevalence of SFP during rearing was 60% in the present study, which resembles that of several other studies: 47% (Lambton et al., 2010) and 54% (Bestman et al., 2009). Feather damage generally occurs at peak of laying (Bright, 2009). Taken together with other studies the high recorded prevalence of SFP, indicating that at least 49% of flocks are affected by this problem, highlights the importance of finding a solution.

We noted that in ISA hens, feather damage was seen for all body regions, while for DW hen's feather damage was mainly seen in the neck and belly region

at laying. Feather damage in the neck and belly region is most likely to be caused by aggressive pecking and vent pecking, respectively, while feather damage to the back region is most likely caused by SFP at the base of the tail. This result suggests that ISA hens show more forms of pecking behaviour at laying while DW hens mainly show aggressive and vent pecking at laying. This suggestion is supported by our study of parent stock hens, where DW hens had more damage to the belly region than ISA hens (de Haas et al., 2013a).

Feather damage during rearing was mainly limited damage to the back region, while feather damage during laying was mainly moderate to severe damage to the neck region. High incidence of severe feather damage to the neck region occurred in 86% of the laying flocks. Specifically, feather damage to the neck area may be attributed to the occurrence of molt at the onset of lay, frustration due to feed-competition and abrasions due to the housing system. Under natural conditions, when hens start laying eggs, this is combined with a temporary cessation in feed intake and the occurrence of molt (Berry, 2003). A few days prior to the onset of lay, feed-intake is reduced and is followed by a rapid increase in feed-intake until stable levels are reached approximately one month after the onset of lay (Hurwitz et al., 1975). During the period of increased feed-intake, aggressive pecking may occur more frequently at the feeding times. Aggressive pecking caused by frustration has been observed when feed is not readily available i.e. when hens needed to use an operant feeder in their home cage (Lindberg and Nicol, 1994). Feather damage to the neck is associated with aggressive pecking (Savory, 1995) but can also be caused by abrasion (Bilčík and Keeling, 1999) against feeders and perches mounted above feeders. These factors may together explain the high incidence of feather damage to the neck area at laying. Feather damage at the back region during rearing and laying

indicates SFP (Bilčík and Keeling, 1999), which can be used by farmers as a criterion for SFP and requests adjustments of management.

6.6. CONCLUSIONS

In this study we aimed to find risk factors for feather damage during laying in laying hens housed in non-cage systems. Fear of humans and SFP during rearing is risk factors for feather damage at laying. Floor housing, a large group size and fear of humans during laying increased levels of feather damage, with ISA flocks having more hens with feather damage to the back compared to DW flocks. During laying, an adjusted management reduced feather damage. An adjusted management can be applied as a preventive measure for feather damage, especially in large flocks housed in floor systems. The overall risk of SFP can thus be reduced when management is optimized at rearing and laying, specifically by providing appropriate foraging substrates and strategies aimed at reducing fearfulness.

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Chapter 7

General Discussion

7. INTRODUCTION

Severe feather pecking (SFP) is a serious behavioural problem in laying hens, leading to impaired welfare and, in extreme cases, to mortality of feather pecked birds. In order to prevent the behaviour, it is important to understand the risk factors in the development of SFP. For that reason, all phases of the laying hen production chain (parent stock, rearing flocks and laying flocks) were studied. The aim of this thesis was to find the risk factors, pre-and post-hatch, for development of SFP in laying hens.

Fearful birds have been found to have a higher tendency to develop SFP (Rodenburg et al., 2004a; 2013; all chapters). In all chapters of this thesis, variables reflecting how birds cope with fear and stress were assessed as potential predictors of SFP. Studies presented in chapters 2 and 3 were conducted to gain insight into the principles of SFP with emphasis on how birds cope with fear and stress. Studies presented in chapters 4, 5 and 6 were conducted to assess the risk factors of SFP in the laying hen production chain. In this chapter, I will discuss the outcome of these studies starting with the principles of SFP followed by application of these principles to the commercial practice of laying hens. Finally, I will end this chapter with the implications of this thesis for the laying hen production chain.

7.1 FEAR AND FEATHER PECKING

Throughout this thesis it was found that hens from lines which differ in tendency to develop SFP also differ in behavioural and physiological traits related to fearfulness, stress-sensitivity and the dopaminergic and serotonergic systems. The results from studies in this thesis complement studies showing genetic correlations between fearfulness, stress responses and SFP (Rodenburg et al.,

2003; 2004; Buitenhuis et al., 2004). The tendency of hens to develop SFP has a strong genetic component i.e. genetic lines differ in predisposition to develop SFP, see Table 7.1. for an overview of studies. Hens of a Dekalb White (DW) cross were consistently more fearful than hens of an ISA Brown (ISA) cross (chapters 4, 5 and 6). In chapters 4 and 5, high fearfulness also coincided with high levels of feather damage on a flock level. Moreover, hens selected on low levels of mortality due to cannibalism in group housing (low mortality line: LML) were less fearful as young (chapter 2) and adult (chapter 3) than hens not selected against mortality (control line: CL). Especially high fearfulness at a young age appears to be a risk for development of SFP, on an individual level (Jones et al., 1995; Rodenburg et al., 2004a) and, as shown in chapters 5 and 6, also on flock level. At an adult age, stress-sensitivity appears higher in lines which have a high tendency to perform SFP (Uitdehaag et al., 2009; 2011; Bolhuis et al., 2009; Rodenburg et al., 2009a, Table 7.1.). Why do fearful birds develop SFP? In chapter 2, 5-week old chicks, which were fearful in the Open Field, had higher corticosterone (CORT) responses to restraint in adulthood, but did not differ in fear response to restraint, compared to chicks which were not fearful in the Open Field. These results indicate a long-term effect of being fearful at a young age on stress-sensitivity in later life. High fearfulness is part of anxiety. Anxiety can be interpreted as a sustained high tendency to be fearful (Korte, 2001; Clément and Chapouthier 1998; Reiss et al., 1986). Anxiety influences an animal's responses under a variety of conditions (Clément and Chapouthier, 1998; Kim et al., 2011) and this trait may be sustained over time and affect an animal's stress-sensitivity, as shown in chapter 2. The tendency to develop SFP in a young anxious bird might derive from deviant neural development caused by repeated or chronic

Table 7.1. Comparisons of lines with a high tendency to perform SFP vs. lines with a low tendency to perform SFP in fearfulness, corticosterone; basal and response to stressors, and involvement of the dopaminergic and serotonergic systems.

Comparison	Type of test/brain measurement	Age of birds d:daysw: wks	Activity in fear test	Corticosterone; basal and response to stressor		Physiological measurements related to dopaminergic (DA)/serotonergic (5-HT) systems			Interpretation fearfulness high vs. low SFP line ++ higher, +/- age dependent	Reference
				basal	response	DA	5-HTc	5-HTp		
HFP vs. LFP	Manual restraint	28 d	↑			↑turn d	↓turn d		+/-	Van Hierden et al., 2004
	Manual restraint	3,14,28,41,56 d		↓		↓turn d	↓turn d			Van Hierden et al., 2002
	Open field	5w	↓							Jones et al., 1995
	Tonic Immobility	5w	=							Korte et al., 1997
	Manual restraint	22w		↓						Kops et al., 2014a
HP vs. LP	Manual restraint	52w								
	Isolation test	8-9d	↑							
	Novel object	23d	↑							
	Human approach	31d	↑							
	Open field test	16w	=							
	Manual restraint	17w	↓							
	HPLC	8w				↓ d	↓ d			
		25w				↑ d	↑ d			Kops et al., 2014a
	Micro dialysis	23w					↑			
	Tonic immobility	25w	=							Kops et al., 2014b
CL vs. LML	Novel object	25w	=							Rodenburg et al., 2010
	Human approach	25w	=							
	Novel plus maze	35w	↑							
	Manual restraint	65-68w		=	↑					De Haas et al., 2010
	Open field	7d	=							Kjaer and Guémene, 2009
	T-maze	12-16d	↓							Nordquist et al., 2011
	Human approach	26d	↓							
	Open field	5-6w	↓		↑					Rodenburg et al., 2009a
	Open field	5-6w	↓							Chapter 2
	Open field	5-6w	↓							

HFP: High Feather Pecking line and LFP: Low Feather Pecking line deriving from commercial White Leghorn Line (Blokhuys and Beuving, 1993);

HP: High pecking line and LP: Low pecking line, derived from divergent selection on feather pecking (Kjaer et al., 2001);

CL: Control line and LML: Low mortality line deriving from commercial White Leghorn line selected on low mortality in sib housing (Ellen et al., 2007);

HPCL: High performance liquid chromatography; ↓ lower level of., ↑ higher level of., = similar level of., d: differences depend on brain area,

DA: Dopamine; 5-HT: 5- hydroxytryptamine (Serotonin), 5-HTc: central 5-HT concentrations, turn: turnover levels ((5-HT turnover 5-HT (5-HIAA/5-HAT), turnover DA ((DOPAC+HVA+3-MT)/DA) see van Hierden et al., 2002), 5-HTp: peripheral 5-HT, 5-HTUp: blood platelets 5-HAT uptake velocity; CORT: corticosterone, *: effects depend on interaction with treatment, Grey filled boxes are tests conducted at rearing period (0-17wks), white filled boxes are tests conducted at laying period >20wks

Table 7.1. Comparisons of lines with a high tendency to perform SFP vs. lines with a low tendency to perform SFP in fearfulness, corticosterone: basal and response to stressors, and involvement of the dopaminergic and serotonergic systems.

Comparison	Type of test/brain measurement	Age of birds d:days w:wks	Activity in fear test	Corticosterone basal and response to stressor		Physiological measurements related to dopaminergic (DA)/serotonergic (5-HT) systems					Interpretation fearfulness high vs. low SFP line ++ higher, +/- age dependent	Reference
				basal	response	DA	5-HTc	5-HTp	5-HT up			
CL vs. LML	Manual restraint	29w	↓		=				↓*	↑*	++	Bolhuis et al., 2009
	Manual restraint	33w	=		↑				↓*			Rodenburg et al., 2009b
	Manual restraint	33w	↓		=							Chapter 2
	Human approach	42w	↓*									Bolhuis et al., 2009
	Immunohisto-chemistry	52w				↑						Nordquist et al., 2013
	HPLC	33w					↑ ↓turn d	=				Chapter 3
WL vs. RIR	Open field	5-6, 7-8	↓								++	Uitdehaag et al., 2008c
	Manual restraint	7-8w	↓									Uitdehaag et al., 2008c, 2009
	Manual restraint	24w	↓									Uitdehaag et al., 2009
	Open field	17-18w	↓									Uitdehaag et al., 2008a
	Novel object	23, 46, 69w	↓									Uitdehaag et al., 2011
	Manual restraint	47w 47w	↓	=	=	↓ ↑turn	↓ ↑turn	↓	↓	↑		Chapter 5, 6
DW vs. ISA	Isolation test	1,5w	=								++	Chapter 5, 6
	Stationary person	1,5,10w	↓									Chapter 5, 6
	Novel object	1,5,10w	=									Chapter 5, 6
	-	15w		=					↓			Chapter 5
	-	40w		=					↓			Chapter 4
	Stationary person	40w	↓									Chapter 4
	Novel object	40w	↑									Chapter 4
	Stationary person	40w	↓									Chapter 6
	Novel object	40w	↓									

CL: Control line and LML: low mortality line deriving from commercial White leghorn line selected on low mortality in sib housing (Ellen et al., 2007);

WL: White leghorn and RIR: Rhode Island Red: commercial pure lines selected on divergent egg production traits (Uitdehaag et al., 2008);

DW: Dekalb White and ISA: ISA brown: hybrids deriving from WL and RIR lines (de Haas et al., 2012, 2013, under review);

HPCL: High performance liquid chromatography; ↓ lower level of..., ↑ higher level of..., = similar level of..., d: differences depend on brain area,

DA: Dopamine; 5-HT: 5- hydroxytryptamine (Serotonin), 5-HTc: central 5-HT concentrations, turn: turnover levels (5-HT turnover 5-HT (5-HIAA/5-HAT), turnover DA ((DOPAC+HVA+3-MT)/DA) see van Hieren et al., 2002), 5-HTup: peripheral 5-HT, 5-HTup: blood platelets 5-HAT uptake velocity; CORT: corticosterone, *: effects depend on interaction with treatment. Grey filled boxes are tests conducted at rearing period (0-17wks). white filled boxes are tests conducted at laying period >20wks

high CORT levels. Repeated activation of the hypothalamus-pituitary-adrenal (HPA) axis by frequent fear responses may in the long term exhaust the HPA-pathway as over-activation of the HPA-axis can cause its negative feed-back loop to become desensitised leading to excessive or flattened CORT responses (Daniels et al., 2004a; 2004b; Heim and Nemeroff, 2001). Repeated HPA (over) activation are detrimental for the individual (McEwen, 2000) and animals with anxiety can develop a pathology (Pryce et al., 2005), such as SFP.

Although brain development was not studied in this thesis, deviant brain development could underlie the differences in neurotransmitter levels in lines differing in fearfulness and mortality levels (chapters 2 and 3). For dopamine activity (DA), CL hens had higher DOPAC and NA levels compared to LML hens, most predominantly in the arcopallium. The arcopallium is a somatomotor area partly consisting of nuclei from the amygdala (Reiner et al., 2004; Cheng et al., 1999) and as such is involved in fear responses (Saint-Dizier et al., 2009). In this specific area, DA levels could be elevated potentially by its involvement in control in fear-related behaviour. In the same lines, tyrosine hydroxylase, the precursor of DA was higher in a brain area related to control of executive functions and planning (nidopallium caudolaterale) in CL hens compared to LML hens (Nordquist et al., 2013). Several studies with other line comparisons show comparable results (Table 7.1.). In the HFP vs. LFP lines, Van Hierden et al. (2004) found higher DA turnover levels in the hippocampus and archistriatum (consisting of both arcopallium and amygdala nuclei; Kuenzel & Masson, 1988; Reiner et al., 2004). Cheng and colleagues also showed that a line selected on high mortality had higher DA levels compared to a line selected on low mortality (Cheng et al., 2002) and compared to the commercially selected DW line (Cheng and Fahey, 2009). The White Leghorn (WL) and the Rhode Island Red (RIR) lines,

the pure lines of the DW and ISA crosses, also differed in fearfulness, feather damage, and DA and 5-HT brain levels (Uitdehaag et al., 2011). WL hens had higher DA and lower 5-HT levels in the rostral brain than RIR hens (Uitdehaag et al., 2011). On the basis of these studies, it appears that activity of the dopaminergic and serotonergic systems relates to high levels of SFP and fearfulness, although age-dependent differences have also been found (van Hierden et al., 2002; 2004; Kops et al., 2014a; 2013b, Table 7.1.). In lines with a high SFP tendency, at a young age lower DA and 5-HT turnover levels in specific brain areas were found (thalamus and medial striatum: Kops et al., 2014a, midbrain: van Hierden et al., 2002) while at an adult age, DA and 5-HT turnover levels were higher compared to lines with a low SFP tendency (thalamus and NCC: Kops et al., 2013a; rostral area: Uitdehaag et al., 2011). Low brain 5-HT activity of young chicks could be predisposing in development of fearfulness as found in other species (Lesch et al., 1996; Migliarini et al., 2012), and thereby influence the development of SFP.

When looking at peripheral 5-HT, DW hens had lower levels than ISA hens (chapters 4 and 5). In the pure lines of DW and ISA similar results were found (Uitdehaag et al., 2011), as well as in the CL vs. LML lines (Bolhuis et al., 2009). Peripheral 5-HT (stored in the blood-platelets) derives from the enterochromaffin cells in the intestine (Ebert-Zavos et al., 2013) of which a major function is gut peristalsis. Although peripheral 5-HT cannot pass the blood-brain barrier, 5-HT from the gut can influence the brain indirectly via the nervus vagus (Berger et al., 2009). Moreover, central and peripheral (platelet) 5-HT are correlated (Uitdehaag et al., 2011; Ursinus et al., 2013) and their reuptake transporters and receptors show similarities (Yubero-Lahoz et al., 2012). In pigs, low levels of peripheral 5-HT have been recorded in pigs during episodes of tail-biting (Ursinus et al., under

revision); a redirected behaviour which shows similarities with SFP (Brunberg et al., 2011; 2013). In hens, peripheral 5-HT may be influenced by feather eating thereby affecting peristalsis and altering gut-micro biota (Meyer et al., 2012; 2013). SFP has been related to feather eating (Harlander-Mataucke et al., 2006; 2007a; 2009), and DW hens had lower 5-HT and higher feather damage levels than ISA hens. In this thesis, also indications were found that feather eating at 15 weeks of age related to high levels of feather damage at 40 weeks of age (chapter 6). Based on these findings it can be speculated that SFP stems from altered brain DA and 5-HT. Secondly, peripheral 5-HT levels appears to relate to a bird's level of fearfulness and can derive from a bird's genetic and epigenetic predisposition. The latter is indicated by the positive relationship between high maternal stress and offspring's SFP and fearfulness at an early age in the DW cross but not in the ISA cross (chapter 5).

Additionally, the higher levels of activity in fearful hens (chapter 2) may induce a tendency to develop SFP. Hens, which develop SFP, frequently show inactivity in fear tests when young (Jones et al., 1995; Rodenburg et al., 2004, on flock level: chapters 5 and 6) and when adult show a high activity in their home pen (Kjær, 2009; Kops, 2014) and in novel settings (de Haas et al., 2010; Forkman et al., 2004; Rodenburg et al., 2003; 2004a). The differences in neurotransmitter levels of LML and CL birds in the arcopallium (chapter 3), a somatomotor area (Reiner et al., 2004), may indicate a reduced ability for inhibition of motor control. There are indications that fearful birds lack ability for inhibition of motor control, which might lead to impulsive responses which also relates to 5-HT (Bizot & Tiebot, 1996; Masaki et al., 2006) and DA activity (Kalenscher et al., 2006). In a two-choice associative learning task, fearful hens showed more rushed - potentially impulsive - actions, such as a short latency to leave the start-box and

to make a choice compared to non-fearful hens (de Haas et al., 2013c). In this task, fearful hens stuck to a preferred side more often than non-fearful hens even when visits were unrewarded (de Haas et al., 2013c). This may derive from a stuck-in-set perseverance type which has been associated with behavioural pathologies (Garner, 2005) such as trichotillomania in humans (Stein et al., 1999; Fineberg et al., 2010; Chamberlain et al., 2009) and self-mutilating pecking in parrots (van Zeeland, 2013) which show similarities with SFP (van Zeeland et al., 2009).

In conclusion, it appears that fearful birds have a higher stress-sensitivity, altered brain DA and 5-HT levels, and low peripheral 5-HT levels. Behaviourally, fearful birds show high activity levels in their home environment, which has previously also been recorded in a line selected on SFP (Kjær, 2009; Kops et al., 2014a). Altogether, these factors may create a risk for fearful birds to develop SFP.

7.2. SOCIAL FACTORS INVOLVED IN FEATHER PECKING

This thesis supplied many indications that social factors play a role in development of SFP (Table 7.2). Laying hens are socially living animals and use information from their group mates about predators, food, and resources (Nicol, 2004; Dawkins, 1989). Hens highly synchronize their behaviour, especially when foraging (Lundberg, 2002).

7.2.1. SOCIAL FACTORS IN RELATION TO FEARFULNESS

In chapter 2, we suggested that fear responses of fearful birds may initiate fear responses in their group mates, whereby group mates become more sensitive to stress. Hysteria of a few hens in response to perceived danger can induce a fear reaction in the whole flock (Van Rött, 1977). Alarm or distress calls are most

Table 7.2. Social influences on behaviour found in this thesis and follow-up study

SOCIAL FACTORS	BEHAVIOURAL EFFECTS				
	Stress sensitivity adult	Severe feather pecking	Feather damage	Fearfulness on group level	Smothering
HOUSING WITH FEARFUL BIRDS	↑ chapter 2			↑ De Haas et al., 2013b	
LARGE GROUP SIZE		↑ chapter 5	↑ chapters 5,6		
SMALL GROUP SIZE					↑ chapter 4
MIXING CAUSED BY THE PROCEDURE OF LITTER DISRUPTION ¹				↑ chapter 5	

¹During litter disruption farmers take away the cardboard paper in the cages of the aviary system. During that procedure birds are taken from cages on the 2nd level to cages on the 1st level of the tier, and are thereby mixed with unknown birds.

frequently used to transmit information about a predator (Evans & Evans, 1999; Evans & Marler, 1994), but also high vigilance in certain individuals (by staying immobile while fixing their attention to a specific location/stimuli) can be interpreted as perceived danger by group mates (Odén et al., 1999). In groups with 50% fearful hens, vigilance behaviour and fear of a novel object in the home pen was higher for all hens in comparison to groups with 0 and 25% fearful hens (de Haas et al., 2013b). These results support the idea that fearful hens can cause group members to become more fearful. Another more direct route by which fearful birds may affect their group mates is by engaging in aggressive interactions. In groups with a fearful bird present and many birds with high levels of comb lesions, pen-average CORT levels were higher compared to other groups (chapter 2). High levels of comb lesions indicate that many aggressive interactions took place in the group (Savory, 1995). In the study in chapter 2 the combination of many birds with comb lesions in a pen and a fearful bird in a pen

resulting in highest pen-average CORT. A high level of aggressive interactions indicates social instability and restlessness, potentially induced by the fearful individual (as explained in chapter 2). In large groups, however, aggression levels are generally lower than in small groups (Odén et al., 2000; Estevez et al., 2003) and the social organization appears different than in small groups (D'Eath & Keeling, 2003; Hughes et al., 1974; Dawkins, 1982). It is also likely that fearful hens have a stronger effect on their group mates when the group is relatively small compared to large groups. Fearful hens may disturb other hens by their high activity levels (chapter 2), which may elicit aggression. Riber and Forkman (2007) showed that inactive chicks are often the target of SFP. It could be possible that highly active birds disturb inactive birds and target SFP to them. This suggestion is partly supported by the findings that the supplementation of a creep area under which (inactive) chicks can hide diminished SFP both under experimental (Jensen et al., 2006) and on-farm conditions (Gilani et al., 2012). Additionally, flocks' activity levels have also been associated with feather damage (Lee et al., 2010).

Altogether these studies give indications on how fearful birds can influence their group mates: 1) by initiating fear responses in their group mates, 2) by engaging in or eliciting aggression due to high activity in the home pen and thereby disturbing group members, and 3) potentially by performing SFP directed to their group members. Additionally, the average fear level of a group was related to the group's post-stress CORT levels (chapter 2) and basal CORT shows to differ between flocks (chapters 4, 5 and 6), indicating that fearfulness and stress-sensitivity can be (come) a group phenomenon.

7.2.2. SOCIAL FACTORS IN RELATION TO SEVERE FEATHER PECKING

A large group size influenced SFP display and level of feather damage (chapters 5 and 6), which has also been shown in other studies, both experimentally (Bilčík & Keeling, 2000; Nicol et al., 1999) and on-farm (Shimmura et al., 2010; Nicol et al., 2006; Bestman and Wagenaar, 2003). SFP is generally performed by approximately 10% of the birds in a flock (8.3%: Bilčík & Keeling, 2000; 9% Keeling, 1994; 12% Wechsler et al., 1998). In a large flock, this means that more hens will perform SFP, but also that 'aggressors' have access to more victims. Additionally, SFP may spread through a flock by attraction of birds to ruffled and damaged feathers (McAdie & Keeling, 2000). In a large flock where more hens peck, there will likely be more hens with damaged feathers and this may initiate SFP in naïve birds. Based on the results from chapter 6, it appears that keeping hens in large flocks can increase the level of feather damage.

Social learning may play a role in development of SFP, especially at an early age. Young chicks use the foraging choices of conspecifics to choose what to ingest (Nicol, 2004), possibly because their own reward mechanism related to food ingestion is not yet strongly developed (Hogan, 1984). The early acquisition of food preferences may thus be conditioned by observations of foraging pecking. Under conditions where foraging is thwarted, SFP can occur at an early age, as seen in the study in chapter 5. If one chick shows SFP, feathers may be misinterpreted by other chicks as edible feed particles. The similarity between fixed action patterns of foraging pecking and SFP (Dixon et al., 2008) may underlie these responses. Having a feather pecking bird in close proximity can also affect other kinds of pecking behaviour. For instance, high levels of foraging pecking were reported in an observer bird which could see a demonstrator bird perform SFP (Sherwin et al., 2004), and high levels of gentle feather pecking by

LFP birds housed with HFP birds (McAdie & Keeling, 2002). These responses probably derive from social facilitation i.e. display of similar behaviour as that performed by conspecifics in close proximity. Social facilitation could also lead to fear responses in group mates housed with a fearful bird as discussed under paragraph 7.2.1. In this thesis, it was repeatedly found that fear responses measured on a flock level correlated to SFP and feather damage (chapters 4, 5 and 6). The social processes explained here likely underpin these findings and influence SFP. In conclusion, social factors, such as group size and group composition can contribute to the development of SFP. On a flock level, associations between SFP, fearfulness and feather damage have been found which can be used to assess flocks at risk of developing SFP.

7.3. FEATHER PECKING IN THE LAYING HEN PRODUCTION CHAIN

In this thesis the whole laying hen production chain has been studied from parent stock (PS) to rearing and laying flocks, yielding information on risk factors in the development of SFP on-farm. It was found that offspring originating from PS with high CORT, high feather damage, and high peripheral 5-HT displayed high fearfulness and SFP at one week of age (chapter 5). Also, flocks exhibiting high SFP at five weeks of age had high levels of feather damage at 40 weeks of age (chapter 6). It thus becomes clear that the propensity to develop SFP in laying hens can originate from previous parts in the chain. In the subsequent paragraphs I will explain how the development of SFP can develop is affected by genetic effects, parental effects, as well as early and late life conditions.

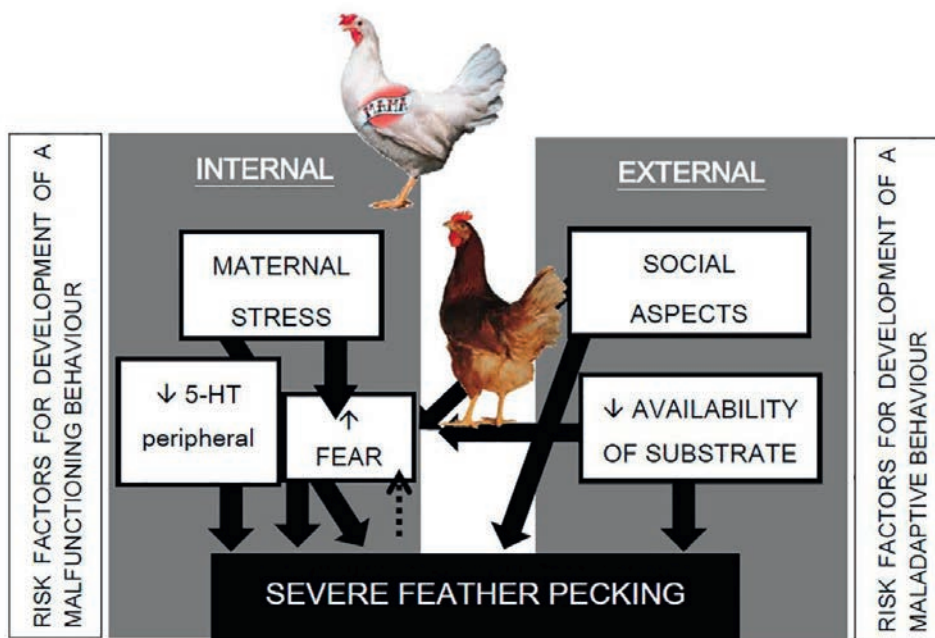
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7.3.1 GENETIC DIFFERENCES IN THE TENDENCY TO DEVELOP FEATHER PECKING

In chapters 4 and 5, DW hens had higher levels of feather damage and lower peripheral 5-HT levels than ISA hens. The differences between DW and ISA hens may derive from selection on different egg production traits. Selection for production traits may have coincided with selection on SFP, as seem to be the case in lines differing in egg production traits (Blokhuis & Beutler, 1992; Blokhuis and Beuving, 1993; Korte et al., 1997; 1999; Jones et al., 1995; van Hierden et al., 2002; 2005; chapters 4 and 5). Supporting these findings are the many correlations we found between fearfulness, CORT, feather damage and production traits (chapter 4). The tendency to develop SFP in hens of different genetic origins can, however, be influenced by specific environmental conditions, as seen in chapters 4 and 5

In the ISA cross, substrate limitation influenced SFP and substrate disruption influenced fearfulness and peripheral 5-HT, whereas in the DW cross under similar conditions these effects were not found (chapter 5). Also, in the ISA PS, a large group size (i.e. social aspects) affected mortality and feed conversion levels (chapter 4), while in DW PS these effects were not found. The manifestation of SFP in hens of a DW cross and hens of an ISA cross can be explained in terms of malfunctioning behaviour and maladaptive behaviour, respectively [see Figure 7.1 for an amendment to the theoretical model]. Malfunctioning behaviour describes a behaviour which fails to have a function while maladaptive behaviour describes a behaviour which occurs when adaptation fails (Mills, 2003).

Figure 7.1. Amendment to the theoretical model (chapter 1) of the risk factors in the development of severe feather pecking (SFP) based on Dekalb White (DW: white hen) and ISA Brown (ISA: brown hens).



DW hens show to have a high (epi)genetic predisposition (see "MAMA" tattoo on white hen) to develop high fear levels and low peripheral 5-HT levels, which creates a risk for development of SFP as a malfunctioning behaviour. ISA hens (brown hen) are strongly influenced by social factors (large group size) and lack of substrate availability which creates a risk for development of SFP as a maladaptive behaviour (chapters 4 and 5). Social aspects (a fearful hen in a group) can influence fear levels (chapter 2) and SFP directly (chapters 5 and 6), see Table 7.2. A lack of availability of substrate can lead to SFP. Solid thick black arrows shows relations found in this thesis, dashed black arrow shows reciprocal effect found by others.

THE DEKALB WHITE AND ITS MALFUNCTIONING BEHAVIOUR

The occurrence of a malfunctioning behaviour is indicative of brain dysfunction, meaning that in any context the behaviour and its neural basis have no functional value (Mills, 2003). Malfunctioning behaviour is also referred to as a (psycho)pathology (Korte, 2001). When we sum the outcome of the studies in this thesis, we see that DW hens have high levels of fear (especially fear of humans: chapters 4, 5 and 6), can be interpreted as being distressed (chapter 4) and have low levels of peripheral 5-HT (chapter 5 and 6) compared to ISA hens. Likewise, Uitdehaag et al. (2009; 2011) recorded that hens from a WL origin (from which DW descends) had higher fear levels in several behavioural tests involving humans compared to RIR hens (from which ISA descends). WL hens also had lower central and peripheral 5-HT levels, higher DA, 5-HT turnover, and 5-HIAA levels which is the metabolite of 5-HT (Uitdehaag et al., 2011). These results may indicate higher metabolic 5-HT activity in adult WL hens compared to adult RIR hens. Besides a genetic propensity to develop SFP, stress in the PS also created high SFP and anxiety in DW chicks (chapter 5). As DW hens showed to be highly fearful of humans (chapters 4, 5 and 6), stress due to negative interactions/associations with humans may induce anxiety and should be diminished to prevent SFP to develop and trans-generational effect of high levels of stress to occur. Trans-generational effects of stress were only recorded in DW hens and not in ISA hens (chapter 5). Altogether, the findings of this thesis indicate that hens descending from a WL origin are more at risk to develop anxiety and become stress-sensitive, and thereby are more prone to develop SFP compared to hens descending from an RIR origin [see Figure 7.1].

THE ISA BROWN AND ITS MALADAPTIVE BEHAVIOUR

Maladaptive behaviour specifically refers to lack of adaptability to an inadequate environment (Mills, 2003). Under lack or limited availability of substrate SFP developed (chapter 5). Especially lack of substrate induced SFP at five weeks, as SFP decreased when lack of substrate was abolished at a later age (chapter 5). A maladaptive behaviour does not necessary persist over time or develop into a malfunctioning behaviour, if the environment provides opportunities for complete adaptation (Garner and Mason, 2002). For example, supplying (foraging) enrichment can reduce the occurrence of maladaptive behaviours (i.e. in mink: Malmkvist et al., 2013; bears: Maslak et al., 2013; pigs: Spoolder et al., 1995; Zonderland et al., 2011; parrots: van Zeeland, 2013). However, enrichment does not always reduce maladaptive behaviour (Newberry, 1995), especially if the behaviour is performed in excess (Mason, 1991; Rushen et al., 1993) and in the case of an extreme outbreak of SFP (Dixon, 2008) by many birds in a flock. Supplying adequate foraging substrate thus shows to be an important preventive measure for SFP to occur as a maladaptive behaviour (chapter 5).

In this thesis, birds from an ISA cross appeared to be more sensitive to develop maladaptive behaviour, while DW birds appeared to be more sensitive to develop malfunctioning behaviour [Figure 7.1]. First of all, ISA pullets but not DW pullets showed higher SFP during rearing when substrate was limited (chapter 5). Second, ISA pullets had higher fearfulness - which may indicate stress or originate from stress - in an environment where substrate was disrupted compared to DW pullets (chapter 5), and thus seem to react more strongly to their inadequate environment. Supporting this statement is the finding that ISA pullets' fear levels were positively correlated to their peripheral 5-HT levels in an environment where substrate was disrupted (chapter 5), indicating that the

environment affected ISA hens physiologically more than it did in the DW hens. Part of the procedure of disruption in substrate consisted of handling and mixing birds: farmers take away the cardboard paper and, during that procedure chicks are taken from 2nd to the 1st level of the tier, and are thus housed with unknown chicks. For both crosses, these procedures would be similar, but only ISA pullets became more anxious as measured in the novel environment test (by longer duration of inactivity). Again this indicates that only ISA pullets, but not DW pullets, were affected by their social environment. Supporting this is the finding that when RIR hens (pure line of ISA) were housed with unknown birds of a WL line, they became more fearful and started SFP directed at the WL birds. These effects, however, appear specific for hens from a RIR background. When mixing a HFP and LFP line originating from a WL background, no differences in SFP have been found in SFP between strains in mixed-housed and single strain-housed groups (McAdie & Keeling, 2002). ISA PS flocks were also affected by their social environment (chapter 4). In ISA PS but not in DW PS flocks, production parameters were affected by group size. In ISA PS flocks there was also an eight-fold higher incidence of smothering compared to DW PS flocks. In chapter 4 it was discussed that smothering may occur due to social adherence. Social adherence can influence behavioural synchrony (a major source of clustering: Appleby et al., 2004; Collins et al., 2011), and inter-bird distance (Keeling & Duncan, 1991). A small inter-bird distance (which can differ between genetic crosses: Keeling & Duncan, 1991) can increase transmission of behaviour (Nicol, 1989; 1995). Subsequently, a small inter-bird-distance in ISA flocks may generate a higher risk for behaviour transmission or social facilitation of SFP than in DW flocks with a supposedly larger inter-bird distance. Altogether these findings in ISA hens indicate that, compared to DW hens, the environment can strongly

affect their behaviour and physiology which leads to a high risk of maladaptive behaviour to occur under inadequate environmental conditions [Figure 7.1].

7.3.2. PARENTAL EFFECTS IN THE DEVELOPMENT OF OFFSPRING BEHAVIOUR

Various authors have addressed the importance of assessing parental effects on behavioural development of commercially housed laying hens (Henriksen et al., 2011b; Henriksen, 2012; Guibert et al., 2013; Rodenburg et al., 2013). To date, very limited attention has been given to this issue in commercial flocks. That there is an underestimation of potential PS effects is shown by the strong effects of maternal stress, 5-HT and feather damage on farm level on offspring early life behaviour (chapter 5). The results of chapters 4 and 5 are therefore not only important for the commercial practitioners dealing with laying hens but also for the scientific community, as these effects could be similar in other oviparous species.

High maternal CORT levels in the PS, which could be a sign of stress, were related to a low egg weight (chapter 4) and to high SFP and high fearfulness in one-week-old DW chicks (chapter 5). High CORT levels in the mother birds can induce high fearfulness and competitiveness in the offspring (Janczak et al., 2007b). The effects of high maternal CORT may help the offspring prepare for living under stressful conditions (the predictive adaptive response theory: Gluckman et al., 2005; Bateson, 2007). High fearfulness can be adaptive in an environment with many predators, while competitiveness can be adaptive in an environment with limited resources. However, this is not the case under commercial rearing conditions. The strong effects of maternal physiology on offspring behaviour found in the DW cross could be transmitted via egg hormones. First, it has been shown that maternal stress can influence yolk-

hormone levels and gene-expression patterns involved in the stress response (hens: Nätt et al., 2009; Henriksen et al., 2011a; Goerlich et al., 2012 and quail: Guibert et al., 2010; 2011; 2012). Second, it is known that when high CORT levels are induced by CORT implants, hens of a WL origin differ from ISA hens in egg hormone levels and production parameters (Henriksen et al., 2011a). Egg hormones have been put forward as a major force behind inheritance of gene-expression patterns involved in the stress response (Guibert et al., 2012; 2013; Nätt et al., 2009; Ho and Burggren, 2010; Goerlich et al., 2012). Preliminary data analysis of testosterone levels in the eggs of PS flocks showed differences between farms which might relate to stress-physiology of the hens and behaviour of the offspring as seen under experimental conditions (Guibert et al., 2010; 2011; 2012; Henriksen et al., 2012; Goerlich et al., 2012; a tendency: Nätt et al., 2009). This indicates that further research on hormonal content of eggs from commercial PS is needed to gain more understanding of the mechanisms of PS effects in the development of SFP and fearfulness. In conclusion, the study in chapter 5 shows, on a farm level, that maternal stress can lead to development of SFP and fearfulness in the offspring (especially for the DW cross), and thus high stress levels should be prevented to reduce the risk of SFP to occur.

High levels of feather damage, fear, and stress in the parental birds may originate from their housing conditions. As seen in the study on the laying farms (chapter 6), a floor housing system can lead to higher flock level of feather damage than an aviary housing system. Aviary systems enable possibilities for exploration and hide-outs on different levels, for example for PS hens in the case of excessive reproductive attempts of roosters and from being feather pecked. Having escape opportunities will likely improve hens' welfare and reduce stress. By reducing stress, the performance of hens may benefit, considering the

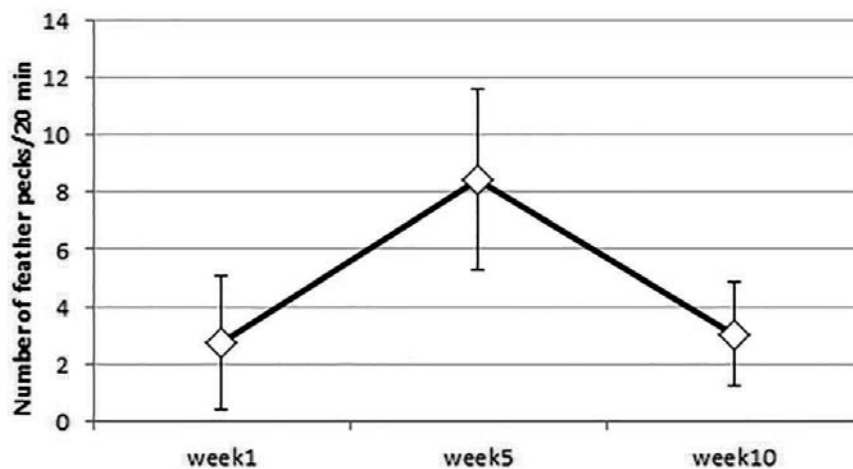
negative correlations found between fear, CORT and egg weight and body weight (chapter 4). Knowledge from the study on commercial laying hen farms (chapter 6) can supply further suggestions for management improvements in PS flocks. For example, laying hen farms where more pecking possibilities were offered (aerated blocks) and where measures were taken to reduce fear (radio playing) resulted in lower feather damage in a flock (chapter 6) than farms with standard management. Providing foraging possibilities has been shown to reduce feather damage on a flock level also in other studies of laying hens (Lambton et al., 2010; 2013; Gilani et al., 2013; Bestman et al., 2009). These measures can potentially be transferred to PS farms to stimulate foraging pecking, and reduce fear and stress thereby improving bird welfare.

Another possibility to reduce fearfulness and stress-sensitivity in PS flocks is the implementation of a temporary separation of hens and roosters. In broiler breeder flocks, a so-called “quality-time” method has been studied in which at certain times roosters are blocked from access to the hens (Van Emous and Gunnink, 2011). In the “quality-time” flocks, more successful matings, more voluntary matings by hens, and less aggression occurred than in standard flocks. At the same time, fearfulness and high levels of stress in the roosters may also influence the offspring. Paternal effects have been reported on offspring behaviour and stress sensitivity (Goerlich et al., 2012), and on gene-expression and DNA-methylation (Nätt et al., 2011). Roosters in PS flocks may also exhibit stress due to living in an unnatural environment under high density (De Jong et al., 2011), by being exposed to humans, housed with many other roosters (Bilčík and Estevez, 2005), and by being the target of SFP by the hens (Riedstra et al., 2013). As fear tests were conducted on flock level, the response to novel object and a stationary person also incorporates the response of the roosters in the flock

(chapter 4). The body weight of the roosters may also be affected by fear levels as was the case in the hens (chapter 4). Roosters' body weight was not accurately recorded by many farmers, which highlights the undervaluation of the importance of the roosters' wellbeing. Catching and handling roosters can be difficult as they tend to be aggressive towards humans, which might also be a reason why their body weight was not recorded by farmers. Nevertheless, as PS flocks consist of roosters and hens, equal care and attention should be given to improve the welfare of both sexes. Fearfulness, stress-sensitivity and SFP in PS flocks can potentially be improved by providing appropriate housing, fear-reducing stimuli (e.g. a radio playing), and aerated blocks for pecking.

7.3.3. EARLY LIFE ENVIRONMENT – REARING PERIOD

During rearing, lack of substrate increased SFP and fearfulness (chapter 5). High SFP and fearfulness were related to high levels of feather damage and fearfulness in later life (chapter 6). These results support the suggestion, given in chapter 1, that early life conditions can have long lasting effects on behaviour. In chapter 1, I addressed the lack of knowledge about a sensitive period for the development of SFP. Feather pecking behaviour was recorded at 1, 5 and 10 weeks of age. At 5 weeks of age, a peak of GFP and SFP was noted (Figure 7.2).

Figure 7.2. Severe feather pecking during the rearing period in age of birds in weeks.

In the studies in this thesis, five weeks of age appears a critical time-point for development of SFP. This is supported by a study of Huber-Eicher and Sebo (2001a), where at 5 weeks of age high frequencies of SFP were recorded (>50 pecks per 30 individuals during 30 min). However, lack of substrate continuation prior to 5 weeks of age did significantly influence SFP and GFP display at 5 weeks of age (chapter 5), as also seen by Huber-Eicher and Sebo (2001b). A lack of substrate in the first 3 weeks of life can increase SFP and GFP during rearing and can lead to high SFP and feather damage in later life (Gunnarsson et al., 1999; Nicol et al., 2001b; Bestman et al., 2009; de Jong et al., 2013; Huber-Eicher and Sebo, 2001a; 2001b). It appears that especially the first 3 weeks of a chick's life are a sensitive period for behavioural development and for brain synapse formation (Rogers, 1994; 1995; Atkinson et al., 2008). As laying hen chicks are precocial, they are rather independent in finding their food, but do use

information from conspecifics. For young chicks, it is thus extremely important that they are provided with a foraging substrate from hatch onwards so they learn where and what to peck, and do not develop a preference for pecking feathers nor learn to perform SFP from others. Prevention of SFP during the rearing period appears very important in prevention of feather damage during rearing and the laying period (i.e. SFP at five weeks of age explained 29% of the variation in feather damage at 40 weeks: chapter 6).

High fear of humans during the rearing period was related to high fear of humans and feather damage during the laying period (chapter 6). Commercial pullets are exposed to many potentially negative interactions with humans i.e. handling, mixing, vaccination and catching for transport. These interactions may lead to sustained high fear of humans. Fear of humans can be reduced by habituation and positive association with humans (Barnett et al., 1994). A measure, which potentially could have a positive effect on feather damage in the laying period by its effect on reducing fearfulness during rearing, is exposure to sounds (chapter 6). This was also found by Gilani et al. (2013). Although, sound exposure during the rearing period did not remain significant in the analysis of feather damage during the laying period (chapter 6), management factors during rearing can influence feather damage during the laying period (Lamton et al., 2010; Bestman et al., 2009; Gilani et al., 2013). It is therefore important to provide an optimal environment during the rearing and laying period, in relation to SFP and prevention/reduction of fearfulness. The latter appears extra important for DW hens while the former appears extra important for ISA hens [see Figure 7.1].

7.3.4 LATER LIFE CONDITIONS – LAYING PERIOD

During the laying period, feather damage was influenced by housing system, group size and management of the farmer (chapter 6). Lower levels of feather damage were found in flocks housed in an aviary system, in small flocks and in flocks receiving an adjusted management compared to flocks housed in a floor system, in large flocks and flocks kept under standard management, respectively. Specifically, when farmers provided extra pecking material (also shown by Gilani et al., 2013; Lambton et al., 2010) and applied measures for fear reduction (radio playing, roosters in the flock (also shown by Bestman and Wagenaar, 2003)), levels of feather damage were lower compared to standard management (chapter 6). These management strategies are likely to be extra important for large flocks housed in a floor housing system.

High fear of humans during the laying period was related to high levels of feather damage during the laying period (chapter 6). In the PS, fear for humans was related to low body weight and egg weight (chapter 4). Fear for humans can negatively affect production (Barnett et al., 1992). Reducing or preventing fear of humans both during the rearing and laying periods can have positive effects on production (Barnett et al., 1994), fearfulness and feather damage. Farmers should be aware of the negative effects of high fearfulness, and adjust their management in order to reduce high fearfulness in their flocks.

Feather damage at 40 weeks of age could not be related directly to substrate conditions on the laying or rearing farm. However, substrate disruption and limitation during the rearing period led to high SFP at five weeks of age, which in turn related to high feather damage at 40 weeks of age. In other studies, it was found that substrate conditions during the rearing as well as laying periods

can influence feather damage (Gunnarsson et al., 1999; Nicol et al., 2001a; 2001b, de Jong et al., 2013). These studies indicate the importance of supplying adequate foraging substrate throughout the life of a hen. Although influence of substrate could not be directly shown to affect feather damage during the laying period (chapter 6), I would hypothesize that when a hen's need to forage is thwarted this leads to frustration which requires adaptability of the hen to deal with a suboptimal environment. As mentioned under maladaptive behaviour (7.2.2), when hens cannot cope with their suboptimal environment (i.e. substrate disruption and limitation) they can develop a maladaptive behaviour like SFP. A maladaptive behaviour is a sign of reduced welfare, and to maintain and improve bird welfare it is important to supply foraging substrate, especially as foraging is the most important behaviour for laying hens (Dawkins, 1989). After all, we house laying hens under quite unnatural conditions whereby we challenge their adaptive capacity already. Therefore it is our responsibility as caretakers to provide the materials hens need to optimally thrive under these conditions and prevent them from developing behaviour (i.e. SFP) caused by suboptimal living conditions.

CONCLUSIONS

This thesis has shown that the tendency to develop SFP can be influenced by genetic origin, maternal stress and the conditions during the rearing and laying periods. It supplied evidence that - also on a flock level - high fearfulness related to the tendency to develop severe feather pecking (SFP), indicating that high fearfulness is a risk factor for the development of SFP under commercial conditions. By comparing genetic strains it was recorded that fearfulness and the dopaminergic and serotonergic systems are involved in the propensity to develop SFP. Additionally, it was found that hens of an ISA brown cross are sensitive to become fearful and develop SFP under suboptimal substrate conditions and an unstable social environment, while hens of a Dekalb White cross have a high risk to become anxious and may develop SFP as a form of malfunctioning behaviour. In DW parental flocks, hens exhibiting high levels of stress and feather damage induced high fearfulness and SFP in their chicks. Early life occurrence of SFP was found to be induced by lack of substrate continuity during the rearing period further increased feather damage during the rearing and laying periods. If all farmers in the chain supply optimal substrate for pecking and implement fear-reducing measures, the risk of SFP to occur will be reduced.



Practical implications for the laying hen production chain

In this thesis, it was shown that the development of severe feather pecking (SFP) in laying hens can derive from previous parts in the laying hen chain as well as from the housing conditions of laying hens. To prevent SFP, farmers, consultants, hatcheries and breeders need to be aware of the risk factors in the development of SFP.

RISK FACTORS

- The risk factors are: stress and feather damage in the parent stock (DW), limitation and disruption of litter during the rearing period, large group size, high local density due to floor or level housing and high fearfulness both during the rearing and laying period.

MANAGEMENT

Management focusing on fear reduction and optimizing foraging behaviour of laying hens should be made common practice in the whole laying hen production chain. As such, in all parts of the laying hen production chain, farmers should provide adequate foraging material and fear reduction measures on their farm.

- Suggestions for management adjustments for improving foraging behaviour are: providing aerated concrete blocks (this thesis), providing litter from early life onwards or outdoor-range access (other studies).
- Suggestions for management adjustments to reduce fearfulness are: sound exposure (radio playing), habituation to humans, positive association with humans (i.e. supplying grain) and improving predictability of the environment (i.e. using cues to predict exposure to humans).

GENETIC BACKGROUND

In this thesis, hens of Dekalb White (DW) origin differed from hens of an Rhode Islans Red (RIR) origin in their predisposition to develop SFP (i.e. DW: high fear and stress sensitivity and RIR: environmental challenges).

- The management of hens should be adapted to the genetic cross of their hens. Further research on genotype*environment interactions could help in assessing breed differences in different environments.

- Breeders and hatcheries should establish appropriate group sizes, in particular for ISA hens.

PARENTAL EFFECTS

In this thesis, it was found that indicators of stress in the parent stock (PS) related to high fear and SFP in their rearing flocks. Breeders, hatcheries, and farmers keeping PS should be aware of these parental effects. Factors to be considered for PS are:

- Fear and stress can reduce egg weight, body weight and feed intake. Information on production parameters could be useful as early indicators of high fear and stress in PS.
- A more elaborate assessment of hatchability of the PS by calculation % of hatched eggs, fertilized eggs, number of hatched and non-hatched males and females, and body weight measures can give more insight in potential PS effects.
- Currently, eggs from different PS flocks from the same farm are not labelled uniquely prior to incubation. However, to assess PS effects more accurately, it should be possible to link rearing flock and parent stock flock. For example, PS flocks from different houses could be incubated separately so as to be able to determine difference between houses and effects on development of the rearing flocks
- Other studies show that roosters can also influence offspring development. Farmers keeping PS flocks should therefore monitor the behaviour, feather cover and body weight of roosters in order to monitor and reduce stress in their roosters.

Summary

Billions of laying hens are kept worldwide. Severe feather pecking (SFP) is a behaviour which occurs with a high prevalence on commercial farms. SFP, the pecking and plucking of feathers of another bird, induces pain and stress and can ultimately lead to cannibalism. Moreover, SFP can occur if a bird is unable to cope with fear and stress and is living in an inappropriate environment. SFP thus reduces the welfare of many laying hens worldwide. To prevent SFP it is essential to know the risk factors in its development. To that aim, first, two experimental studies were conducted to gain insight in the principles of SFP, and three on-farm studies were conducted to assess the risk factors of SFP under commercial conditions.

THE PRINCIPLES

Factors which relate to SFP are high fearfulness as young and low levels of brain and peripheral serotonin (5-HT) and brain dopamine (DA). Furthermore, commercial laying hen lines can differ in SFP tendencies and associated traits indicating that SFP has a genetic component. In chapters 2 and 3, fear response as young and adult, and stress response, 5-HT and DA brain levels as adult were compared in hens of two lines: the low mortality line (LML) selected on low levels of mortality due to cannibalism and individual performance vs. the control line (CL) which was selected on individual performance only. Hens were exposed to an Open Field (OF) test at 5 weeks of age and a Manual Restraint (MR) test at 33 weeks of age. At 33 weeks of age, levels of corticosterone (CORT) post MR and 5-HT and DA levels in four brain areas were determined. Hens of the LML were less fearful at both ages and had lower levels of DA in the arcopallium, a somatomotor area involved in fear and motor control, compared to hens of the CL. In chapter 2, it was also shown that fearful chicks had higher levels of CORT and higher activity levels as adult, compared to non-fearful chicks. Moreover, presence of fearful animals in the group was related to average CORT levels of their pen members. Fearful hens may induce social instability in a group, and thereby affecting the stress-sensitivity of their group mates. These results indicate that groups differ in levels of fear and stress-sensitivity, and that fearfulness at a young age can lead to stress-sensitivity as adults, which create a risk for development of SFP.

THE PRACTICE

In chapters 4, 5 and 6, the laying hen production chain consisting of parent stock, rearing flocks and laying flocks was studied. Risk factors for SFP could originate from previous parts in the chain. Therefore, in all on-farm studies, measurements of SFP, fearfulness, basal CORT and peripheral 5-HT system were obtained, and related to housing conditions and to previous parts in the chain. Fearfulness was assessed, on a flock level, by distance to a stationary person (SP) test and latency of bird to approach a novel object (NO). Dekalb White (DW) and ISA brown (ISA) crosses whose pure lines differ in levels of fear, CORT, 5-HT and DA, were compared. First, parent stock (PS) flocks were studied and associations between production performance and measurements of fear, stress and 5-HT were conducted and related to group size conditions (chapter 4). Second, rearing flocks originating from PS flocks were studied throughout the rearing period (chapter 5). High levels of feather damage, CORT and 5-HT in the mothers were related to fearfulness and SFP in their offspring at flock level. Especially, a large flock size and limitation and/or disruption in litter supply affected SFP and levels of fearfulness and 5-HT (chapter 5). Finally, high levels of feather damage during the laying period were related to high SFP rearing, and high fearfulness during rearing and laying (chapter 6). These studies together aimed to determine the risk factors for the development of SFP and the resulting feather damage. The main outcomes of these studies are as follows.

➤ Parent stock flocks

DW flocks were more fearful of an SP and hens had higher levels of feather damage than in ISA flocks. ISA flocks, in turn, were more fearful of the NO and hens had higher 5-HT levels than in DW flocks. A small flock size led to higher feed conversion, mortality levels, and smothering events in ISA but not in DW flocks. These results indicate that DW and ISA PS flocks differ in levels of fear and feather damage, and respond differently to their social environment. For both crosses, fear of an SP related to high mortality and fear of the NO related to low hen body weight, egg weight, and feed intake. High basal CORT related to low egg weight. High fear and stress levels in PS flocks may, thus, negatively affect

(re)production, and thereby potentially negatively influence the developing embryo.

➤ Rearing flocks

In the DW cross, high CORT, feather damage, and 5-HT of mother hens related to high SFP and fearfulness of their rearing flocks at 1 week of age. At 5 weeks of age, a peak in both gentle feather pecking (GFP) and SFP was recorded, coinciding with a disruption in substrate availability (i.e. a temporal absence of substrate) and a limitation of substrate (i.e. limited amounts of substrate provided) in some of the farms. Especially, ISA pullets showed higher SFP under substrate limitation and became more fearful under substrate disruption than DW pullets. ISA pullets had higher 5-HT levels than DW pullets. Only in the ISA cross, high 5-HT related to high fearfulness, specifically under substrate disruption. For both crosses, high fearfulness was related to high feather damage. Furthermore, in a level system (floor system where levels are gradually added) higher levels of SFP and feather damage were found compared to an aviary system (a tier-system with cages and litter area). These results highlight that; 1) parental effects exist in the development of fearfulness and SFP, 2) disruption and limitation in substrate availability can lead to high SFP at 5 weeks of age, 3) ISA pullets are more strongly influenced by environmental conditions than DW pullets and 4) a level housing, which coincided with a large group size, increase the risk of SFP and feather damage during rearing.

➤ Laying flocks

In our sample, 49% of the laying flocks had severe damage at 40 weeks of age, compared with 71%, 65% and 53% of the rearing flocks at 15, 10 and 5 weeks of age, respectively. High fear of a SP at rearing and high SFP at 5 weeks of age related to high levels of feather damage at lay. In a floor system and at a large flock size higher levels of feather damage were recorded than in an aviary system and at a small flock size. An adjusted management on the laying farm (i.e. aerated blocks, presence of roosters or a radio playing) reduced levels of feather damage compared to standard management. DW flocks were more fearful of the SP and NO than ISA flocks. This study showed that factors during rearing and laying contributed to feather damage at 40 weeks of age.

Summary

With the knowledge from the experimental and on-farm studies in this thesis, an assessment of the risk factors for SFP could be established. Risk factors for SFP are: high fear, stress and feather damage in DW parent stock, high fear of humans, especially for DW hens, litter disruption or limitation during rearing, large group sizes, and a floor or level system.

Samenvatting

Wereldwijd worden er biljoenen leghennen gehouden voor de productie van eieren. Ernstig verenpikken (EV) is een gedrag dat voorkomt met een hoge prevalentie op commerciële legghenbedrijven. Het pikken en plukken aan veren, en het uittrekken van veren van een groepsgenoot, veroorzaakt pijn en stress in het slachtoffer en kan uiteindelijk leiden tot kannibalisme. Daarnaast komt EV voor wanneer een kip niet kan omgaan met de stress en angst van het leven in een onnatuurlijk omgeving. EV reduceert dus het welzijn voor een groot aantal leghennen wereldwijd. Om EV te voorkomen is het essentieel om te weten wat de risico factoren zijn in de ontwikkeling ervan. Om dat doel te behalen, werden er twee experimentele onderzoeken uitgevoerd om meer inzicht te verkrijgen in de principes onderliggend aan EV, en drie bedrijfsonderzoeken werden uitgevoerd om de risico factoren van EV onder commerciële condities in kaart te brengen.

DE PRINCIPES

Factoren die gerelateerd zijn aan EV bij leghennen zijn hoge angstigheid en een verlaagd niveau van brein en perifere serotonine (5-HT) en brein dopamine (DA). Verder blijkt dat commerciële legghen kruisingen kunnen verschillen in de gevoeligheid tot het ontwikkelen van EV, wat aantoont dat EV een genetische component heeft. In hoofdstuk 2 en 3, werden de angstreacties bij jonge en volwassen hennen vergeleken, alsmede de stress-reactie, 5-HT en DA niveaus in twee genetische kippen lijnen: de lage uitval lijn (low mortality line: LML) en een controle lijn (CL). De LML kippen waren geselecteerd op individuele eiproduktie en lage uitval door lage niveaus van kannibalisme, terwijl de CL kippen alleen op individuele eiproduktie waren geselecteerd. De kippen werden blootgesteld aan een Open Veld (Open Field: OF) test op 5 weken leeftijd en een Manuele bewegingsbeperkingstest (Manual Restraint, MR) op 33 weken leeftijd. Het niveau van het stress-hormoon corticosterone (CORT) in het bloedplasma als gevolg van de MR test en niveaus van 5-HT en DA in vijf brein gebieden werden bepaald. Hennen van de LML waren, in vergelijking met de hennen van de CL, minder angstig zowel als jong als volwassen hen, en hadden lagere niveaus van DA in het arcopallium, een breingebied welke controle van angst reacties regelt. In hoofdstuk 2 werd ook aangetoond dat angstige kuikens een hoger niveau van CORT hadden en actiever waren als volwassen kip, in vergelijking met minder

angstige kuikens. Daarnaast werd aangetoond dat de aanwezigheid van een angstig dier in de groep, het groeps-gemiddelde van het stresshormoon CORT verhoogd. De oorzaak hiervan kan zijn dat angstige dieren sociale instabiliteit veroorzaken in de groep en daarmee de stress-gevoeligheid van hun groepsgenoten beïnvloed. Deze resultaten laten zien dat groepen verschillen in angstigheid en stress-gevoeligheid en dat hoge angstigheid op jonge leeftijd kan leiden tot verhoogde stress-gevoeligheid wat een risico kan veroorzaken in de ontwikkeling van EV.

DE PRAKTIJK

In hoofdstuk 4,5 en 6 werd onderzoek gedaan naar EV in de legghen-productie keten. De keten bestaat uit ouderdier bedrijven, opfok bedrijven en legbedrijven. Risico factoren die EV kunnen veroorzaken kunnen voortkomen uit de voorgaande schakels in de keten. Daarom werden in alle bedrijfsbezoeken metingen uitgevoerd gerelateerd aan EV, angstigheid, stress-gevoeligheid en het serotonerge systeem. Deze metingen werden gerelateerd aan huisvestings-condities en aan de voorgaande schakels in de keten. Op koppel niveau werden angst testen uitgevoerd, waarbij de latentie tijd die kippen nodig hebben om een vreemd voorwerp (novel object: NO) en stilstaand persoon (SP) te naderen, werd bepaald. Twee commerciële kruisingen, de Dekalb White (DW) en ISA brown (ISA), werden vergeleken, waarvan is bekend dat de pure lijnen (voorouders) verschillen in angstigheid, 5-HT en DA en stress gevoeligheid.

Allereerst werden de ouderdier bedrijven onderzocht, en associaties tussen productie parameters en metingen van angst, stress and 5-HT werden uitgevoerd. Deze metingen werden gerelateerd aan de groepsgrootte van de koppel (hoofdstuk 4). Hieropvolgend werden de opfokkoppels, afkomstig van de ouderdier bedrijven, onderzocht tijdens de opfokperiode (hoofdstuk 5). Hoge niveaus van veerschade als gevolg van EV, CORT en 5-HT bij de moeder kippen relateerde aan hoge angstigheid en EV bij de kuikens. In het bijzonder bleek dat een grote groepsgrootte en een tijdelijke beperking of limitatie in scharrelmateriaal EV, alswel niveaus van angst, verergerde bij de kuikens (hoofdstuk 5). Daarnaast bleek dat ernstige veerschade tijdens de legperiode gerelateerd was aan een hoog niveau van EV en angstigheid tijdens de opfokperiode (hoofdstuk 6).

Deze studies tezamen hebben inzicht verschaft in de risicofactoren van EV en de daaruitvolgende veerschade. De belangrijkste bevindingen zijn als volgt:

➤ Ouderdierbedrijven

Koppels van de witte kruising (DW) waren angstiger voor een vreemd persoon en hadden meer hennen met ernstigere veerschade dan koppels van de bruine kruising (ISA). Daar tegenover waren ISA koppels angstiger voor het vreemde object en hadden hogere niveaus van 5-HT dan DW koppels. Een kleine groepsgrootte leidde tot een hogere voedselconversie, uitvalpercentage en incidenties van verdrukkingen in ISA koppels maar niet in DW koppels. Deze resultaten laten zien dat DW en ISA ouderdierkoppels verschillen in niveaus van angst, veerschade, en dat zij verschillend reageren op hun sociale omgeving. Voor de ISA kruising bleek dat angst voor een vreemd persoon gerelateerd was aan hogere uitval en voor de DW kruising bleek angst voor een vreemd voorwerp gerelateerd aan laag lichaamsgewicht voor de hennen, eigewicht en voedsel opname. Een hoog basaal niveau van CORT relateerde aan een laag gemiddeld eigewicht. Hoge niveaus van angst en stress in ouderdierkoppels kunnen dus een negatief effect hebben op de (re)productie en daarbij potentieel een negatieve invloed hebben op het ontwikkelende embryo in het ei.

➤ Opfokkoppels

In de DW kruising bleek een hoog niveau van CORT, veerschade en 5-HT in de moederdieren te relateren aan hoog EV en angstigheid bij de kuikens op één week leeftijd. Op vijf weken leeftijd vonden we een piek in zowel zacht- als ernstig verenpikken, wat samenviel met een tijdelijke onderbreking en beperking van scharrel materiaal in de eerste vier weken. In het bijzonder werden de ISA kuikens hierdoor beïnvloedt. Zij vertoonden meer EV onder beperking van substraat en werden angstiger door een tijdelijke onderbreking van substraat. De ISA kuikens hadden ook hogere 5-HT niveaus dan DW kuikens. En, alleen in de ISA kuikens relateerde 5-HT aan angstigheid, specifiek onder de condities van substraat beperking. In beide kruisingen werd gevonden dat angstigheid gerelateerd is aan EV. Daarnaast werden hogere niveaus van EV gevonden in een niveau-varia systeem (een grond stal waarin plateau's geleidelijk worden toegevoegd) in vergelijking met een traditioneel voliere systeem (een battery kooi

systeem in rijen, welke geopend wordt en een scharrelruimte wordt gecreeërd in de loopgang tussen de rijen). Deze resultaten belichten dat; 1) ouderdieren effect kunnen hebben op de ontwikkeling van angstigheid en EV bij hun kuikens, 2) dat een onderbreking en beperking van sustraat in het vroege leven kan leiden tot hoog verenpikken tijdens de opfok, 3) dat ISA kuikens sterker worden beïnvloed door de omgevingscondities in vergelijking met DW kuikens, en 4) dat het huisvestingsstelsel, wat mogelijk samenhangt met een grote groepsgrootte en hoge dichtheid, een risico kan vormen tot het ontstaan van EV tijdens de opfokperiode.

➤ Legkoppels

In dit onderzoek bleek dat 49% van de bezochte legkoppels ernstige veerschade had op 40 weken leeftijd, in vergelijking met opfokkoppels was dit 71%, 65% en 53% op 15, 10 en 5 weken leeftijd. Hoge niveaus van EV op 5 weken leeftijd en hoge niveaus van angst tijdens de opfokperiode relateerde aan hoge niveaus van veerschade tijdens de legperiode. In een grond systeem en in grote koppels was er meer veerschade dan in een voliere systeem en kleine koppels. Een aangepast management op het legbedrijf (zoals het verspreiden van gas beton blokken, het houden van hanen in de koppel en het hebben van een radio-geluid) reduceerde het niveau van veerschade in vergelijking met standaard management. De DW koppels waren angstiger voor zowel het vreemde voorwerp als de vreemde persoon in vergelijking met ISA koppels. Deze studie toont aan dat factoren tijdens de opfok en leg periode tezamen bijdragen aan veerschade op 40 weken leeftijd bij leghennen.

Met de kennis opgedaan uit dit project is het mogelijk om risicofactoren voor EV aan te tonen. De risico factoren zijn, hoge niveaus van angst, stress en veerschade in de ouderdieren, met name in DW ouderdierkoppels. Voor beide kruisingen: angst voor mensen, onderbreking en beperking van scharrel materiaal tijdens de opfok, grote groepsgrootte en het huisvesten in een grond systeem. Hieruit blijkt dat alle schakels in de keten een invloed hebben op EV en dat daarom ook samen moet worden gewerkt aan de oplossing ervan.

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- De Haas E.N., Bolhuis J.E., de Jong I.C., Kemp B., Janczak A.M., Rodenburg T.B.. Is it the past or is it present? Effects of the rearing environment and laying environment on feather damage in commercially housed laying hens. Submitted to Applied Animal Behaviour Science (17-03-2014). Under review (Chapter 6)

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UNPUBLISHED PEER REVIEWED ABSTRACTS OF SCIENTIFIC PRESENTATIONS

- De Haas E.N. Pro-active coping style – a predisposition for feather pecking in laying hens? Annual Meeting of the Netherlands Society of Behavioural Biology Netherlands, Dalfsen, The Netherlands. 25-27 November, 2009.
- De Haas E.N. Nielsen B.L., Buitenhuis A.J., Rodenburg T.B. Peckers like to peck. Selection for feather pecking leads to increased pecking motivation in laying hens. WIAS Science Day, Wageningen, The Netherlands, 28th of January, 2010.

List of publications

De Haas E.N. Extrapolate scientific knowledge into practice, in order to prevent feather pecking in laying hens. Welfare seminar: Scientific Research in Animal Welfare: Do we make a difference? Wageningen, The Netherlands, January 18th, 2011.

De Haas E.N., ten Napel J., Rodenburg T.B. Integrating science and practice, in order to reduce feather pecking in laying hens. WIAS Science day, Wageningen, The Netherlands, 2nd of February, 2012. *NZV travel grant for best poster.*

De Haas E.N. Differences in fearfulness between two commercial hybrids of laying hens. Minding Animals Conference, Utrecht, The Netherlands, 4-6 June, 2012.

De Haas E.N., Rodenburg T.B., ten Napel J., Kemp B. Behavioural development of feather pecking in commercial laying hens – the past or the present? UFAW: Animal Welfare Conference, Recent advances in Animal Welfare Science II, York, Merchant Adventurers' Hall UK, 21th of June, 2012.

De Haas, E.N., Rodenburg T.B. Feather pecking in laying hens during rearing and laying in non-cage systems, management practices and farmers opinions. ISAE: 48th conference of the International Society of Applied Ethology. Victoria-Gasteiz, Spain. 29th July – 2nd August, 2014. *Accepted. Avialter Award.*

OTHER PUBLICATIONS

Niekerk, T.G.C.M. van; Jong, I.C. de; Krimpen, M.M. van; Reuvekamp, B.F.J.; De Haas E.N. Invloed van UV-licht, vezelrijk voer of strooisel in de vroege opfok op verenpikken in opfok en legperiode. Effect of UV-light, high fiber feed or litter provision in early rearing on feather pecking in rearing and laying period. Lelystad, Wageningen UR Livestock Research, 2013. Rapport / Wageningen UR Livestock Research P. 671 - 37.

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Bange kip sleept buurvrouw mee. Boerderij Vandaag, 14 September, 2012, p.13.

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Waardering voor dierwelzijn. Paspoort voor hen. Nieuwe oogst, 14 June, 2014, p. 17

Verenpikken zit in synapsen en darmflora. Bionieuws 11, 21 June, 2014

AWARDS AND TRAVEL GRANTS

Award “ best young researcher” on the theme human-animal-environment by NZV (Dutch Zootechnical Association), WIAS Science day 2012, Wageningen University, The Netherlands NZV.

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Mijn naam is (de) **Haas** en ik ben trots dat ik uit een familie kom met zeer getalenteerde, creatieve, lieve, positieve mensen, welke mijn oprechte bewondering hebben o.a. **oom Hubert, oom Theo & tante Joke, Har & Ing, nicht Gerda** en **mijn vader**. Op mijn bagagedrager en in mijn hart draag ik mijn vader's levenswijsheid mee, welke mij enorm hebben gesterkt en gestuurd in

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About the author



Elizabeth Nicole (Elske, i.e. me) was born on the 21st of June 1982 in Leiderdorp, the Netherlands. Since a child I adored animals and wanted to become a veterinarian. We had dogs, cats, rabbits, hamsters at home and from my 14th year onwards I took care and owned (until this day) two Shetland ponies. From the lower agricultural school, followed by a study for veterinarian assistant whilst working at a veterinarian and completing my school of higher secondary education (HAVA), I got enrolled in the BSc professional higher agricultural college in Delft (BSc Animal Husbandry). Here I got acquainted with the Wageningen university by doing external courses of statistics and animal genetics, an internship at the Centre of Genetic Resources in Wageningen (with Dr. Kor Oldenbroek), and a thesis at the Livestock Research Centre of Wageningen University in Lelystad (with Dr. Marc Bracke). After my BSc, I got enrolled in the MSc programme of Animal Science at the Wageningen University with specialization of Animal Health and Behaviour. My major thesis was on effects of enrichment on cognition in pigs with Dr. Liesbeth Bolhuis and Dr. Marije Oostindjer in Wageningen, after which I went to Denmark (Aarhus University) for a fruitful project on feather pecking in laying hens working with Dr. Bart Buitenhuis, Dr. Birte Nielsen while being supervised by Dr. Bas Rodenburg of the Wageningen University. Luckily, after finalizing my MSc I could continue working with animals, as a research assistant, in the PhD project on pigs of Dr. Carol Souza da Silva. In November 2009, I got accepted in the PhD project on feather pecking in laying hens supported by NWO's programme on the value of Animal Welfare. During my PhD I have visited many farms throughout the Netherlands and spoke to many farmers. I had the opportunity to work with excellent inspiring people, and was able to travel, do courses and develop my knowledge and skills. In these years I became a better scientist and more capable person. At this moment I work at the INRA research institute in France, together with Dr. Aline Bertin on maternal effects on chicken behaviour, and I hope to be somehow involved in animal welfare throughout my life.

You can reach me by email endehaas@gmail.com

Educational certificate

Courses of the research programme "The value of animal welfare"	5.0 ECTS
Sustainable Animal Production, Lunteren (the Netherlands)	2010
Ethics and Animal Welfare, Lunteren (the Netherlands)	2011
Animal Behaviour & Society Behaviour, Lunteren (the Netherlands)	2011
Market and Animal Welfare, Spaarnwoude (the Netherlands)	2012
International conferences	9.2 ECTS
UFAW, Recent avances in Animal Welfare Science, York (UK)	2010, 2012, 2014
44 th , 45 th , 47 th , 48 th International Congress of the International Society for Applied Ethology (Sweden, Austria, USA, Spain)	2010-2014
5 th International Conference on the Assessment of Animal Welfare at Farm & Group Level (WAFL), Guelph (Canada)	2011
Minding Animals Conference, Utrecht (the Netherlands)	2012
Behaviour, Newcastle (UK)	2013
International conference on Individual Differences, Groningen (the Netherlands)	2013
Presentations	17.0 ECTS
Oral presentation Nederlandse vereniging voor Gedragsbiologie, Dalfsen/Soesterberg (the Netherlands)	2009, 2011
Poster presentation WIAS Science Day, Wageningen (the Netherlands)	2010
Poster presentation 44 th and 46 th International Society Applied Ethology, Uppsala (Sweden) and Vienna (Austria) resp.	2010, 2012
Oral presentation Welfare seminar: Scientific Research in Animal Welfare: Do we make a difference? Wageningen (the Netherlands)	2011
Oral presentation 45 th and 47 th International Society for Applied Ethology, Indianapolis (USA) and Vitoria (Spain) resp.	2011, 2014
Poster presentation 5 th International Conference on the Assessment of Animal Welfare at Farm and Group level, Guelph (Canada)	2011
Oral Presentation UFAW, recent avances in animal welfare science, York (UK)	2012, 2014
Oral presentation Minding Animals Conference, Utrecht (the Netherlands)	2012
Poster presentation on the IX European Symposium on Poultry Welfare Uppsala (Sweden)	2013
Poster presentation on the International Conference on Individual Differences, Groningen (the Netherlands)	2013
Oral presentation on the Benelux International Society of Applied Ethology conference, Sterkstel (the Netherlands)	2013

Educational certificate

Seminars and workshops	5.2 ECTS
How to design a good behavioural experiment? and What are mathematical models for in biology? Nederlandse vereniging voor Gedragsbiologie, Dalfsen/Soesterberg (the Netherlands)	2009, 2012
WIAS Science Day, Wageningen (the Netherlands)	2010 - 2012
Welfare seminar: Scientific Research in Animal Welfare: Do we make a difference? Wageningen (the Netherlands)	2011
5th combined workshop on Fundamental Physiology and Perinatal Development in Poultry, Wageningen (the Netherlands)	2011
In-depth courses	8.8 ECTS
Trends in Stress Biology, interpretation of stress response, Viborg (Denmark)	2011
Epigenesis and epigenetics, Wageningen (the Netherlands)	2011
International Workshop Perinatal Effects shaping Individual Phenotypes, Linköping (Sweden)	2011
Crane seminar: The good, the bad and the ugly sides of the human animal interaction, Lundsbrunn (Sweden)	2012
Advanced courses	3.0 ECTS
Advanced statistics, design of animal experiments	2010
Statistics for the Life Sciences	2011
Professional Skills Support Courses	3.0 ECTS
Techniques for Writing and Presenting Scientific papers	2011
Writing a paper with impact	2012
Project and Time management	2013
Last Stretch of the PhD programme	2013
Research Skills Training	2.0 ECTS
External period at the Animal Welfare Centre, Melbourne, Australia	2012, 2013
Didactic skills	14.6 ECTS
Lecturing in BSc course Adaptation Physiology-1	2012, 2013
Lecturing in MSc course Health Welfare and Management	2011, 2012
Assisting practical Inleiding Dierwetenschappen	2011, 2012
Discussion group (Animal Welfare)	2010-2012
Coaching groups in MSc course Adaptation Physiology - 2	2010
Coaching and grading papers in MSc course Research Master Cluster	2010, 2014
Supervising 6 BSc and 2 MSc students	2010-2014
Management skills training	4.0 ECTS
Organization WIAS science day	2011, 2012
Board member WIAS council	2011, 2012

Colophon

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Glossary

DA: Dopamine
 DOPAC 3, 4-dihydroxyphenylacetic acid
 DW: Dekalb White (hybrid originating of crosses with White leghorn)
 CORT: corticosterone
 ECD: electro chemical detection
 F: Freezing bird in the Open Field test (chapter 2)
 FP: Feather pecking
 GFP: Gentle feather pecking
 HFP: High feather pecking line
 HPLC: High Performance Liquid Chromatography
 HVA homovanillic acid
 ISA: ISA Brown (hybrid originating of crosses with Rhode Island Red hens)
 LFP: Low feather pecking line
 LML: Low mortality line (White leghorn line selected on low levels of mortality in group housing and individual egg performance)
 MR: Manual restraint test
 NA: Noradrenaline
 NCL: Nidopallium caudolaterale
 NET: Novel environment test
 NOT: Novel object test
 OF: Open Field test
 PCA: Principle Component Analysis
 QBA: Qualitative Behavioural Assessment
 RIR: Rhode Island Red
 SFP: Severe feather pecking
 SPT: Stationary person test
 5-HT: Serotonin (5-hydroxytryptamine)
 W: Walking bird in the Open Field test (chapter 2)
 V: Vocalizing bird in the Open Field test (chapter 2)
 WV: Walking and vocalizing bird in the Open Field test (chapter 2)
 PS: Parent stock (flocks containing roosters and hens)
 3-MT: Dopamine metabolite 3-methoxytyramine
 5-HIAA 5-hydroxyindoleacetic acid
 WL: White Leghorn