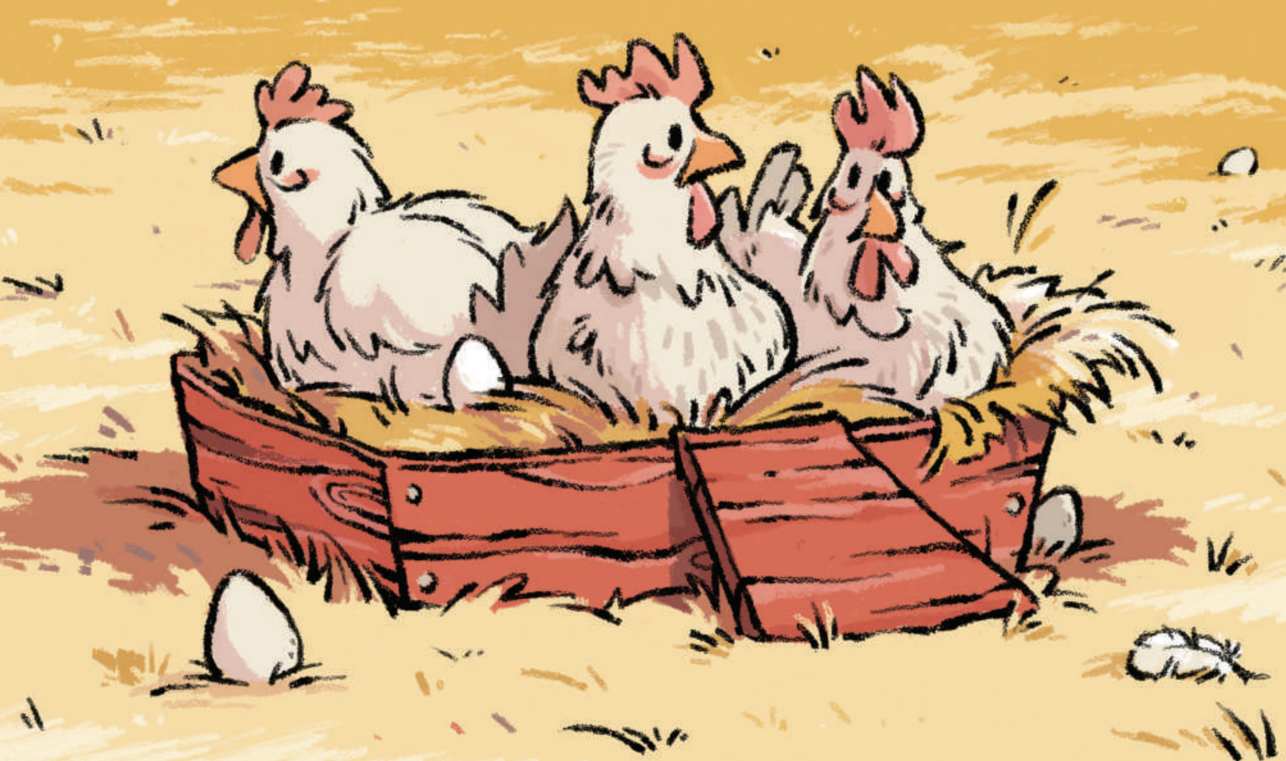
Anne van den Oever

WHAT AFFECTS NESTING BEHAVIOUR IN BROILER BREEDERS?

The role of motivation, physical ability and social opportunity.



Propositions

1. When given the choice, broiler breeders prefer wooden nests over plastic nests.
   (this thesis)

2. Nest visits in broiler breeders are not limited by suboptimal leg health, but by excessive gregarious nesting.
   (this thesis)

3. If results have biological relevance, statistics are overrated.

4. Designing experiments that are scientifically sound, but are also relevant for commercial practice is the biggest challenge for a PhD candidate working within industry.

5. The fact that consumers' perception of livestock husbandry is largely based on shreds of biased information complicates the public debate.

6. Improving animal welfare without increasing environmental pressure is only possible if future meat consumption decreases drastically.

Propositions belonging to the thesis, entitled

What affects nesting behaviour in broiler breeders? The role of motivation, physical ability and social opportunity

Anna Cornelia Maria (Anne) van den Oever
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What affects nesting behaviour in broiler breeders?

The role of motivation, physical ability and social opportunity

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What affects nesting behaviour in broiler breeders?

The role of motivation, physical ability and social opportunity

Anna Cornelia Maria (Anne) van den Oever

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Chapter 1

General introduction

Reproductive success in birds is mainly affected by predation pressure, weather conditions, human disturbance and food constraints (Burger, 1982). The location and design of a nest can help protect the eggs and brooding birds from these environmental threats. The ancestor of the domestic chicken, the red jungle fowl (*Gallus gallus*), lives in secondary forests in South East Asia that consist of grassland with some shrubs and trees (Collias and Saichuae, 1967). The red jungle fowl separates herself from the flock for egg laying and incubation. No real nest is built by the red jungle fowl, the nest is an indentation in the ground that is made by the hen or was already present and is surrounded by tall grass or larger vegetation (Collias and Collias, 1967). This vegetation conceals the nest from predators and humans, while also providing seeds, fruits and insects for feeding. Domestic hens (*Gallus gallus domesticus*) nest in similar locations when released in the wild, suggesting that the preference for a concealed nest site has been maintained during the domestication of chickens (Duncan et al., 1978). The animal husbandry systems in which most domestic hens are kept are considerably different from the natural environment with limited or no vegetation and predation, large flock sizes and an automatic feed supply. While the large majority of domestic hens worldwide are kept in conventional cages, an increasing number of countries are moving towards systems that are better suited for the hens' needs through legislation or initiatives from the food industry (Hartcher and Jones, 2017; Ochs et al., 2019). Hens are provided with more space and provisions, such as nests, perches and scratch pads in colony or aviary housing. It has been shown experimentally that laying hens are willing to work to gain access to these nests, as they provide a concealed site to lay eggs (Cooper and Appleby, 1996), which will be discussed more in detail later in this introduction. The nest is therefore an important housing feature for the hen, but it is also essential for the poultry husbandry. Eggs that are laid in the nest can be collected automatically, thus reducing labour for the farmer, and are kept safe from dirt or damage, optimising their saleability (van den Brand et al., 2016). Nesting behaviour including nest site selection has been extensively studied in laying hens, but only limited research has been done on nesting behaviour in broiler breeders.

Broiler breeder paradox

Due to an increasing world population and changing consumption patterns, meat production worldwide has increased from 71 Mt to 342 Mt between 1961 and 2018 (FAO, 2020). Poultry meat production has increased more relative to other meat sources, as this is more affordable both for producers due to low production costs and for consumers due to low product prices (OECD/FAO, 2019). Furthermore, chicken meat does not have to overcome religious or cultural objections, while also having dietary and nutritional benefits over red meat (Magdelaine et al., 2008). To meet this increased demand for poultry meat, flock sizes in broiler production systems and output per broiler have been increasing. Broilers in 1960 had an average live weight of 1.52 kg at the slaughter age of 42 days, where current conventional broilers weigh 2.87 kg average at the same age (National Chicken Council, 2020). A combination of improved environmental factors, such as housing and nutrition, and successful genetic selection for high body weight gain are the main drivers behind this tremendous change (Tallentire et al., 2016).

When selecting for high body weight at the age of 8 weeks, other correlated traits are also affected. Amongst other biological systems, the reproductive system was found to be affected by the selection for a high body weight in chickens (Dunnington and Siegel, 1996). Selection experiments show that mainly the fecundity of females was negatively affected by the selection for high body weight at 8 days of age. After 54 generations of selection, the hen's age at sexual maturity was delayed by 34 days compared to control lines (Jambui et al., 2017). The frequency of chromosomal abnormalities in embryos and the number of defective eggs (mainly double-yolked eggs) were higher after 15-17 generations as well as the percentage of unhatched fertile eggs, which is probably caused by chromosomal aberrations (Reddy and Siegel, 1977; Dunnington and Siegel, 1985, 1996). It is possible that the reproductive system might not meet its demands when more resources are used for growth, as stated by the resource allocation theory (Beilharz et al., 1993).

The physiological mechanisms between high body weight or obesity and reproductive dysfunctions have been studied mostly in mammals, which can be partially translated to chickens (Walzem and Chen, 2014). Obese broiler hens have higher insulin and leptin concentrations, exhibit changes in lipid and lipoprotein metabolism and experience increased systemic inflammation. These characteristics are similar to women with polycystic ovary syndrome, a reproductive problem commonly seen in obese women (Goodarzi et al., 2011). The changes in lipid metabolism caused by a high body weight provokes inflammation, granulose cell apoptosis and altered timing of ovulation, which impairs egg formation in chickens (Xie et al., 2012). Furthermore, selection for growth in chickens causes an increase of insulin-like growth factor I (IGF-I), which increases the number of yellow follicles in the ovary (Waddington et al.,

1989). When these growth selected lines are fed ad libitum, multiple follicles develop simultaneously, resulting in erratic and multiple ovulations and thus increasing the number of defective eggs (Onagbesan et al., 1999). Also, ad libitum fed birds that are selected for growth have a lower production of progesterone, causing follicles to become easily atretic. When feed restricted, the interaction of IGF-I with LH/FSH ensures that the follicular hierarchy is maintained and thus increases regular egg production.
This negative correlation between body weight and reproduction causes challenges for the husbandry of the parent stock of the broilers, the broiler breeders. Broiler breeders have been genetically selected for high body weight gain of their offspring, while they are also expected to have good reproductive performance in order to lay enough hatching eggs. This is at the heart of the so-called 'broiler breeder paradox' (Decuypere et al., 2010). When broiler breeders are allowed to reach their full genetic growth potential by feeding them ad libitum, the number of eggs per hen and the hatchability of these eggs will decrease to economically unviable levels. Attempts are made to repair this low reproductive performance by both genetic selection and management adjustments. Specialised sire and dam lines are used for breeding, where the focus of the males lines is on growth and the female lines focus more on reproduction to maximise reproductive performance of broiler breeder hens (Emmerson, 2003). Nevertheless, broiler breeders are fed restrictively to constrain their growth (Leeson and Summers, 2000). This management practice has proven to be successful to maintain reproductive performance at an acceptable level for economic viability, although broiler breeders are consequently chronically hungry (Savory et al., 1993). Broiler breeders are therefore highly motivated for feeding, which seems to have affected other behavioural motivations, including the motivation for seeking the concealment of a nest for egg laying (Sheppard and Duncan, 2011). Two nesting behaviours are observed in broiler breeders that reflect this: floor laying and gregarious nesting.

Floor laying

Eggs laid in locations other than the nests, i.e. on the litter or slatted floors (often referred to as floor eggs), is a major issue within the broiler breeder industry. The percentage of floor eggs is generally high at the start of egg production, but the percentages in broiler breeder flocks remain much higher than 1-2% reported in laying flocks during the remainder of the production cycle (Zupan et al., 2008; Sheppard and Duncan, 2011). Percentages up to 30% have been reported in broiler breeder research (Appleby et al., 1984a), although these numbers are probably outdated. Floor eggs are undesired for several reasons. First, floor eggs can be a sign of reduced welfare as hens are naturally inclined to seek a sheltered space for egg laying (Struelens et al., 2008). So, when a hen lays her egg outside a nest, this is a sign that the provided nests are not found suitable by the hen or the hen is unable to get in the nest. Laying hens show clear signs of frustration when housed without nests or when they are pushed out of nests, such as pacing, displacement preening and high-pitched calls (Zimmerman et al., 2000; Yue and Duncan, 2003). Second, the collection of floor eggs is time consuming and compromises the usefulness of automatic egg collection systems. Last, the profitability of broiler breeder companies is mainly dependent on the number of settable (i.e. suitable for incubation) hatching eggs produced. Floor eggs are often dirty and broken, contain more bacteria on the eggshell and have more cracks than clean nest eggs (Berrang et al., 1997). The contamination with bacteria includes *Salmonella* bacteria, which has been identified as a critical point of salmonellae contamination of broilers and thus is considered to be a public health risk (Cox et al., 2000). This reduces the hatchability and saleability of floor eggs, even after washing them (van den Brand et al., 2016).

Eggs that are unsuitable for incubation can be processed in the other industries, but these eggs are bought at a lower price from the farmer. In the Netherlands, a farmer gets €0.19 (€0.17-0.21) for an hatching egg, but the price for these second grade eggs is €0.02 (Wageningen UR Livestock Research, 2020), resulting in large losses for the farmer. In some cases eggs cannot be used in any industry and are discarded, which means that the farmer loses the entire income of those eggs. Furthermore, the resources in terms of food, water and energy that were used for the creation of this egg are wasted. A broiler breeder hen lays approximately 174 (164-184) eggs during a complete production cycle of 40 weeks of which 10 eggs are considered unsuitable for incubation (Wageningen UR Livestock Research, 2020). Of these 10 eggs, 5.5 eggs are assumed to be floor eggs, while the other eggs have physiological deviations, such as double-yolked eggs, small eggs or disformed eggs (F. Leijten, personal communication, 26 October 2020). To produce these eggs, the hen consumes around 160 (150-170) gram of feed per egg (Wageningen UR Livestock Research, 2020). A farm with 40,000 hens will thus have approximately 220,000 floor eggs that create a loss of €37,400 for the farmer, while also wasting 35,200 kg of feed. Not only the eggs are lost, but the subsequent hatch of a chick with all its value is lost as well. With an average hatchability of 80% (Wageningen UR Livestock Research, 2020), 176,000 chicks could have hatched from these floor eggs.

Many factors have been suggested to be causing floor laying behaviour (for a review see Appleby, 1983). Genetic effects are evident from differences between genetic strains in laying hens, as well as the previously mentioned difference in floor egg percentage between broiler breeders and laying hens (McGibbon, 1976; Campo et al., 2007; Singh et al., 2009; Heinrich et al., 2014). Several studies report consistent floor laying individuals in laying hen flocks, suggesting that individual differences in behavioural strategies or perception of what an optimal nest site looks like might play a role in floor laying behaviour (Rietveld-Piepers et al., 1985; Cooper and Appleby, 1995; Zupan et al., 2008). Social status also has an influence on nest site selection. Subordinate hens are reported to get disturbed in their nesting behaviour by other hens (Freire et al., 1998; Ringgenberg et al., 2015a), which leads to avoidance of dominant hens on their route towards the nest (Freire et al., 1997).

The management of the birds can influence nest site selection and thus floor laying behaviour. Less than 14-16h of light in houses increases floor eggs in broiler breeder stocks, probably caused by an increasing proportion of eggs being laid before lights-on (Lewis and Gous, 2006; Lewis et al., 2010). Feeding during the laying peak of the day is found to be disadvantageous due to the high feeding motivation in broiler breeders, even though no effect on floor egg percentage was found under experimental conditions (Sheppard and Duncan, 2011). In this experiment, groups of 10 broiler breeders were fed on alternating days at lights-on or at the moment 2-3 hens per group were in the nest. Practically all hens left the nest when feeding initiated during laying, but 73% of the hens returned to the nest to lay an egg and only 4% laid an egg on the floor. The effect of stocking density on floor egg percentage was studied in broiler breeders by comparing densities of 10 and 5.5 birds/m^2 during the rearing and/or production phase (de Jong et al., 2011). Broiler breeders housed at the high density in the production phase had a higher percentage of floor eggs and this was more pronounced for the birds housed at a low density during the rearing. Similar results were found for laying hens, where hens housed at 10 birds/m^2 had a higher floor egg percentage than the hens housed at 5, 6 or 7 birds/m^2 (Kang et al., 2016). It is important to remark that housing broiler breeders or laying hens at 10 birds/m^2 is not permitted in the European Union, which limits the density at 7.7 and 9.0 birds/m^2 for broiler breeders and laying hens respectively.

Another manner floor laying behaviour can be influenced is by adjusting housing features. Including perches in the housing design during rearing or production phases can help to decrease the number of floor eggs in laying hen and broiler breeder flocks, although this is probably only the case when nests are raised and require perching behaviour to access the nest (Appleby et al., 1986, 1988; Brake, 1987). Slat material (wood or plastic) and proportion of slats (fully slatted or two-thirds slats, one-third litter) have not been found to influence the incidence of floor eggs of broiler breeders (Newcombe et al., 1991). Placing mesh panels in the litter area resulted in less floor eggs in broiler breeder flocks (Leone and Estévez, 2008), as the panels attract many birds and this increased bird traffic is thought to deter floor laying. A lot of research has been done on the preference for nest design as a means to minimise floor eggs mostly with laying hens but also some with broiler breeders, which will be discussed later in this chapter. Despite the extensive research on this topic, the exact combination of design features to minimise floor laying has yet to be discovered. Another behaviour related to nest site selection also requires more research in order to minimise its negative consequences for bird welfare and egg quality, namely gregarious nesting.

Gregarious nesting behaviour

Broiler breeder hens as well as laying hens are frequently observed choosing to enter occupied nests instead of empty nests, which is called gregarious nesting (Appleby et al., 1984b; Riber, 2010, 2012; Clausen and Riber, 2012; Ringgenberg et al., 2015a). This behaviour leads to an uneven distribution of hens over the provided nests, which can become problematic when too many hens exhibit gregarious nesting. Bird welfare is at risk when hens use excessive energy to enter overcrowding nests (Appleby, 1986), which often leads to scratches that can get infected (Clausen and Riber, 2012). In extreme cases hens have also been reported to suffocate in overcrowded nests (Lentfer et al., 2011). In addition to these welfare risks, egg quality is also likely to be affected by excessive gregarious nesting. Eggs may get damaged due to insufficient egg belt capacity as a consequence of this uneven distribution or eggs get dirty when they remain in the nest instead of rolling away due to crowding of the nest.
In order to minimise these negative effects of overcrowded nests, it is necessary to understand why the hens are drawn to nests that already have hens in them. Even though the phenomenon of gregarious nesting has been studied in laying hens for some years, the exact cause of this behaviour is still unknown. The red

jungle fowl does not nest communally (Collias and Collias, 1967), so this behaviour is probably the result of selection for productivity or tameness. However, it has been suggested that gregarious nesting is an anti-predator response (Riber, 2012), in line with the natural nesting behaviour of concealing from predators when nesting. Joining other hens in a nest is seen as a risk-dilution strategy, decreasing the chance that the hen or her egg is predated on due to other hens and eggs being present. Shared preferences for nest design or higher recognisability of certain nests might also be an explanation (Clausen and Riber, 2012). Hens are known to be consistent in laying site, which probably stems from the desire to lay a clutch of eggs to brood (Romanov et al., 2002). Commercial poultry houses usually have long lines of identical nests, which makes the nests at the end of the row or in a corner more easily found again. The nests in these location are often observed to be preferred to nests in the middle of the row or house (Riber, 2010; Lentfer et al., 2011; Clausen and Riber, 2012; Ringgenberg et al., 2015a). However, increasing the heterogeneity of nests to make the nests more easily recognisable did not reduce the occurrence of gregarious nesting behaviour (Clausen and Riber, 2012). Previous research has only studied gregarious nesting in laying hens and not in broiler breeders. Furthermore, it is unknown whether there is a genetic background to this behaviour. In conversations with farmers it is apparent that some breeds of broiler breeders also perform this behaviour with the aforementioned welfare and economic risks.

Aims & approach

This thesis aims to understand the nest site selection of broiler breeder hens by investigating which factors contribute to floor laying behaviour and gregarious nesting. To disentangle these factors, the set-up of the experimental work was inspired by a framework called the Motivation-Ability-Opportunity (MAO) model (Maclnnis and Jaworski, 1989). This model is mainly used within social sciences to understand human behaviour, communication and decision making, but it is also suitable for animal behaviour research. The MAO model proposes that the intrinsic motivation to perform a behaviour is modified by a combination of ability and opportunity (Figure 1). Motivation describes the drive to engage in a certain behaviour, while ability refers to the skills necessary to perform it. Opportunity describes situational constraints, so whether the environment allows the performance of the behaviour. The three components

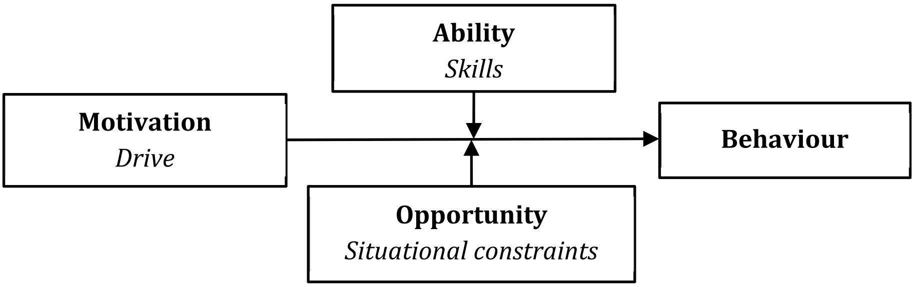


Figure 1. Visualisation of the MAO model (MacInnis and Jaworski, 1989), which shows the proposed relationship between motivation, ability, opportunity and behaviour.

will now be described within the frame of this thesis, where the studied behaviour is laying an egg in a nest.

Motivation

The component 'motivation' is used to describe the willingness of an animal to engage in a behaviour (Kirkden and Pajor, 2006). Motivation can be both positive and negative, so the desire to either do or avoid something. Furthermore, the motivation to perform a specific behaviour will differ in strength relative to other behavioural motivations depending on the importance of the behaviour to the animal, which varies over time. Time of oviposition in chickens is mainly controlled by the circadian rhythm of dark and light periods. Oviposition takes place 6-8 hours after LH surges, which is triggered by the start of the dark period (Wilson, 1964). The behavioural motivation of finding a suitable nest site is highest just before oviposition and when this nest site is found, the motivation decreases rapidly (Duncan and Hughes, 1988; Cooper and Appleby, 2003). If no suitable nest site is found, then hens show signs of frustration in the form of pacing and high-pitched vocalisations (Zimmerman et al., 2000; Yue and Duncan, 2003).

Measuring preferences and motivational strengths are of value in animal behaviour and animal welfare research, as this can give an insight into what is important for the studied animal. Preference tests offer multiple options to groups of animals and by actively choosing or avoiding these options, the animals show their preference. In terms of nest site selection, the most commonly studied read out parameter of preference is the number of eggs at a location. However, the behaviour shown in a nest can also be indicative of preference, for which the term 'settled' nesting behaviour has been coined. Originally the term settled was used for a higher portion of time spent in the final laying position (Appleby, 1990; Appleby et al., 1993). More time sitting in the nest, especially in fewer bouts, is currently used more often as a measure for settled behaviour (Cronin et al., 2012). It has also been expanded to the searching phase, where fewer nest inspections and visits per egg are interpreted as more settled nesting behaviour (Freire et al., 1996; Hunniford and Widowski, 2018).

To test motivational strength, the animal must have limited time, work for or pay a price to access a resource, also known as the consumer demand theory (Dawkins, 1983). When time is limited, it is expected that 'luxury' behaviour will drop out of the repertoire and time is only spent on behaviours that are needed. Laying hens have been taught to work for access to a resource, by pecking at a key, pushing against a weighted door or by passing through a narrowing tunnel in previous consumer demand studies (Duncan, 1991). When the number of pecks required to gain access or the weight of the door is increased, the hens will continue to do the required task until the reward is considered not worth the work. Using these techniques, laying hens are willing to work for access to a nest by passing through tunnels as narrow as 14 cm (Cooper and Appleby, 1996) and pushing against a door with more than 10 N resistance (Kruschwitz et al., 2008) to access a nest. Furthermore, laying hens are willing to work over five times harder 20 minutes before expected time of oviposition compared to 60 minutes before (Cooper and Appleby, 2003).

As it has been established that domestic hens are motivated to lay their egg in a nest, this willingness is known to be affected by nest design preference. Earlier work on nest design preference has studied nest size, which affects the maximum number of hens that can nest gregariously. A preference for small metal nests (0.75 m^2) was found in comparison to larger wooden nests (1.2 m^2) in broiler breeders, although it is not clear whether nest size, nest wall material or its combination was the decisive factor (Holcman et al., 2007). Laying hens were also found to prefer a smaller nest of 0.43 m^2 to a nest of 0.86 m^2, which were both wooden (Ringgenberg et al., 2014). Research with laying hens has shown that providing concealment via nest curtains makes the pre-laying behaviour more settled (Hunniford and Widowski, 2018), although whether the curtains were made of one piece or thin strips did not affect preference (Struelens et al., 2008; Stämpfli et al., 2012). Making the nest interior more dark by painting metal nest walls black, did not increase nest preference compared to unpainted metal nest walls in broiler breeders (Brake, 1985). Similar to the natural nest of chickens, concave nest floors were preferred over flat floors in broiler breeders (Brake, 1985). Offering pine shavings as a nest floor was not preferred over a plastic nest pad without shavings in one study with broiler breeders (Brake, 1985), although littered nests were preferred to non-littered nests in another study with broiler breeders (Holcman et al., 2007). These contrasting results could be (partially) caused by the fact that the genetics of broiler breeders was altered significantly between 1985 and 2007, when these studies were performed. Broiler breeders preferred grey nest pads over brown, green and black and this was suggested to be caused by conditioning to grey metal equipment in commercial housing or an innate preference for this colour (Brake, 1993). Nest floors are sloped in commercial nests to ensure that eggs roll out of the nest, although this slope might be uncomfortable for hens to sit and stand on. Research comparing nest floor slopes of 12 and 18% found that both slopes were accepted by laying hens, as the number of eggs and the number of nest visits per egg did not differ between the two nest designs (Stämpfli et al., 2011).

In the current broiler breeder husbandry, a variety of nest materials, sizes and design is used worldwide. This thesis aims to update and expand our knowledge on preferred nest design for modern broiler breeders by looking at unstudied aspects of nest design, including the comparison of plastic nests to other materials and the microclimate inside the nest. Plastic is an often used material for commercial nests because it is easy to mould in production, durable and easily cleaned leading to improved hygiene in the poultry house, but its effects on nest acceptance has not been studied. In colder climates chickens are observed to get disturbed by air flows in the nests as they might be sensed as draughts due to the lower temperature (Wim Peters, personal communication, 21 July 2017), but this has yet to be confirmed in experimental conditions. By increasing our knowledge on what is regarded as a suitable nest by broiler breeders, the housing can be improved to increase bird welfare as well as decrease the economic costs of floor eggs.

Ability

The word ‘ability’ could refer to having the skills or intelligence to perform certain behaviour, but in this thesis it refers to physical abilities. When the hen is motivated to go to the nest to lay an egg, she must be physically able to reach this nest. Leg health and mobility in chickens have received increasing attention from animal welfare scientists over the last decades, especially in research on broilers. Decreased mobility can be caused by both contact dermatitis, an acute inflammation of the skin due to contact with irritating substances (Greene et al., 1985), and leg weakness. Poor litter quality, where a high litter moisture level causes more ammonia release, is the most common risk factor found for foot pad dermatitis (Martland, 1985). The incidence of contact dermatitis in fast-growing broiler flocks at slaughter age is up to 65% for the foot pad and 41% for severe hock burns (Haslam et al., 2007; de Jong et al., 2012; Bassler et al., 2013). Leg weakness or lameness has a prevalence of 14-30% in flocks of fast-growing broilers (Sanotra et al., 2003; Bassler et al., 2013).

The risk factors for developing contact dermatitis in broilers are also present in the broiler breeder industry, such as poor litter quality, genetic predisposition and high body weight (Shepherd and Fairchild, 2010). Contact dermatitis is likely a painful condition (Ekstrand et al., 1998; de Jong and Guémené, 2011) and together with leg weakness, these conditions could limit the hen's ability to reach the nest. However, little research has been done on the prevalence of this contact dermatitis in broiler breeders (Wolanski et al., 2004; Renema et al., 2007; Kaukonen et al., 2016) and no study has looked into leg weakness or the relationship between leg health and floor eggs. A reduced physical ability to reach the nest, while the hens are motivated to reach the nest, is expected to lead to frustration and hence low broiler breeder welfare. Furthermore, this could increase the number of floor eggs with its previously described economic consequences.

The lay-out of broiler breeder houses varies throughout the world, including the proportion of the house that is slatted, the height of the raised slatted areas and the material of the slats. Slats provide the broiler breeders an opportunity to rest without their feet coming into contact with the litter, but a raised slatted area requires jumping which might be difficult for the birds. As the nests are usually provided on the raised slatted areas, the chance of floor eggs could also increase if the broiler breeders are unable to jump unto these slats. This thesis aims to describe the prevalence of contact dermatitis in our current broiler breeders housed in the most common lay-out in the Netherlands, namely with a partially raised plastic slatted area. Furthermore, it is investigated whether the presence or absence of a raised slatted area in front of the nests affects leg health, body weight and floor eggs.

Opportunity

The word 'opportunity' describes the situational constraints to perform the behaviour of laying an egg in the nest and the focus in this thesis is on constraints caused by other hens and roosters. When the hen is motivated to get into the nest and is physically able to reach the nest, the other birds must allow this hen to enter the nest. Entrance to the nest could be impossible, when too many other hens are present in the nest. Assuming that the total nest space (surface per hen) is sufficient for the number of hens in the house when adhering to minimal housing requirement laws, specific locations might still have insufficient space due to crowding. If many hens within a flock show gregarious nesting behaviour, this might limit the opportunity of other hens to enter the nest. Some possible explanations behind this behaviour have been studied in laying hens as described in the paragraph on gregarious nesting, but not yet in broiler breeders.

Another situational limitation to reaching the nest, could be the presence of males. Research with small groups of laying hens found that adding a male to the group resulted in males guiding the females to the nest and thus reducing the number of floor eggs (Rietveld-Piepers et al., 1985). However, broiler breeders males are known to be aggressive in their mating behaviour and females tend to avoid them (de Jong and Guémené, 2011). In most European farms, the nests are accessible from a slatted area. Males hardly spend time on slatted areas (de Jong and Guémené, 2011), which could result in the slatted area being a safe space for hens to rest and enter the nests. When the nests are accessible directly from the litter, which is the case in many other regions of the world, males might disrupt hens in their way towards the nests.

A comparison of mating and nesting behaviour between providing nests on a raised slatted area and on the litter has not been made to our knowledge. This information could however lead to housing adjustments to improve broiler breeder hen welfare and possibly decrease floor eggs. Therefore, this thesis aims to fill this gap of knowledge. Furthermore, the occurrence of gregarious nesting in broiler breeders is studied together with possible related factors such as genetics, general spatial distribution and fearfulness. Whether excessive gregarious nesting can also cause increased floor eggs is studied as well to get insight into the economic costs of this behaviour.

Thesis outline

Figure 2 indicates the interpretation of each component of the MAO model for this thesis and how this relates to each chapter. To motivate the broiler breeder hens to lay the eggs in a nest, the nest design must be according to their preference (motivation). If the hens are motivated to get into the nest, their leg health must be good enough to be able to reach the nest (ability). Lastly, when the hen reaches the nest, other hens and roosters must give the hen the opportunity to enter the nest (opportunity). Each component is studied in a separate chapter (chapters 2-4), while all components are combined in an experiment that formed the basis of chapter 5.

Chapter 2 describes a preference test as part of the 'motivation' component, offering four different nest designs to groups of broiler breeders. Preference during the first 12 weeks of egg production was measured by counting the number of eggs laid in each nest. The behaviour performed inside the nests was observed to investigate whether the nesting behaviour was more 'settled' (i.e. more sitting, less nest inspections and visits per egg and less agonistic behaviour) inside the nest that is preferred in terms of number of eggs. A second experiment forms the basis for chapters 3 and 4. Chapter 3 focusses on the leg health of large groups of broiler breeders of different genetic lines during the entire 40 weeks of a production cycle by scoring for foot pad dermatitis, hock burn and gait. Body weight and mortality were included as possible related factors and the relationship with floor egg percentage was investigated to gain insight into the 'ability' component. In chapter 4, the component 'opportunity' was investigated by measuring the distribution of eggs over the available nests as a reflection of the amount of gregarious nesting behaviour in these large groups of broiler breeders of different genetic lines. The relation to the percentage of floor eggs and possible causes behind gregarious nesting were studied as well, including the genetic effects, general spatial behaviour, fearfulness and the consequences of mating behaviour. All three components of the MAO-model are combined in chapter 5. The two most preferred nest designs from chapter 2 are offered in a new preference test in this experiment (motivation), while also studying the effects of providing a raised slatted area in front of the nest on leg health (ability), mating behaviour (opportunity) and floor egg percentage. By spending time on the slatted area, the hens limit the contact of their feet with the litter and thus decreasing the chance of foot pad dermatitis. Furthermore, the males avoid slatted areas and this could influence the frequency of mating behaviour and nesting behaviour. In the general discussion in chapter 6, the results from the previous chapters are discussed and integrated to gain insight in the effect of these factors on nest site selection in broiler breeders. The validity of using the MAO-model to study this behaviour is argued as well.

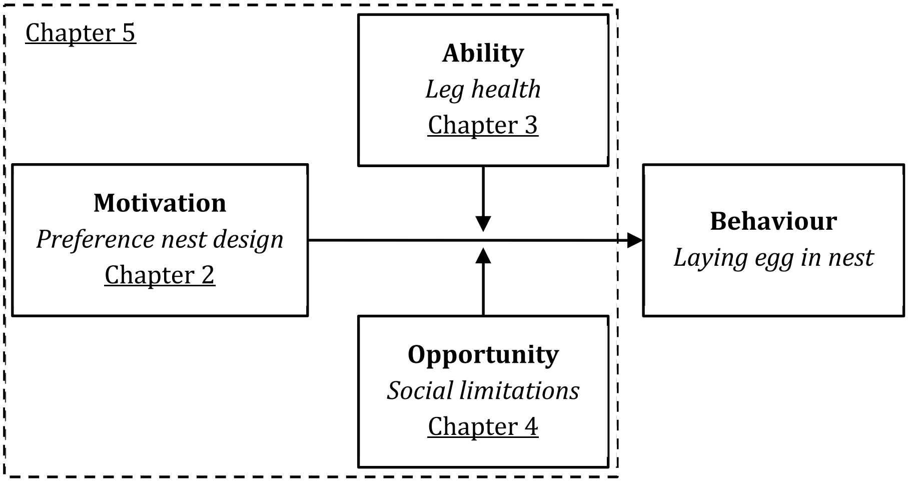
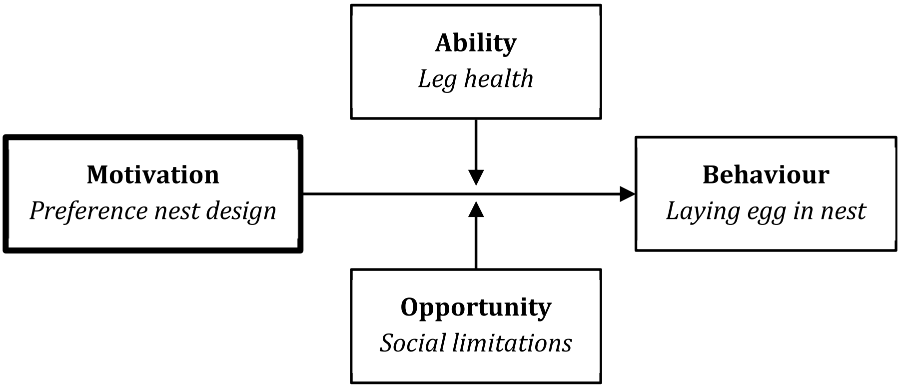


Figure 2. Implementation of the MAO model in this thesis, based on the behaviour of laying an egg in a nest. The outline of the thesis is indicated, where each chapter captures one or more components of the MAO model.

Chapter 2

Relative preference for wooden nests affects nesting behaviour of broiler breeders



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Abstract

Optimising nest design for broiler breeders has benefits for both the animals and the producers. The welfare of the hens will increase by providing preferred housing, while also reducing eggs laid outside the nests. These floor eggs cause economic losses by compromised automatic egg collection and reduced saleability and hatchability. Attractiveness of nests can involve factors such as seclusion, material and microclimate. In this study, four nest box designs were offered in a relative preference test: a plastic control nest, a plastic nest with a partition to divide the nest in two areas, a plastic nest with a ventilator underneath to create air flow inside the nest and a wooden nest. Six groups of 100 hens and 9 roosters had access to these four nests in a randomised location during the ages of 20 to 34 weeks. Nest and floor eggs were collected five days a week. Camera images from inside the nests made during the ages of 24-25 weeks and 26-27 weeks were analysed for behaviour. This included general activity, nest inspections, nest visits and social interactions. At 32 weeks of age the wooden nests were closed, and the subsequent response of the hens was monitored in terms of number of eggs. We found a clear preference in number of eggs for the wooden nest (69.3 ± 1.0%) compared to the control nest (15.1 ± 0.8%), partition nest (10.2 ± 0.5%) and the ventilator nest (5.4 ± 0.4%; $p<0.0001$ for difference between all nest designs). The preference for the wooden nest was also reflected in an increased time spent sitting, together with fewer nest inspections and visits per egg laid in the wooden nest. The preference for the wooden nest led to crowding, which caused an increased amount of piling, nest displacement, aggression and head shaking. The fact that the hens were willing to accept the crowded circumstances in these nests, underlines the strength of this preference. After the wooden nests were closed, the hens chose a new nest based on a combination of nest design and location. The control nest was still preferred over the other two plastic designs, although the neighbouring nests were overall preferred to the non-neighbouring nests. This study shows how the material used for nests is an important factor in suitability and should therefore be taken into account when designing nests.

Keywords: broiler breeder, nest design, preference test, behaviour, welfare

1 Introduction

When attempting to optimise housing conditions for chickens kept on commercial farms, the main question is what does the hen prefer. Providing nests with a preferred design has benefits for the hens as well as for the producer. The welfare of the hens will likely be increased by meeting their needs and this could also increase the number of eggs laid in the nests as opposed to other locations. Eggs outside the nests are undesirable due to the time consuming manual collection and the reduced hatchability and saleability (van den Brand et al., 2016). This is caused by the fact that floor eggs are often dirty and broken, contain more bacteria on the eggshell and have more cracks than clean nest eggs (Berrang et al., 1997). 2

Relative preference or choice tests are the most used method to gain insight into the preference of the hens. Hens are offered two or more designs at the same time and their response to this is monitored for a certain amount of time. This response can be studied in terms of different parameters. The number of eggs laid in the nests is often used as a main parameter, as this clearly reflects a choice of the hens. Behaviour exhibited in and around the nest can also be used as a parameter. The term 'settled' has been used for describing nesting behaviour that would reflect a preference. Originally the term settled was used for a higher portion of time spent in the final laying position (Appleby, 1990; Appleby et al., 1993). More time sitting in the nest, especially in fewer bouts, is currently used more often as a measure for settled behaviour (Cronin et al., 2012). It has also been expanded to the searching phase, where fewer nest inspections and visits per egg are interpreted as more settled nesting behaviour (Freire et al., 1996; Hunniford and Widowski, 2018). Unsettled nesting behaviour can also be caused by negative social interactions, rather than a disliking of the environment. Aggression and displacement behaviour have previously been described in laying hens and can be disruptive to settled nesting behaviour (Freire et al., 1996; Struelens et al., 2008).

Previous research on the nest design preference of broiler breeders has shown that there is a preference for smaller metal nests compared to larger wooden nests, with unpainted rather than black painted walls (Brake, 1985; Holcman et al., 2007). Plastic materials, although commonly used in commercial practice, have not been compared to other materials before. Preferred nest size has also been studied, where smaller nests have been preferred over larger nests by broiler breeders as well as laying hens (Holcman et al., 2007; Ringgenberg et al., 2014). Although the preference of broiler breeders for type of bedding material was inconsistent, concave nest floors were preferred over flat floors and grey nest pads were chosen over other colours (Brake, 1985, 1993; Holcman et al., 2007). Seclusion is thought to be important for laying hens as pre-laying behaviour is more settled in the presence of nest curtains compared to no nest curtains, although no difference in number of eggs was found when comparing nests with sliced curtains to nests with one-piece curtains (Struelens et al., 2008; Stämpfli et al., 2012). In warmer climates, nests are often equipped with perforated nest floors to allow for air flow inside the nest. However, in colder climates chickens are observed to get disturbed by air flows in the nests as they might be sensed as draughts due to the lower temperature (Wim Peters, personal communication, 21 July 2017).

When the preferred nest has been found by hens, they tend to return to this nest every day. This conservatism in nest location has often been described (Appleby et al., 1984a; Duncan and Kite, 1989; Riber, 2010; Riber and Nielsen, 2013), which has been suggested to be caused by the last remainders of broodiness, so as to form a clutch of eggs in the same location (Riber, 2010). To our knowledge, no study has investigated the response of hens when their usual nesting location becomes unavailable.

This study aims to compare four nest designs in a relative preference test. The most preferred nest was expected to be a nest with a partition in the middle, as this provides a smaller nest size and more seclusion. After that, we expected that the nest with wooden walls would be preferred over the control nest with plastic walls due to the natural properties of the material. The nest with a ventilator underneath was expected to be least preferred, as this creates an undesired air flow in the nest. Preference was hypothesized to be apparent in number of eggs laid in the respective nests, but also in more settled nesting behaviour. When closing the preferred nest at a later age, the hens were expected to move to the nest nearest their preferred nest independent of the nest design, which reflects their conservatism in location.

2 Materials and Methods

Animals and housing

The relative preference for nest design was tested with Ross 308 broiler breeders during the winter of 2017/2018. A total of 600 females and 54 males, all non-beak trimmed, were purchased from a commercial rearing farm with raised slatted areas at the age of 20 weeks. The chickens were randomly assigned to six pens, resulting in 100 females and 9 males per pen. The pens were identical in size (3.4 x 4.6 x 2.0 m, length x width x height) and layout and were placed in one row. The pens had wire mesh walls, which allowed the animals from different pens to see each other. The litter area (1.8 x 4.6 m) was covered with saw dust and gave access to two feeding lines, which were partially covered with grids to create separate female and male feeding areas. The slatted area (1.15 x 4.6 m) was raised by 0.5 m and gave access to 25 drinking nipples and a row of four nests. The four nests were of a different design (see below) and placed in a different location in each pen using block randomisation to resolve location preference in the pen. The lay-out of the pen was according to commercial practice in the Netherlands, although the stocking density (7 birds per m^2), nest space per hen (207 cm^2 per hen) and birds per drinking nipple (4 birds per nipple) were lower than what is seen in commercial practice to be in accordance with the Dutch Experiments on Animals Act (IVD Utrecht, 2019). This study was approved by the Dutch Central Authority for Scientific Procedures on Animals (CCD) and is registered under application number AVD1040020173027.

The group nests were of a rollaway type, designed specifically for this study. The nests were 1.15 m wide, 0.45 m deep and measured 0.6 m high at the front and 1.0 m high at the back. The nests were raised by 10 cm from the slats, so the birds had easy access to the nests. All nests had a green rubber nest floor slanting towards the front. The front of the nest was closed by two solid red nest curtains, made of PVC coated fabric, leaving an opening of 20 x 23 cm in the middle. Four types of nests were tested: the control, partition and ventilator nest all had black HDPE plastic side and back walls, whereas the wooden nest had dark brown epoxy coated birch plywood side and back walls. The partition nest had a plastic partition made of the same material as the wall in the middle of the nest floor of 20cm high. All wall materials were smooth, solid and 12 mm thick. The ventilator nest had a low noise ventilator (Tristar VE-5904) underneath the nest to create an air flow (0.2 m/s) inside the nest, as air could pass between the walls and the floor of the nest.
The house was lit with artificial LED-lighting, creating a photoperiod schedule according to commercial practice. At 20 weeks of age, the animals had 10 h of light (8:00 to 18:00 h) at 10 lx measured at bird height. This was gradually increased with age to 14 h of light (6:00 to 20:00 h) at 60 lx at bird height at the age of 26 weeks. The temperature was maintained at 18 ± 1 ^{0}C, according to the management guidelines. Food was provided at lights-on, given at a restricted amount according to commercial practice. At 20 weeks of age the animals received 105 g per individual per day, which gradually increased to 165 g per individual per day at the age of 26 weeks. Random samples of 60 birds were

weighed weekly. Water was provided at lights-on for 3 h and for 30 min in the afternoon. The nests were opened to the hens from 30 min before lights-on until 30 min after lights-out, from the day after the first egg was found (23 weeks of age). The birds were kept until the age of 34 weeks.

Data collection

In order to assess preference for the different nest designs, our primary outcome variable was the number of eggs laid in each nest. Eggs were collected separately from each nest and from other areas of the pen (noted as floor eggs). This was done three times a day, five days per week between 8:00 and 16:00 h. Collection continued until the experiment was terminated at 34 weeks of age.

Behavioural data were recorded from video images, using four infrared cameras IPC-BT508V-20SC (Techage, Shenzhen, China) placed through a hole in the ceiling of the nests to film inside the four nests of each pen simultaneously. Digital cameras FI9803EP (Foscam, Shenzhen, China) were also mounted on the pen walls recording the entrances of the nests. After a pen was filmed for one day, the cameras were moved to the next pen. This allowed for filming three pens per week, taking two weeks to film all pens involved in this study. The recordings were made between 24-27 weeks of age, resulting in two days per pen observed with two weeks between the two days for each pen. Recordings were made from the moment the nests were opened in the morning until 17:00 h.

Behavioural observations inside the nest were done between 9:00-11:00 h and 14:00-16:00 h, so as to include both a period during and after the peak of egg laying. Behaviours as listed in Table 1 were observed using scan sampling with a 10-min interval. Frequencies of behaviours as listed in Table 2 were observed using a behavioural sampling method in which 5 min per 30 min of video recordings were analysed continuously.

At the age of 32 weeks a preference for the wooden nest was found in 5 out of 6 pens, so the wooden nests in all pens were closed in order to study subsequent preference. The number of eggs laid in each nest as well as floor eggs were then recorded for 9 days as described before. One pen was randomly chosen and excluded from this part of the experiment, as it was needed for other research purposes.

In order to explore what factors correlated to the differences in preference for the nest designs, the nests were physically characterised. The light intensity, rounded to the nearest lx, inside each nest was measured with lux meter 540 (Testo, Almere, The Netherlands) held facing up at 20cm height from the nest floor in the middle of the nest. Air temperature with a precision of 0.01 ^{0}C, was measured inside the nests of two pens every 10 min for 48 h using data loggers 174 (Testo, Almere, The Netherlands). The electrostatic properties of the control and wooden nest were measured using the electrostatic field meter EFM51 (Wolfgang Warmbier, Hilzingen, Germany; precision of 1 V) and surface resistance meter METRISO 2000 – 541C (Wolfgang Warmbier, Hilzingen, Germany; precision of 1 MΩ) with two resistance probes model 850.

Table 1. Ethogram of behaviours inside the nest recorded using a 10 min scan sampling method.

Behaviour	**Description**
Resting	Sitting with neck folded backwards with head tucked between feathers
Sitting	Sitting on the nest floor
Standing	Standing in an upright position
Walking	Moving at least two steps
Piling	Sitting on top or under a conspecific with at least one body part (head, wing, rump, tail)

Table 2. Ethogram of behaviours inside the nest recorded (as frequencies) using the behaviour sampling method.

Behaviour	**Description**
Head shaking	Shaking only the head, counted in bouts of 5 s
Head pecking	Pecking to the head or neck of conspecifics, counted in bouts of 5 s
Feather pecking	Pecking the feathers of conspecifics (gentle or severe), counted in bouts of 5 s
Displacement	Forcing another hen to move (including threats without physical contact)
Nest inspection	Placing head in the nest box without entering it
Nest visit	Entering the nest box with both feet

Statistical analysis

All statistical analyses were performed with SAS (version 9.4). P values below 0.05 were considered significant and the MIXED procedure was used to perform general linear mixed models (and the GLIMMIX procedure where necessary). The assumptions of homogeneity of variance and normally distributed errors were examined visually using the conditional studentized residuals plots. In order to satisfy these assumptions, the number of eggs per nest and the behaviours walking, resting, piling, nest visits, nest inspections, displacement, feather pecking, head pecking and head shaking were square root transformed and the MIXED procedure was used. Results are shown as non-transformed means with corresponding standard errors. For the behaviours nest displacement, feather peck and head peck the GLIMMIX procedure was used with a Poisson distribution. For pairwise comparisons, Tukey's post hoc test was performed.

Behavioural observations between 9:00-11:00 h were summed and named AM, while observations between 14:00-16:00 h were summed as PM. As fixed effects nest design, age and time of day were included, as well as the interactions between these variables. Pen was included as a random effect in all models. Sitting, standing, walking, resting and piling were analysed as the percentages of total number of observations. For the data on eggs laid per nest after closing the wooden nests, the remaining nests were given a proximity label. Nests directly adjacent to the wooden nests were labelled 'neighbour' and the other nests were labelled as 'non-neighbour'. If the wooden nest was in the corner, there was one neighbouring nest, otherwise two. The model for eggs per nest after closing the wooden nest included this proximity label as a fixed effect besides nest design, age and time of day, as well as the interactions between these variables. Pen was included as a random effect. Percentages of floor eggs were calculated for 9 days before closing the wooden nests and 9 days after closing these nests. Nest open or closed was included as a fixed effect, pen as a random effect.

For light intensity and electrostatic measurements no statistical analysis was performed, as all light intensity measurements were equal and the electrostatic measures were measured without replication. Air temperature was analysed with nest design and nest closed or open as fixed effects, while including pen as a random effect.

3 Results

Distribution of eggs

During the course of the experiment 31,223 eggs were laid in the nests. The percentage of floor eggs over the entire experiment was 5.8 ± 2.8%. For the development of percentages of eggs in nests over time, see Figure 1. The interaction between nest design and age was found to be significant ($F_{27,2696}=3.03$, $p<0.0001$). In week 23 the wooden nest did not differ significantly yet from the partition nest, but from then onwards there was a strong preference for the wooden nest. The overall percentage of eggs in the four nest designs were found to all differ significantly from each other (Tukey test, $p<0.0001$). Most of the eggs were laid in the wooden nest (69.3 ± 1.0%), followed by the control nest (15.1 ± 0.8 %), the partition nest (10.2 ± 0.5%) and the ventilator nest (5.4 ± 0.4%) ($F_{3,2696}=738.1$, $p<0.0001$). Percentage of egg laying hens was on average 51.2 ± 6.1% and 86.9 ± 5.7% during the behavioural observation weeks 24-25 and 26-27 respectively. The proportion of eggs laid between 8:00-12:00 h was 51.0 ± 3.0% and between 12:00-16:00 h was 15.4 ± 1.5%, so the chosen behavioural observation time frames fell during and after peak laying time.

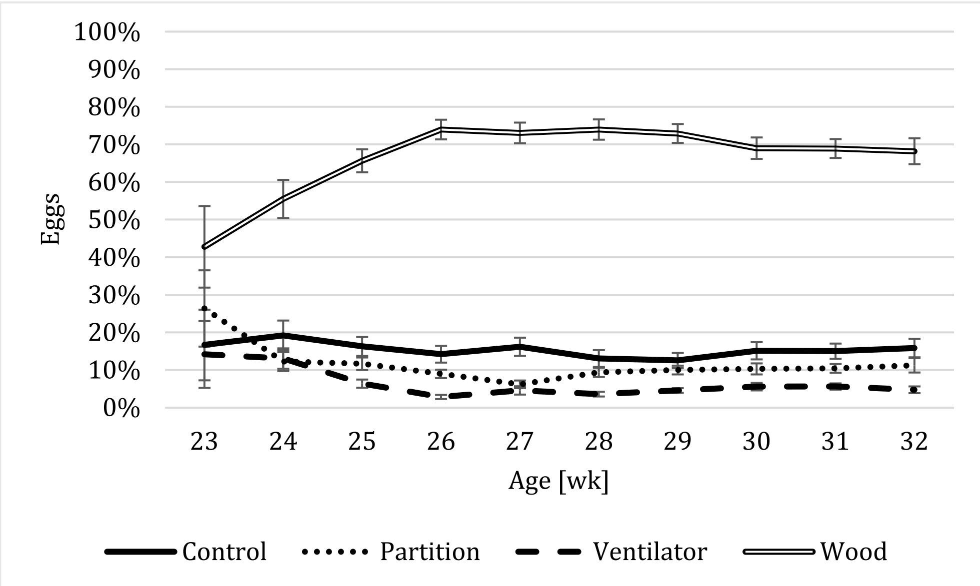

Figure 1. The distribution of eggs (%) over the four nest designs per week of age. Error bars depict the standard error.

Behaviour at age 24-27 weeks

For the time budget, the only behaviour differing between the nest designs was sitting (Table 3). More sitting was found in the wooden nest compared to the partition and ventilator nest, although no significant difference was found with the control nest. The number of inspections and visits expressed per egg was significantly lower in the wooden nest and significantly higher in the ventilator nest compared to the other nest designs (Table 4). Behaviours piling, displacement, feather pecking, head pecking and head shaking were all observed significantly more in the wooden nest compared to the other nest designs (Table 5). Piling behaviour was significantly lower in the partition and ventilator nest compared to the other nest designs. The maximum number of hens seen in the nests were 4, 5, 5 and 11 for respectively the ventilator, partition, control and wooden nest.

The number of nest inspections per nest visit declined with age ($F_{1,232}$=16.2, p<0.0001) from 2.19 ± 0.15 at age 24-25 weeks to 1.58 ± 1.15 at 26-27 weeks of age.

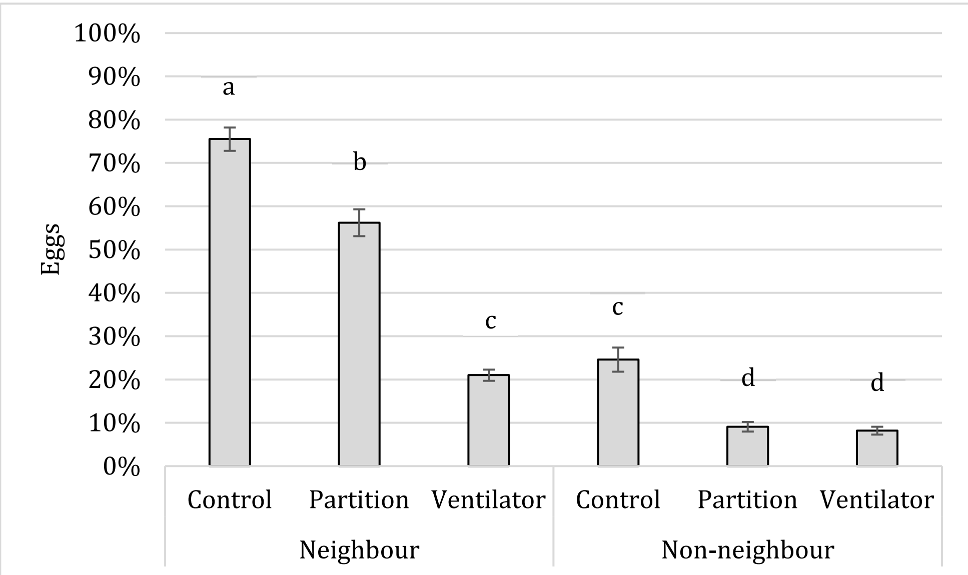

Figure 2. The distribution of eggs (%) over the remaining three nest designs after closing the wooden nests, separated for the proximity labels 'neighbour' and 'non-neighbour'. Error bars depict the standard error. Bars lacking a common superscript differ significantly (P < 0.01).

Table 3. Mean and standard error of percentage of activity budget observations (observed between 9:00-11:00 and 14:00-16:00 h) per nest design, including the F statistic and corresponding P value.

Parameter	Control	Partition	Ventilator	Wood	F statistic	P value
Sitting (%)	35.6 ± 7.0^{ab}	32.8 ± 6.3^{b}	29.2 ± 6.2^{b}	52.1 ± 3.6^{a}	$F_{3,89}=3.72$	0.0142
Walking (%)	33.3 ± 7.1	39.7 ± 6.6	35.8 ± 6.5	36.2 ± 3.4	$F_{3,89}=2.07$	0.1104
Standing (%)	9.0 ± 4.2	18.6 ± 5.7	8.3 ± 3.3	7.4 ± 1.8	$F_{3,89}=0.24$	0.8705
Resting (%)	1.3 ± 1.0	0.6 ± 0.6	5.9 ± 4.2	0.2 ± 0.2	$F_{3,89}=1.45$	0.2339

Table 4. Mean and standard error of nest interest observations (observed between 9:00-11:00 and 14:00-16:00 h) per nest design per egg, including the F statistic and corresponding P value.

Parameter	Control	Partition	Ventilator	Wood	F statistic	P value
Inspections per egg	9.4 ± 3.5^{b}	8.0 ± 2.8^{b}	12.2 ± 2.1^{a}	1.6 ± 0.3^{c}	$F_{3,42}=10.79$	<0.0001
Visits per egg	3.4 ± 0.8^{b}	4.1 ± 1.2^{ab}	6.2 ± 1.1^{a}	1.0 ± 0.2^{c}	$F_{3,42}=11.68$	<0.0001

Table 5. Mean and standard error of negative behaviours per nest design (observed between 9:00-11:00 and 14:00-16:00 h), including the F statistic and corresponding P value. Piling is expressed in percentage of total scan observations, the other parameters as frequency per 5-min observation.

Parameter	Control	Partition	Ventilator	Wood	F statistic	P value
Piling	8.75 ± 1.51^{b}	0.97 ± 0.57^{c}	1.88 ± 0.79^{c}	28.75 ± 2.82^{a}	$F_{3,3593}=50.45$	<0.0001
Displacement	0.17 ± 0.05^{b}	0.13 ± 0.04^{b}	0.08 ± 0.04^{b}	0.95 ± 0.21^{a}	$F_{3,377}=16.17$	<0.0001
Feather pecking	0.64 ± 0.18^{b}	0.28 ± 0.09^{b}	0.64 ± 0.21^{b}	1.55 ± 0.24^{a}	$F_{3,377}=16.40$	<0.0001
Head pecking	0.58 ± 0.16^{b}	0.27 ± 0.11^{b}	0.29 ± 0.11^{b}	2.34 ± 0.41^{a}	$F_{3,377}=27.58$	<0.0001
Head shaking	1.26 ± 0.33^{b}	0.72 ± 0.18^{b}	0.99 ± 0.23^{b}	3.83 ± 0.93^{a}	$F_{3,377}=14.26$	<0.0001

^{a,b,c} *Means lacking a common superscript within a row differ ($p<0.05$).*

Closing of the wooden nest
After closing the wooden nests, the percentage of eggs found in the remaining nests was the result of an interaction between nest design and proximity label ($F_{2,321}$=18.6, p<0.0001). Most eggs were found in the neighbouring control nest, followed by the neighbouring partition nest (Figure 2). Fewer eggs were found in the neighbouring ventilator nest and non-neighbouring control nest. The non-neighbouring partition and ventilator nest received the fewest eggs. Closing the wooden nests did not significantly affect percentage of floor eggs, which was found to be 2.6 ± 0.3% before and 2.9 ± 0.2% after closing the wooden nests ($F_{1,96}$=0.5, p=0.48). Percentage of egg laying hens was on average 73.5 ± 3.6% during this period.

Physical characteristics
Light intensity inside the nests was found to be 0 lx in all nests. Air temperature inside the nests was found to be the result of the interaction between nest design and whether the nest was open or closed ($F_{3,2295}$=190.7, p<0.0001). The temperature in the wooden nest was 1.42 ± 0.06 ^{0}C higher during the time the nests were open to the birds compared to the other nest designs, but this difference was not found during the time the nests were closed. The electrostatic field measurements in the control nest were numerically higher than in the wooden nest, which can be found in Table 6. The surface resistance of the plastic wall of the control nest was measured at 1,000,000 MΩ, while the wooden wall was found to be 4 MΩ.

4 Discussion

This study shows a strong relative preference of the broiler breeder hens for the wooden nest. The large majority of the eggs were laid in the wooden nest and this suggests that the hens found this nest design best suited for egg laying, which is the ultimate purpose of a nest. The lower number of nest inspections

Table 6. Electrostatic fields measured in one control nest and one wooden nest, expressed in volts.

Part of nest	Control	Wood
Entrance	8	7
Nest floor	7	4
Nest curtain	67	9
Side wall	75	8

(looking into the nest) and nest visits (entering the nest) per egg in the wooden nest compared to the other nest designs strengthens the suggestion that this nest is found to be more suitable by the hens (Appleby and Hughes, 1995; Freire et al., 1996). Furthermore, an increased amount of sitting behaviour was observed in the wooden nests compared to the other nest designs. More sitting behaviour has been linked to more settled nesting as well (Freire et al., 1996; Cronin et al., 2012). Since chickens are known to be gregarious in their nesting behaviour, the question remains whether all individuals prefer the wooden nests or that the first hens chose this nest design and the other hens joined them (Appleby and McRae, 1986). The fact that the wooden nest was preferred in 5 out of 6 pens does suggest that the majority of hens prefer this nest design, regardless of gregarious motivations.

Contrary to our predictions, the partition nest design was not preferred by our broiler breeder hens. The partition was thought to create two smaller, more secluded areas for the birds. Smaller sized nests have previously been found to be preferred by broiler breeders as well as laying hens (Holcman et al., 2007; Ringgenberg et al., 2014). More secluded nests in terms of nest curtains also resulted in more settled nesting behaviour in laying hens (Struelens et al., 2008; Stämpfli et al., 2012; Ringgenberg et al., 2015b). In this study however, the partition nest was found to be less preferred than the wooden and control nest by the hens. The partition was meant to create extra corners for the hens to sit in, but the partition was placed in line with the nest entrance and hens sitting against this partition would be in clear view of their pen mates. So while creating extra corners, these were perhaps unattractive due to their limited seclusion. In addition, the fact that the partition was only sitting hen height might be insufficient to create the idea of a more secluded nest.

The ventilator nest was least preferred, which is in line with our predictions. The percentage of eggs found in the ventilator nest was significantly lower than in the other nests and the number of nest visits and inspections per egg were significantly higher. Both findings are signs of disliking the nest. Good ventilation in poultry houses is essential to regulate air quality for the welfare of the chickens as well as the farm workers (Whyte, 1993; Kocaman et al., 2006). Depending on the temperature and speed of the air, ventilation can also cause heat loss and thermoregulatory responses to prevent heat loss. Although the air flow in our experiment was relatively low compared to these studies, the temperature in our experiment was set to only 18 ^{0}C and the animals were fed restrictively. These values combined will likely create a nest with the feeling of a draught, which the hens found unattractive for an egg laying location. It is unlikely that noise or vibration caused by the ventilator influenced nest choice, as the ventilator was selected on the characteristics of low-noise and did not have any contact with the nest to cause vibrations.

As for the preference of wood compared to the plastic material of the other nest designs, we included some characterising measurements to tease apart possible reasons behind the preference for the wooden material. The nest designs were all providing equally dark spaces, so this cannot explain the preference for wood. Since the nests were so dark, we assume that the colour difference of dark brown wood versus black plastic was not involved in nest choice. The air temperature was higher in the wooden nests during the time that the nests were open, which is likely caused by the increased number of hens using the nest compared to the other nests. The increased temperature could also be explained by the fact that wood is a better insulator. When the nests were closed, the nest designs did not significantly differ in air temperature and this also seems to suggest that temperature is not a factor in preference for wood. Albeit measured in one nest of each design only, our measurements indicate a potential difference in terms of electrostatic properties between the two materials. The plastic control nest had higher electrostatic fields and surface resistance than the wooden nest, although we could not analyse this difference statistically. Only two studies have been done on the effects of electric fields on poultry behaviour and performance. The results of these studies were inconsistent and focussed on low stray voltage between 0-18V (Vivaldi et al., 1996; Worley and Wilson, 2000). In this study we found electrostatic fields of over 70 V in the plastic nest, which raises the question on whether this could be attributed to the lower preference for this material. More research is needed to clarify the effects of electrostatic properties of materials on chickens as well as other properties not included in this study, such as smell, sound and light reflection. These factors are known to be well sensed by chickens and might be involved in selecting the most attractive nest (Collias and Joos, 1953; Jones and Roper, 1997; Prescott and Wathes, 1999).

The strong preference for the wooden nests caused crowding in these nests. This is reflected by an increased frequency of piling behaviour. The crowding also seemed to have led to increased aggressive behaviour between the hens in the form of nest displacement, feather and head pecking. Head shaking was also observed more in the wooden nest and this behaviour is known to be increasingly performed during conflict situations or in stressful environments (Hughes, 1983; Mason, 1991). Altogether it seems that the wooden nests turned into a negative environment due to the crowding. However, the hens did not change their location for oviposition in response to this crowding, but consistently returned to the wooden nest. We interpret this as a sign of the strength of their preference for this nest design. One of the ways to measure the strength of preference, is by making the animals work or pay a high price for their desired goal (Dawkins, 1983; Duncan, 1991). The biological equivalent of 'paying a high price' could be the amount of aversion an animal is willing to accept in order to reach its desired goal. So when these hens were willing to accept the negative circumstances of crowding in order to lay their egg in the wooden nest, this shows a strong preference for this nest design. It should be noted, though, that the frequencies of behaviours observed were not corrected for the number of hens per nest, and therefore the negative social interactions encountered per hen may have been overestimated in the preferred nests. Furthermore, the crowding could have affected nest choice as the wooden nest was inaccessible for hens at busy moments.

Where a clear preference is seen at the earlier ages, location of the nests starts to become a factor in nest choice at a later age. When the wooden nests were closed at the age of 32 weeks, the hens showed a preference for nest design depending on location. When comparing the same nest design, the nests neighbouring the wooden nest received significantly more eggs than the nests further away. This is in line with previous studies on nesting location with laying hens, reporting a conservatism in egg laying location (Appleby et al., 1984b; Duncan and Kite, 1989; Riber, 2010; Riber and Nielsen, 2013). The finding that the number of nest inspections per nest visit decreased with age also suggests that the hens had made their choice and nest exploration was kept to a minimum. Tracking individual nest choices would be needed to confirm this suggestion. The preference for the wooden nest increased from 23 to 26 weeks of age and thereafter remained stable. This initial period of nest exploration at the onset of lay therefore determines the nest location for the rest of the production period, which is an important message for commercial producers. Once hens have found a nest location, however unsuitable for egg quality, it proves to be difficult to change this preference.

Conclusion

From this study we conclude that a strong preference of broiler breeder hens for wooden nests over plastic nests is apparent, when offered in a relative choice test. This preference was demonstrated by a higher proportion of eggs laid in this nest and more settled nesting behaviour. It also led to crowding, causing piling and aggressive behaviour in the wooden nest. The hens were willing to accept this environment as they continuously returned to the nest. Closing the wooden nest led to a new nest choice, which was the result of an interaction between nest design and proximity to the closed wooden nest. This study shows how the material used for nests is an important factor in suitability and should therefore be taken into account when designing nests.

Acknowledgements

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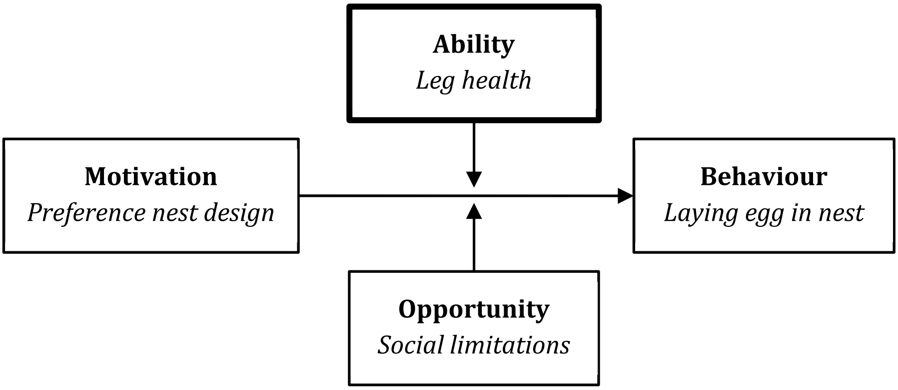
Chapter 3

High levels of contact dermatitis and decreased mobility in broiler breeders, but neither have a relationship with floor eggs



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Abstract

Contact dermatitis, both on the foot pads and hocks, is a well-known health issue in broilers. Less is known about contact dermatitis in broiler breeders, however, although they have many risk factors for developing leg health problems in common with broilers. This study aimed to describe the prevalence and severity of contact dermatitis during the production cycle in 5 lines of broiler breeders, investigate possible causes of contact dermatitis and study its relationship with gait, egg production and floor egg percentage. Five commercially available genetic lines of broiler breeders were housed in 21 pens of 550 females and 50 males from 20 to 60 weeks of age. Every 10 weeks litter quality, leg health measurements (foot pad dermatitis, hock burn, gait) and body weight were assessed of 50 random hens per pen. Total number of eggs, number of eggs laid outside the nest (floor eggs) and mortality were recorded daily per pen. Prevalence of foot pad dermatitis, hock burn and gait problems increased with age. Litter quality started to decrease at 50 weeks of age. Prevalence of foot pad dermatitis was affected by litter quality, while genetic line had little effect. One genetic line was more prone to developing hock burns, though generally the prevalence of hock burn (13%) was much lower than that of foot pad dermatitis (74%). The percentage of broiler breeders with gait problems increased up to 24% with age, but this was not related to the prevalence of contact dermatitis. The lines differed in body weight from 32 weeks of age onwards and a higher body weight was related to lower egg production and higher cumulative mortality. The percentage of floor eggs was not related to leg health parameters or genetic line. Broiler breeders thus have similar leg health problems as broilers, but these problems are not related to the percentage of floor eggs, suggesting that other factors are involved in the undesirable behaviour of floor laying.

Key words: broiler breeder, genetic line, leg health, foot pad dermatitis, floor egg

1 Introduction

Foot pad dermatitis and hock burns in broilers pose a major welfare problem and have received much attention over the last decades, but little is known on these conditions in broiler breeders. Both conditions are a form of contact dermatitis, where the skin becomes acutely inflamed when it comes into contact with irritating material (Greene et al., 1985). The incidence of foot pad dermatitis in fast-growing broiler flocks has been found to be up to 65% at slaughter age, while the incidence of severe hock burn at this age can be as high as 41% (Haslam et al., 2007; de Jong et al., 2012; Bassler et al., 2013). Limited research has been done on the prevalence of contact dermatitis in broiler breeders, but it seems that this condition is also prevalent in the parent stock (Wolanski et al., 2004; Renema et al., 2007; Kaukonen et al., 2016).

The risk factors for developing contact dermatitis that have been identified in broilers, are present in the broiler breeder husbandry as well. Poor litter quality, where a high litter moisture level causes more ammonia release, is the most common risk factor found for foot pad dermatitis (Martland, 1985). Broiler breeder flocks are kept over six times longer in the production house than broilers and during this period very little fresh litter is usually added, which results in accumulating litter (manure) and increasing litter moisture (Lien et al., 1998; Maguire et al., 2006). However, broiler breeder farms usually have slatted areas, which give the birds an opportunity to rest in a nonlitter area and this might have beneficial effects on contact dermatitis (Sander et al., 2003). Genetic predisposition and high body weight are other risk factors in broilers (Shepherd and Fairchild, 2010) and may also play a role in the development of contact dermatitis in broiler breeders.

Contact dermatitis is likely a painful condition, which is associated with health and performance problems (Ekstrand et al., 1998; de Jong and Guémené, 2011). Lame broilers were found to consume more feed containing analgesic drugs than unaffected broilers and contact dermatitis is one of the conditions that can cause lameness (Danbury et al., 2000). Broiler have been found to have a decreased feed intake and a higher chance of infection with microorganisms such as Staphylococcus aureus with increasing foot pad dermatitis (Martland, 1985; Hester, 1994). In broiler breeders, painful contact dermatitis could reduce the hen's ability of reaching the nest and lead to an increased risk of eggs laid outside the nest, so-called floor eggs. Floor eggs are an economic problem as they require manual collection and are often dirty, which results in a lower saleability and hatchability (van den Brand et al., 2016). Floor eggs may also reflect a welfare problem as chickens are inclined to lay their egg in a secluded nest (Zupan et al., 2008; Buchwalder and Fröhlich, 2011), which suggests that a hen laying her egg elsewhere is constrained by for example reduced mobility due to contact dermatitis.

To our knowledge, little is known about the prevalence of leg health issues and their consequences for production and welfare in broiler breeders. The first aim of this study was therefore to assess the prevalence and severity of contact dermatitis in broiler breeders during the production cycle. Furthermore, we investigated possible risk factors associated with contact dermatitis by comparing different commercially available genetic lines, measuring body weight and scoring for gait and litter quality. Lastly, the relationships between contact dermatitis, gait, egg production and floor egg percentage were investigated. We expected an increase in prevalence and severity of both foot pad dermatitis and hock burns with age. Genetics, body weight and litter quality were expected to have an effect on both forms of contact dermatitis. Foot pad dermatitis was expected to be related to increased gait problems, which in turn was predicted to be related to a higher percentage of floor eggs.

2 Materials and Methods

Animals and housing

The experiment took place from June 2018 to March 2019 at a breeding station. A total of 11,550 females and 1,050 males, all non-beak trimmed, were moved from their rearing facilities located at the same breeding station at the age of 20 weeks. Five commercially available fast-growing genetic lines were represented in different numbers. The chickens were assigned to 21 pens of 550 females and 50 males of the same genetic line, resulting in six pens for lines 1 and 2 (3,300 females and 300 males per line), five pens for line 3 (2,750 females and 250 males) and two pens for lines 4 and 5 (1,100 females and 100 males per line). The position of the genetic lines in the house was randomized using a block design. The pens were identical in size (12 x 6.5 x 2.0 m, length x width x height) and lay-out, and were placed in four rows. Animal density was 7.7 birds/m^2, which is comparable to commercial practice. The pens had wire mesh walls, which allowed the animals from different pens to see each other. The litter area (12 x 3.7 m) was covered with wood shavings and the slatted area (12 x 2.3 m) was raised by 0.5 m and gave access to 9 bell drinkers and 10 nests. No wood shavings were added, removed or otherwise treated during the duration of the experiment. The group-nests were of a rollaway type (Vencomatic©), measuring 1.15 x 0.52 x 0.53 m (l x w x h). All nests had a green rubber nest floor slanting towards the back and red nest curtains with an entry

in the middle. The feeding line for the females was placed partially on the slats and partially in the litter area, while the male feeding line was positioned in the litter area.
The house was lit with artificial LED-lighting. At 20 weeks of age, the animals had 8 h of light (7:00 to 15:00 h) at 10 lx measured at bird height. This was gradually increased with age and laying percentage of the flock to 14 h of light (2:00 to 16:00 h) at 60 lx at bird height. The temperature was maintained at 21 ± 1 ^{0}C, using a combination of air intake ventilators, exhaust valves and a heat exchanger. Food was provided at 8:30 h, giving a restricted amount according to the management guide of the breeding companies ranging from 100 to 164 g. Birds were continuously weighed automatically with a poultry scale hanging in the litter area. Water was provided from 8:30 to 12:30 h and from 15:30 to 16:00 h. The nests were available to the hens from 1 h before lights-on until 30 min before lights-off, from the day after the first egg was found (23 weeks of age). The birds were kept until the age of 60 weeks and then slaughtered for human consumption.

Data collection

Leg health

Approximately every 10 weeks (21, 32, 40, 50 and 60 weeks of age) a random selection of 50 hens per pen was hand-weighed and scored for foot pad dermatitis and hock burn according to the Welfare Quality® Assessment Protocol for Poultry (2009), see Figure 1. A score of 0 represents no dermatitis, 1-2 mild dermatitis and 3-4 severe dermatitis. Litter quality was recorded according to the score described in the Welfare Quality® Assessment Protocol for Poultry (2009), ranging from 0 for dry and flaky litter to 4 for solid litter covered with a crust. Gait was scored according to a scale adapted from Garner et al. (2002), see Table 1. Any mortalities were noted.

Production

Starting at 24 weeks of age until the birds were depopulated, the number of floor and nest eggs were recorded daily per pen. Floor eggs were collected three times per day and nest eggs were collected once a day. Eggs laid on the slatted area were prevented from rolling into the litter with a 18mm plastic tube, which allowed for separate recording of litter eggs and eggs laid on the slats. This plastic tube had to be removed at 45 weeks of age for manure management, after which no distinction could be made between floor eggs laid on the slats or in the litter.

This study was not considered to be an animal experiment under the Law on Animal Experiments, as confirmed by the local Animal Welfare Body (3 June 2018, Wageningen, The Netherlands).

Statistical analysis

Egg production percentage per pen was calculated by dividing the total number of eggs by the number of hens present. Floor egg percentage was calculated by dividing the number of floor eggs over the total number of eggs laid per pen per week, whereas litter egg percentage was calculated by dividing the number of eggs laid in the litter over the total number of floor eggs. Mortality percentage per pen was calculated by dividing the number of dead hens over total number of hens placed. All leg health parameters were analysed as mean per pen per observation week. The percentage of birds was calculated for presence of foot pad dermatitis, hock burn and gait (score ≥ 1) as well as for severe foot pad dermatitis, hock burn and gait (scores ≥ 3).

All statistical analyses were performed with SAS (version 9.4). The MIXED procedure was used to perform repeated general linear mixed models in order to investigate differences between lines and weeks of age. Fixed effects included line and week of age and their interaction and pen within line was included as a random effect. The assumptions of homogeneity of variance and normally distributed residuals were examined visually using the conditional studentized

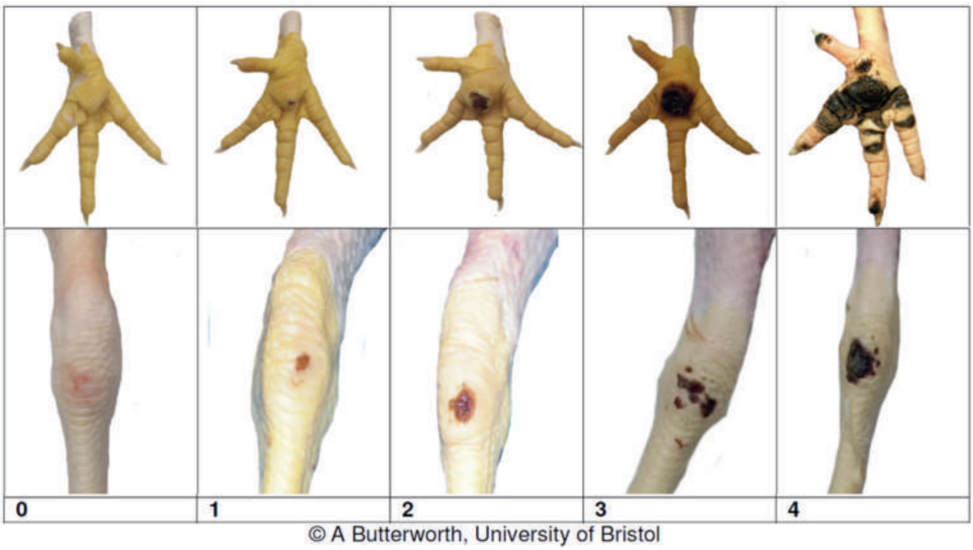

Figure 1. Visual scales for scoring foot pad dermatitis and hock burn (Welfare Quality®, 2009).

Table 1. Scale for scoring gait, adapted from Garner et al. (2002).

Gait score	Description
0	Bird moves fluidly.
1	Bird has an unsteady, wobbling walk. Problem leg cannot be detected.
2	Bird walks for more than 10 seconds. Problem leg can be detected.
3	Bird walks away spontaneously, but squats within 10 seconds.
4	Bird only walks away when approached or nudged.
5	Bird cannot walk.

residuals plots. Pearson correlations were calculated between traits using the CORR procedure. Results are shown as non-transformed means with corresponding standard errors and p-values below 0.05 were considered significant. Tukey's post hoc test was performed to investigate significant differences between test groups.

3 Results

Leg health

For an overview of the results of all measured leg health indicators specified per genetic line, see Figure 2. For an overview of leg health indicators, body weight and litter quality over age, see Table 2.

The condition of foot pads significantly deteriorated as the hens aged, illustrated by higher scores, until it stabilized at the age of 50 and 60 weeks ($F_{4,64}$ = 127.65, P<0.0001). Foot pad condition was not affected by genetic line ($F_{4,16}$ = 0.24, P=0.9) or its interaction with age ($F_{16,64}$ = 1.62, P=0.08). In line with the increasing foot pad lesion scores, the percentage of hens with severe foot pad dermatitis (score 3 or 4) increased from 32 to 50 weeks age, after which it stabilized ($F_{4,64}$ = 34.63, P<0.001), with no significant differences between the lines ($F_{4,16}$ = 0.70, P=0.6) and no line by age interaction ($F_{16,64}$ = 1.41, P=0.2). Average foot pad dermatitis score per pen correlated with litter quality scores (r=0.47, P=0.034), with most problems in pens with the poorest litter. Foot pad score was not significantly correlated with body weight at the individual level.

The mean hock burn score was affected by the interaction between age and line ($F_{16,64}$ = 3.00, P=0.0009) and the main effect of age ($F_{4,64}$ = 23.02, P<0.0001), but not by the main effect of line ($F_{4,16}$ = 1.65, P=0.2). Only at 50 and 60 weeks of age, line 4 had a higher score (0.6 ± 0.1 and 0.4 ± 0.1 respectively) than the other lines (0.1-0.3 and 0.1-0.2 respectively), while the lines did not differ at the earlier ages. The mean hock burn score increased until the age of 50 weeks after which it stabilized. The low mean hock burn score is reflecting of the low severity of hock burn, maximum 0.4 ± 0.2% of the birds received a score of 3, while none received a score of 4. Hock burn score was not significantly correlated with body weight at the individual level or with litter quality score at pen level.

Mean gait score was affected by the interaction between age and line ($F_{16,64}$=2.2, P=0.014) and the main effect of age ($F_{4,64}$ = 22.2, P<0.0001) as well as genetic line ($F_{4,16}$ = 4.29, P=0.02). Line 1 had a higher gait score than line 3 at the age of 40 weeks (0.4 ± 0.0 compared to 0.2 ± 0.0) and line 1 had a higher gait score than line 5 at 60 weeks of age (0.5 ± 0.1 compared to 0.1 ± 0.0). All other lines had intermediate scores. Independent of line, the mean gait score increased to 0.3 ± 0.0 at the age of 40 weeks, after which it stabilized. When looking at the overall differences between lines, line 1 (0.29 ± 0.02) had a higher mean gait score than line 5 (0.07 ± 0.01) and all other lines had intermediate scores. The percentage of birds with severe gait scores (scores 3-5) increased with age ($F_{4,64}$= 3.7, P=0.01), but was not affected by genetic line ($F_{4,16}$= 1.5, P=0.3) or its interaction with age ($F_{16,64}$= 1.4, P=0.2). At 40 and 60 weeks of age, the highest percentages of 2.3 ± 0.5% and 2.7 ± 0.5% respectively were observed. Gait score was not significantly correlated with foot pad score, hock burn score or body weight within individuals.

Body weight was affected by the interaction between line and age ($F_{16,64}$ = 9.34, P<0.0001). The differences between the lines started at the age of 32 weeks, when line 1 (3832 ± 19 g) had a significantly higher body weight than line 5 (3478 ± 27 g) with line 2 (3810 ± 20 g), line 3 (3758 ± 19 g) and 4 (3745 ± 32 g) in between. At 40 weeks of age, both line 1 and 2 (4133 ± 24 g and 4126 ± 22 g respectively) were significantly heavier than line 5 (3692 ± 33 g), while at 50 weeks of age lines 1-3 (4391 ± 27 g, 4301 ± 27 g and 4373 ± 28 g respectively) were significantly heavier than line 4 and 5 (3934 ± 38 g and 3789 ± 30 g respectively). At the age of 60 weeks, lines 1-3 (4489 ± 30 g, 4411 ± 27 g and 4323 ± 32 g respectively) had a significantly higher body weight than line 5 (3768 ± 36 g), while line 4 had an intermediate body weight (4026 ± 42 g).

Litter quality deteriorated with the age of the birds ($F_{4,64}$ = 173.96, P<0.0001), but was not affected by line ($F_{4,16}$ = 1.54, P=0.2) or the interaction between age and line ($F_{16,64}$ = 1.56, P=0.1). The mean litter score increased significantly from 50 weeks of age and 60 weeks of age compared to 21, 32 and 40 weeks of age.

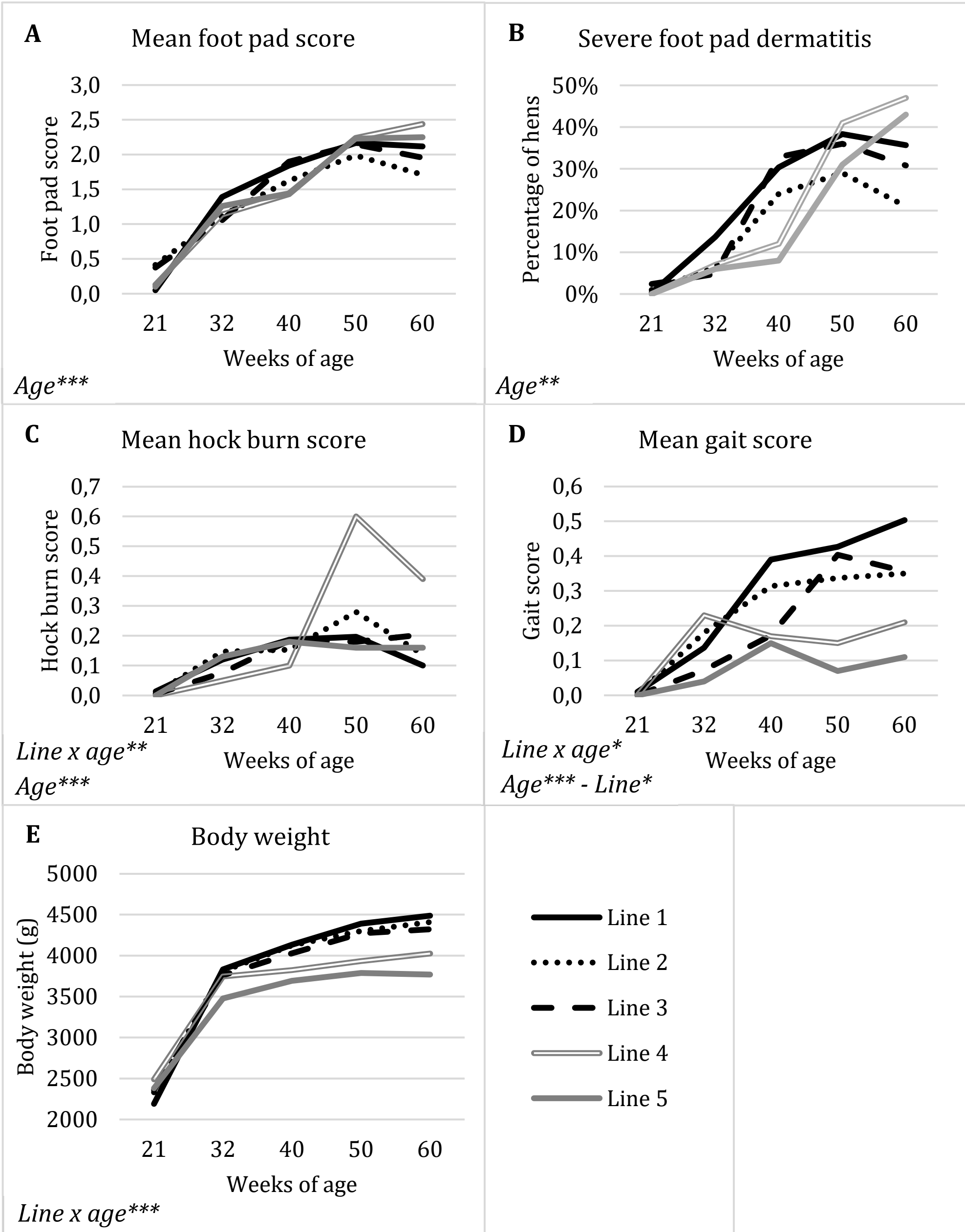

Figure 2. Overview of the development of leg health parameters and body weight with age, specified per genetic line. A) The mean foot pad dermatitis score. B) The percentage of hens with a severe foot pad dermatitis score of 3-4. C) The mean hock burn score. D) The mean gait score. E) The mean body weight in grams. Significant effects of line, age or the interaction between line and age are noted in italic in the bottom left corner with an indication of the P-value (<0.05; ** < 0.01; *** < 0.001). Scores of 0 indicate unaffected birds, while scores of 3 and higher are considered severe.*

Table 2. Mean values and standard errors of leg health variables measured, specified per age. Severe foot pad score, hock burns and gait problems indicate the percentage of hens affected. Scores of 0 indicate unaffected birds, while scores of 3 and higher

Item	21 weeks	32 weeks	40 weeks	50 weeks	60 weeks
Mean foot pad score	0.3 ± 0.0^{d}	1.2 ± 0.1^{c}	1.7 ± 0.1^{b}	2.1 ± 0.1^{a}	2.0 ± 0.1^{a}
Foot pad score ≥ 1 [% of birds]	17.5 ± 1.2^{d}	77.5 ± 1.3^{c}	85.1 ± 1.1^{b}	95.0 ± 0.7^{a}	93.0 ± 0.8^{a}
Foot pad score 3-4 [% of birds]	1.0 ± 0.3^{d}	8.1 ± 0.8^{c}	25.2 ± 1.3^{b}	34.7 ± 1.5^{a}	32.1 ± 1.4^{a}
Mean hock burn score	0.0 ± 0.0^{d}	0.1 ± 0.0^{c}	0.2 ± 0.0^{bc}	0.3 ± 0.0^{a}	0.2 ± 0.0^{ab}
Hock burn score ≥ 1 [% of birds]	0.7 ± 0.3^{d}	10.8 ± 1.0^{c}	15.4 ± 1.1^{b}	20.9 ± 1.3^{a}	16.0 ± 1.1^{b}
Hock burn score 3-4 [% of birds]	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.3 ± 0.2^{a}	0.2 ± 0.1^{a}	0.4 ± 0.2^{a}
Mean gait score	0.0 ± 0.0^{c}	0.1 ± 0.0^{b}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.0^{a}
Gait score ≥ 1 [% of birds]	0.4 ± 0.2^{d}	8.7 ± 0.9^{c}	18.8 ± 1.2^{b}	24.4 ± 1.3^{a}	23.3 ± 1.3^{ab}
Gait score 3-5 [% of birds]	0.0 ± 0.0^{b}	1.0 ± 0.3^{ab}	2.3 ± 0.5^{a}	1.0 ± 0.3^{ab}	2.7 ± 0.5^{a}
Body weight [g]	2319 ± 12^{e}	3766 ± 10^{d}	4035 ± 12^{c}	4236 ± 15^{b}	4314 ± 16^{a}
Litter quality	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.3 ± 0.1^{c}	1.6 ± 0.1^{b}	3.2 ± 0.2^{a}

$^{a\text{-}e}$ *Means lacking a common superscript within a row differ ($P < 0.05$).*

The cumulative mortality differed between the lines ($F_{4,16}$ = 8.65, P=0.0006). Line 1 had a higher mortality (10.3 ± 1.9%) than lines 2 (6.9 ± 0.4%), 3 (4.8 ± 0.4%) and 5 (4.3 ± 0.9%), while line 4 had an intermediate level of mortality (7.5 ± 0.2%). Cumulative mortality was positively correlated with average body weight per pen (r= 0.43, P=0.052), with higher mortality in pens with heavier birds.

Production

Egg production percentage increased rapidly after the onset of lay and then declined again after the age of 30 weeks, see Figure 3. An interaction between line and age was found, where lines differed at earlier ages but not at the age of 60 weeks ($F_{140,5000}$ = 23.66, P<0.0001). Line 5 had a significantly higher egg production percentage than line 1 from the onset of lay up to 50 weeks of age, while line 4 only had a significantly higher egg production percentage than line 1 during ages 40 to 50 weeks. Egg production percentage was negatively correlated with average body weight per pen (r= -0.50, P=0.022).

The percentage of floor eggs was affected by the interaction between line and age ($F_{136,4849}$ = 11.28, P<0.0001), see Figure 4. The percentage of floor eggs of all genetic lines fluctuated with age and no clear pattern could be distinguished in this interaction. Most of the floor eggs were found in the litter area. The percentage of litter eggs (expressed as a percentage of floor eggs) increased significantly from 78.3 ± 2.0% at 26 weeks of age to 88.9 ± 1.1% at 30 weeks of

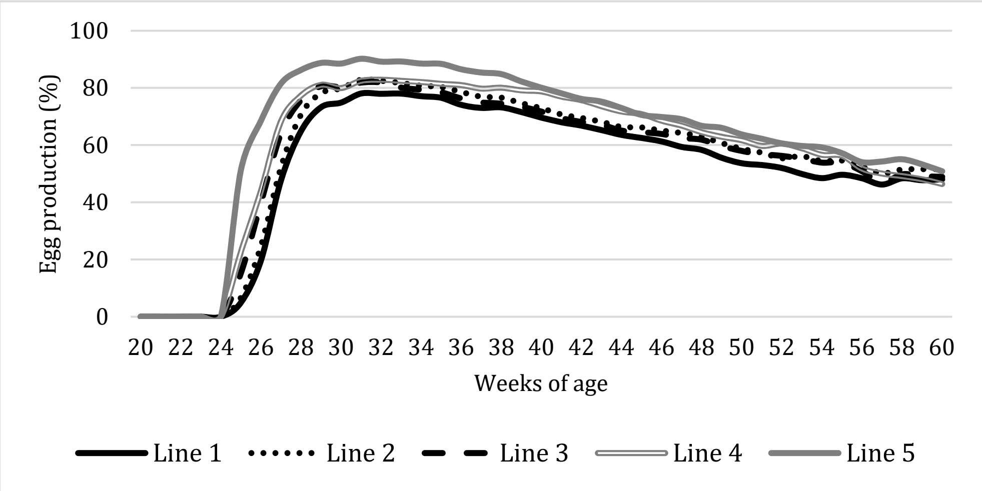

Figure 3. The development of egg production percentage with age, specified for the different genetic lines.

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age ($F_{20,2840}$ = 15.0, P<0.0001) after which it did not increase anymore. The lines did not significantly differ in percentage of litter eggs. No significant correlations between floor egg percentage and leg health parameters or body weight were found.

4 Discussion

This study aimed to provide descriptive information on the prevalence and severity of contact dermatitis in broiler breeders during the production cycle, which we found to increase with age. Hock burn was less prevalent and less severe than foot pad dermatitis, both of which were not correlated to body weight. Body weight did correlate to egg production, which differed significantly between the commercially available genetic lines. The percentage of floor eggs was, against our hypothesis, not correlated to contact dermatitis or gait problems.

Leg health

In accordance with our expectations, the incidence and severity of all indicators for leg health problems increased with age. Our expectations were mainly based on broiler research, where the prevalence of severe foot pad dermatitis in commercial flocks ranges from 38-72% at slaughter age (Haslam et al., 2007; de Jong et al., 2012). Only one study has been published describing leg health during the production cycle of broiler breeders, which found 0-5.5% of birds

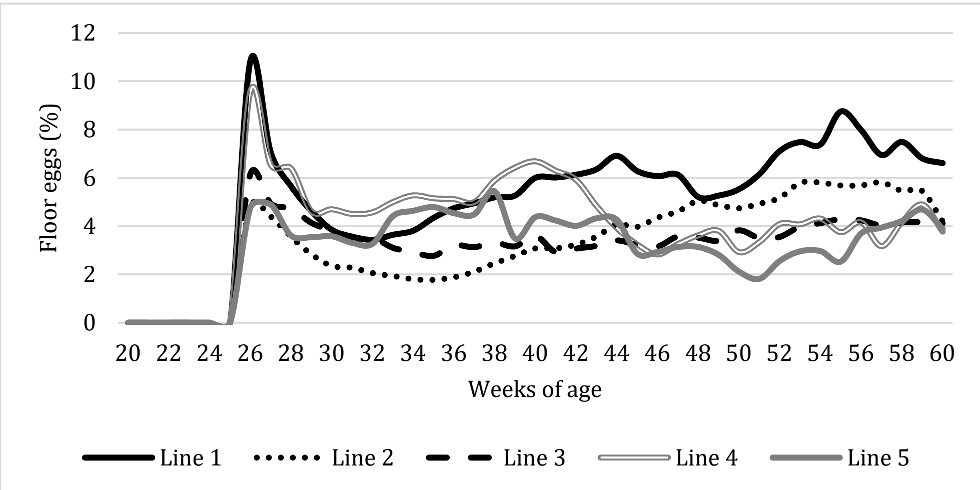

Figure 4. The development of percentage of floor eggs with age, specified for the different genetic lines.

had severe foot pad dermatitis at 19, 24 and 36 weeks of age, after which it significantly increased to 25% at the age of 48 weeks and increased further towards 64% at 60 weeks of age (Kaukonen et al., 2016). This is largely in accordance with our findings, although we did not find the an increased percentage of birds with severe foot pad dermatitis between 50 and 60 weeks of age (35% and 32% respectively). Kaukonen et al. (2016) stressed the large variation between flocks of severe foot pad dermatitis prevalence at slaughter age, ranging from 51% to 83% in their study. This could explain the difference between our findings and the study of Kaukonen and co-workers at later ages. Our study was performed with large groups housed under commercial, but controlled, conditions. This allowed us to gather information that reflects the commercial industry without the large variety in management and environmental factors of a field study. Both our study and that of Kaukonen et al. (2016) report that at least 30% of broiler breeder hens have severe foot pad dermatitis at a certain point in the production cycle, indicating that this is an important problem in the breeder industry.

The severity of foot pad dermatitis was correlated to decreasing litter quality from the age of 40 weeks onwards. This relation between foot pad dermatitis and litter quality has been well established in broiler research, as well as in the previously mentioned studies with broiler breeders (Shepherd and Fairchild, 2010; Kaukonen et al., 2016). The moisture of litter is the most important factor in the development of foot pad dermatitis (Martland, 1985). The scoring system for litter quality used in this study is designed to reflect the level of moisture in the litter. However, Kaukonen et al. (2016) used the same scoring system and found that litter with a higher score had a lower moisture content. Although litter moisture content was not measured in this study, the litter appeared more moist with a higher litter score.

The higher incidence of hock burn in line 4 at later ages was not related to deteriorating litter quality or gait, which suggests that this line has a genetic predisposition for hock burn development. Earlier studies with broilers have also found differences between genetic strains for the prevalence of hock burns (Kestin et al., 1999; Haslam et al., 2007; Ask, 2010). Lines 4 and 5 were represented with a lower amount of birds than lines 1-3 due to constraints in the experimental set-up, so these results should be carefully interpreted and more research is needed on the genetic influence of contact dermatitis.

The severity of hock burns was very low in this experiment with 0.4% of the birds having severe hock burn at the age of 60 weeks. Even so, this is a higher severity compared to the research of Kaukonen et al. (2016), who did not find any broiler breeders with severe hock burn. This could be due to differences in genetic line, stocking density or litter quality compared to our study. However, the percentage of birds with severe hock burn in our study is still much lower than the average percentage of broilers found to have severe hock burn before slaughter. Findings range from an average of 1.3-7.9%, with some farms having more than 40% of the birds affected with severe hock burn (Haslam et al., 2007; Bassler et al., 2013).

Hock burns in broilers have been found to correlate with body weight, as it is thought that the heavier broilers spend more time sitting. This increased amount of time of hocks spent in contact with the litter increases the incidence of hock burn (Kjaer et al., 2006; Haslam et al., 2007). We did not find a correlation between hock burn and body weight, however. It is possible that broiler breeders do not alter their sitting behaviour with increasing body weight as they grow more gradually than broilers. Another explanation would be that the birds mostly use the slatted areas to rest on and decrease the contact with litter in this manner. Our study did not include any activity measurements to validate this suggestion.

The incidence of gait problems increased with age and differed between the genetic lines, which was also found in another study with different genetic lines of broilers (Kestin et al., 1999). The severity of gait problems was low in our experiment with a maximum of 2.7% of the hens having severe gait problems. No other studies with broiler breeders are available for comparison, but our results can be compared with studies on commercial broilers. Two studies found that on average 14-30% of the broilers showed gait problems with some flocks having 50% of the birds affected (Sanotra et al., 2003; Bassler et al., 2013). Against expectations we did not find correlations between foot pad dermatitis and gait score, so it seems that (also) other factors are at the basis of gait problems, such as activity of the birds. Walking ability has been shown to improve by increasing the activity of broilers, as this enhances tibiotarsal bone thickness and decreases vasculature abnormalities of bone extremities (Sherlock et al., 2010). This is likely also the case for broiler breeders, although future studies are necessary to confirm this suggestion.

Although all lines were given the same amount of feed, their body weights differed significantly from 32 weeks of age. This suggests a difference in feed conversion ratio that has a genetic basis, due to selective breeding for desired traits at offspring level (Dawkins and Layton, 2012). A higher body weight has mainly negative consequences for the health of the birds. This is illustrated by the positive correlation between body weight and cumulative mortality in our study. It should, however, be noted that it is also possible that a higher mortality allowed the remaining birds to grow faster due to increased (feeding) space.

Production

Egg production percentage followed a pattern as commonly seen in commercial practice (Zuidhof et al., 2007), although the genetic lines differed significantly. After an initial steep increase until the age of 30 weeks with a mean egg production of 83%, the percentage slowly decreased again to 50% at the slaughter age of 60 weeks. Clear differences between the genetic lines were visible at all ages, except 60 weeks of age. Differences in egg production percentage between genetic strains is a well-known phenomenon, since this is directly related to balancing different breeding goals for broilers in terms of growth and body weight (Dawkins and Layton, 2012).

While egg production percentage can thus be partially explained by genetic potential, we also found a negative correlation with body weight until the age of 50 weeks. It has been well established that broiler breeders have to be fed restrictively in order to maximize egg production (Decuypere et al., 2010). The effect of body weight on reproductive traits has mainly been studied by comparing ab libitum and restrictively fed broiler breeder hens. Ad libitum fed hens have a lower egg production percentage than restrictively fed hens, mainly due to an increased number of defective eggs (for a review see Robinson et al., 1993). Furthermore, ad libitum fed hens were found to have a shorter laying sequence length than restrictively fed hens, which is indicative for erratic oviposition (Renema and Robinson, 2005). The relation between body weight and egg production could also be explained in the opposite direction, as a lower energy expenditure in egg production leaves more energy allocation for growth (Robinson et al., 1993).

The percentage of floor eggs did not differ between the genetic lines, which suggests that this is not a behaviour directly affected by genetic selection but rather the outcome of a combination of other factors. Although the severity of foot pad dermatitis and the prevalence of gait problems increased with age, no correlation was found between either contact dermatitis or gait and the percentage of floor eggs. This suggests that decreased mobility is not the only factor involved in floor laying and further research could focus on other possible explanations for floor laying behaviour, such as the influence of social behaviour and general activity levels. These relationships were studied in the same birds and are described in Chapter 4.

In conclusion, this study shows that deteriorated leg health is an issue of similar size within the broiler breeder industry as it is in the broiler industry and should therefore receive more attention. It is confirmed that litter quality is related to the severity of foot pad dermatitis, while body weight only seems to be related to egg production and not to contact dermatitis. The genetic lines differ in some of the parameters measured. The percentage of floor eggs could not be attributed to genetic line, foot pad dermatitis, hock burn or gait, which means that also other factors are involved in the development of the undesirable floor laying behaviour.

Acknowledgements

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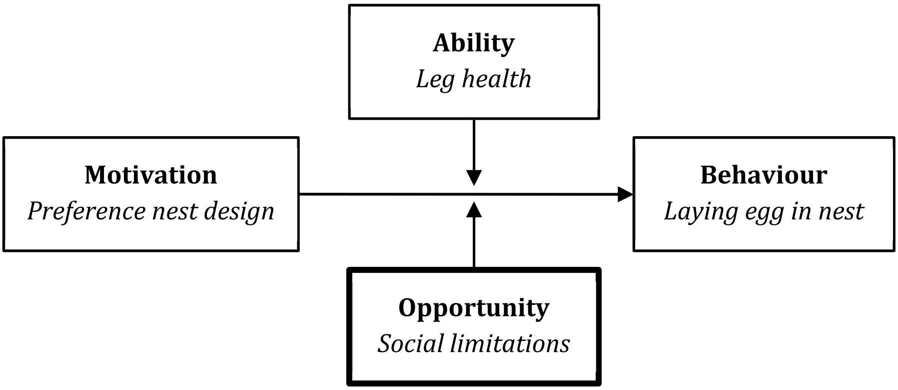
Chapter 4

Gregarious nesting in relation to floor eggs in broiler breeders



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Abstract

Gregarious nesting has often been observed in laying hens, where hens prefer to visit a nest already occupied by other hens over empty nests. This may result in overcrowding of the nests which is considered a welfare issue, and, moreover, can increase the economic issue of floor eggs. This study aimed to describe gregarious nesting and spatial behaviour in broiler breeders and how this relates to genetic background, fearfulness and mating behaviour. Five commercially available genetic lines of broiler breeders were housed in 21 pens of 550 females and 50 males (six pens for lines 1 and 2, five pens for line 3 and two pens for lines 4 and 5) during the ages 20-60 weeks. Every 10 weeks the plumage condition and wounds were assessed of 50 random hens per pen. Avoidance distance and novel object tests were performed to assess fearfulness at four time points. Distribution of eggs over nests was observed for 6 weeks at the onset of egg production at 26 weeks of age and use of space was recorded at four time points, while (floor) egg production was noted daily per pen. We found differences between genetic lines over time in plumage condition and prevalence of wounds. Fear of humans was highest at the earliest age tested and did not correlate with general fearfulness as assessed by the novel object test. The distribution of eggs over nests was related to genetic background, was more uneven at the earliest age compared to later ages and a more uneven distribution was correlated to an increased percentage of floor eggs. Distribution of birds over the litter area differed between the genetic lines and less use of the litter area was correlated to an increased fear of humans and presence of wounds, suggesting an association with aggressive mating behaviour. This difference in distribution of the birds could also explain the correlation between increased presence of wounds and decreased percentage of floor eggs. It is concluded that broiler breeders do show gregarious nesting, which is affected by genetic background. Both increased gregarious nesting and wounds are related to increased floor egg percentage, which should be studied further in broiler breeder research. Genetic selection for even use of the available nests and of the litter and slatted area would therefore support both broiler breeder welfare and performance.

1 Introduction

Choosing to enter an occupied nest over an unoccupied nest is called gregarious nesting, which has often been observed in laying hens and can result in welfare and production problems (Appleby and McRae, 1986; Riber, 2010; Tahamtani et al., 2018). When many hens in a flock exhibit gregarious nesting, other hens can have an excessive energy expenditure when repeatedly trying to enter a nest which is overcrowded (Kite et al., 1980 as cited by Riber, 2010). Increased aggression has been observed in front of nests when multiple hens wanted to enter, as well as inside occupied nests after entering (Meijsser and Hughes, 1989; Appleby and Hughes, 1991). Gregarious nesting also has economic consequences as eggs might break if the number of eggs exceeds the egg belt capacity. Furthermore, when the nests are too full to enter, hens might lay their eggs outside the nests (also known as floor eggs). Floor eggs require manual collection and have a lower hatchability and saleability due to the fact that they are often dirty or broken (van den Brand et al., 2016).

Several possible causes for gregarious nesting have been suggested. It could be that many hens share their preference for nests in certain locations, mostly nests at the end of the row or in a corner (Riber, 2010; Ringgenberg et al., 2015b). Corners might be attractive due to a difference in microclimate or a lower light intensity, but they are also more easily recognized (Appleby and McRae, 1986; Riber, 2010). Nests are often presented in long rows at commercial farms, so the nests at the end of the row are more easily found again than a nest in the middle of the row. However, offering heterogenous nests, which should be easier to recognize, did not decrease the occurrence of gregarious nesting (Clausen and Riber, 2012). Gregarious nesting has also been suggested to be an anti-predator strategy (Riber, 2012) or the result of a lack of nesting experience in younger hens (Riber, 2010), but seems to be unrelated to dominance status (Tahamtani et al., 2018).

Gregarious nesting has only been described in laying hens and it is unknown whether broiler breeders also exhibit this behavior, while studying this behavior in broiler breeders is interesting for several reasons. First, different genetic lines of broiler breeders have been selected for different combinations of goals, which might also affect unselected characteristics such as gregarious behavior (Dawkins and Layton, 2012). Furthermore, it has been shown that the mere presence of males reduced floor eggs in a small experimental study, although the exact reasons remain unknown (Rietveld-Piepers et al., 1985). It is suggested that broiler breeder males might influence spatial distribution of the females and that this in turn affects floor laying behavior. Broiler breeder males use the slatted areas less than the litter area and are known to be aggressive in their mating behavior, causing feather loss and wounds in females (de Jong and Guémené, 2011). Females have been observed spending more time on slatted areas to avoid aggressive males and as the nests are accessed from this slatted area, this could affect nesting and floor laying behavior. Finally, fearfulness could also affect the use of raised areas and thereby nesting behavior. Less fearful laying hens have been found to make more use of raised areas and perches (Brantsæter et al., 2016), although it is unknown whether this is also the case for broiler breeders.

In this study we aim to investigate how much gregarious nesting behavior is performed by broiler breeders and to understand the background of this behavior. This was part of a larger study with the same animals and the results of the other part, which focused on leg health, are reported in Chapter 3. Therefore, we investigated possible relationships between gregarious nesting and use of space with plumage condition, presence of wounds, fearfulness and genetic background. Further, we studied whether these factors are correlated with egg production and floor egg percentage. The magnitude of gregarious nesting, presence of wounds and fearfulness were expected to differ between the different commercially available genetic lines. A more uneven distribution over the nests was expected to be related to a more uneven use of space, while wounds and fearfulness were thought to alter the relative use of slatted and litter area. Floor eggs percentage was hypothesized to increase with a more uneven use of nests, more wounds and lower fearfulness.

2 Materials and Methods

Animals and housing

The experiment took place from June 2018 to March 2019 at a breeding station, where both gregarious nesting (this chapter) and leg health (Chapter 3) were investigated. A total of 11,550 females were reared in 3 groups of mixed genetic lines with raised platforms, while 1050 males (despurred and toe-clipped) were reared in a separate group with raised platforms. All birds were non-beak trimmed and moved from their rearing facilities into the production house located at the same farm at the age of 20 weeks. Five commercially available genetic lines, all fast-growing, were represented in different numbers. The chickens were assigned to 21 pens of 550 females and 50 males of the same genetic line, resulting in six pens for lines 1 and 2 (3300 females and 300 males per line), five pens for line 3 (2750 females and 250 males) and two pens for lines 4 and 5 (1100 females and 100 males per line). The position of the genetic lines in the house was randomized using a block design with 6 blocks, each line was present maximum once per block. The pens were identical in size (12 x 6.5 x 2.0 m, length x width x height) and lay-out, and were placed in four rows (see Figure 1). The animal density was 7.7 birds/m^2, which is comparable to commercial practice. The pens had wire mesh walls, which allowed the animals from different pens to see each other. The litter area (12 x 3.7 m) was covered with wood shavings and the slatted area (12 x 2.3 m) was raised by 0.5 m and gave access to 9 bell drinkers and 10 nests. The group-nests were of a rollaway type (Vencomatic©), measuring 1.15 x 0.52 x 0.53 m. All nests had a green rubber nest floor slanting towards the back and red nest curtains with an entry point in the middle. The feeding line for the females was placed partially on the slats and partially in the litter area, while the male feeding line was positioned in the litter area.

The management of the birds was the same for the current study as well as the study focusing on leg health (Chapter 3). The house was lit with artificial LED-lighting. At 20 weeks of age, the animals had 8 h of light (7:00 to 15:00 h) at 10 lx measured at bird height. This was gradually increased to 14 h of light (2:00 to 16:00 h) at 60 lx at bird height at 27 weeks of age. The temperature was maintained at 21 ± 1 °C. Food was provided at 8:30 h, giving a restricted amount

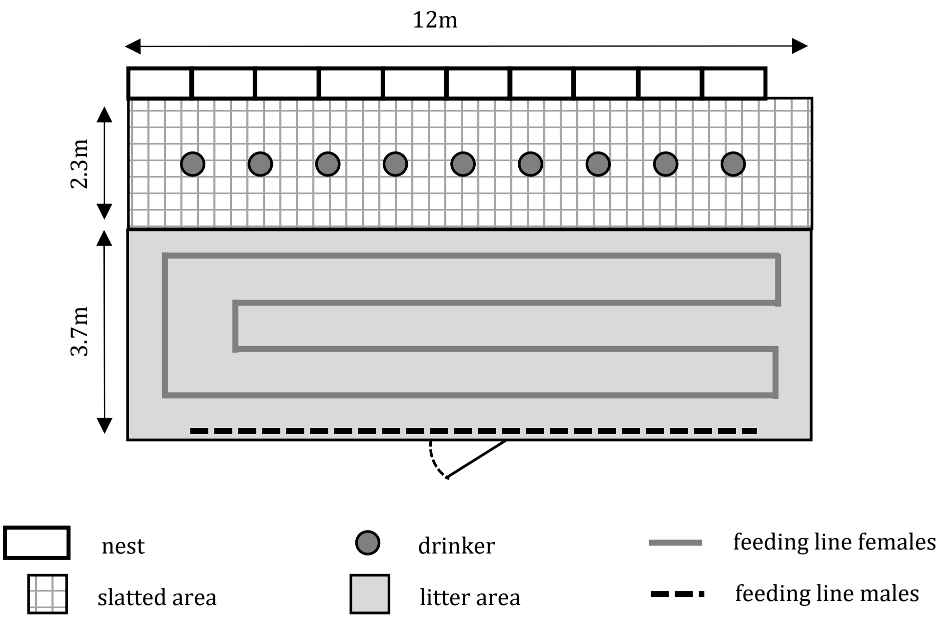

Figure 1. Top view of a pen used for housing the broiler breeders.

according to commercial practice schedule and ranging from 100 to 165 g per female and from 100 to 130 g per male. Bird weight was continuously monitored with hanging poultry scales. Water was provided from 8:30 to 12:30 h and from 15:30 to 16:00 h. The nests were available to the hens from 1 h before lights-on until 30 min before lights-off, from the day after the first egg was found (23 weeks of age). The birds were kept until the age of 60 weeks and then slaughtered for human consumption.

Data collection

Gregarious nesting behaviour and use of space

In order to assess the distribution of hens over the available nests, the number of eggs per nest was counted one day per week during the ages of 26-31 weeks. At 29, 38, 47 and 56 weeks of age the spatial distribution of the birds over the pen was assessed by live observations from the passage between the rows of pens. This was done by counting the number of empty slatted areas sized 1.15 x 1.15 m (total 20 areas) and litter areas sized 1.15 x 1.30 m (total 20 areas). The width of the areas was chosen according to the width of the nests (1.15 m), which could be easily distinguished from a distance. The length of the areas was chosen as half of the total litter or slat length. Observations were done within a few seconds by one observer and the birds hardly moved within this time, especially when they noticed they were being observed. Assuming that an average breeder hen measures 30 x 15 cm, this means that 29 birds fit into a slatted area and 33 birds into a litter area. The number of areas containing fewer than 7 birds in the slatted area or 8 birds in the litter area was noted as well, which meant that 75% of the area was empty. These measurements were repeated five times per day, starting at 3:00 h with an interval of 2 h until 13:00 h. The measurements of 9:00 h were discarded as the birds were eating and therefore all equally distributed along the feeding line.

Plumage condition and wounds

Approximately every 10 weeks (21, 32, 40, 50 and 59 weeks of age) a random selection of 50 hens per pen was scored for feather damage and wounds on the back and rump (adapted from the laying hen protocol of Welfare Quality®, 2009). The presence of wounds was scored on a 3-point scale: 0 for no wounds, 1 for at least one wound smaller than 1 cm and 2 for at least one wound larger than 1 cm. Feather damage was also scored on a 3-point scale: 0 for no feather damage, 1 for areas of ruffled feathers or without feathers smaller than 5 cm in diameter and 2 for areas of ruffled feathers or without feathers larger than 5 cm in diameter.

Fearfulness

To investigate the relationship between fear of humans, gregarious nesting and use of space, a random selection of 20 hens per pen were subjected to an avoidance distance test at 22, 31, 41 and 52 weeks of age (Welfare Quality®, 2009). The observer walked parallel to the slatted area at a distance of 1.5 m and turned to approach the hens sitting on the edge of the slatted area with the hand in front of the body. The distance between the hand of the observer and the hen was noted (rounded to the nearest 10 cm), when the hen retreated. At 22 weeks of age a novel object test was performed to measure general fearfulness. After placing the novel object (a coloured rod) in the centre of the home pen, the observer withdrew for 1.5m and started recording. The number of hens approaching the novel object within bird's distance was noted every 10 s for 2 min in total, in order to determine the latency of 7 hens to approach the novel object and the maximum number of hens that approached the novel object. The benchmark of 7 hens was chosen after performing the test, this was the overall average number of hens approaching the novel object test.

Production

Production data were collected for the purpose of this study as well as the study focusing on leg health (Chapter 3). Starting at 24 weeks of age until the end of the trial, the number of floor and nest eggs was recorded daily per pen. The number of broken nest eggs was counted as well. Floor eggs were collected three times per day and nest eggs were collected once a day. Eggs laid on the slatted area were prevented from rolling into the litter with a 18mm plastic tube, which allowed for separate recording of litter eggs and eggs laid on the slats. This plastic tube had to be removed at 45 weeks of age for manure management, after which no distinction could be made between floor eggs laid on the slats or in the litter.

Statistical analysis

Egg production percentage was calculated by dividing the total number of eggs by the number of present hens. Floor egg and broken egg percentages were calculated by dividing the number of floor or broken eggs over the total number of eggs laid per pen per week, whereas litter egg percentage was calculated by dividing the number of eggs laid in the litter over the total number of floor eggs.

Number of eggs per nest was used to calculate a distribution index using the following formula:

$$SPI = \frac{\sum_{i=1}^{n} |N_i - \frac{T}{n}|}{2 * (T - \frac{T}{n})} = \frac{\sum_{i=1}^{10} |N_i - \frac{T}{10}|}{2 * T * 0.9}$$

where N_i was the number of eggs laid in each nest, T the total number of eggs in the pen and n the number of nests (which is 10) (adapted from Dickens, 1955). A distribution index of 0 indicates that the eggs are spread equally over all nests available and 1 indicates that all eggs are laid in one nest. The number of 75% and 100% empty slatted and litter areas was calculated into total percentage of empty slatted and litter surfaces per pen. These measurements were averaged before and after feeding and analysed separately, to investigate differences between main laying time and the rest of the day. Percentage of empty slatted, litter and total surface were analysed separately. The measurements of the avoidance distance test at 31 weeks of age were discarded as the majority of observations were disturbed by aggressive males. The novel object latency times and avoidance distance were analysed as mean per pen per observation week, whereas the wound score was analysed as the percentage of birds with wounds.

All statistical analyses were performed with SAS (version 9.4). The MIXED procedure was used to perform general linear mixed models in order to investigate differences between lines and ages. Fixed effects included line and age and their interaction, pen within line was included as a random effect. The assumptions of homogeneity of variance and normally distributed residuals were examined visually using the conditional studentized residuals plots. In order to satisfy these assumptions, the percentage of broken eggs was log transformed. Pearson correlations were calculated between traits using the CORR procedure, except for correlations with percentage of broken eggs for which the Spearman's rank order correlations were calculated. Results are shown as non-transformed means with corresponding standard errors and p-values below 0.05 were considered significant. Tukey's post hoc test was performed to investigate significant pairwise differences between test groups, which are reported in the results section if $P<0.05$. The results of pair-wise comparisons between the lines can be found in the Supplementary Tables at the end of this chapter.

3 Results

Gregarious nesting behaviour and use of space

The distribution index of eggs over the nests provided was affected by line ($F_{4,16}$ = 42.15, P<0.0001) and by age ($F_{5,80}$=9.6, P<0.0001), but not by their interaction. Line 4 had the most uneven distribution (0.45 ± 0.01), line 5 had an intermediate level of distribution (0.29 ± 0.02) and lines 1, 2, and 3 the most even distribution (0.11 ± 0.01). Figure 2 illustrates these indices by showing the percentage of eggs laid in each nest per genetic line. The distribution was more uneven at the age of 26 weeks in comparison to the following weeks.

The use of space differed between the lines, see Figure 3. Before feeding the total percentage of empty space was equal for all lines, but lines 1, 2 and 3 left less slatted area empty ($F_{4,16}$ = 18.1, P<0.0001) and more litter area empty ($F_{4,16}$ = 5.1, P=0.008) compared to lines 4 and 5. The total percentage of empty space was lower after feeding, which was caused by a lower percentage of empty slatted areas ($F_{4,16}$ = 7.7, P=0.001). Also after feeding lines 1, 2 and 3 left less slatted area and more litter area empty compared to lines 4 and 5. There was a tendency for more empty litter area with a higher percentage of wounded hens per pen at the ages of 32 and 40 weeks (r=0.38, P=0.09 and r=0.38, P=0.09 respectively). Average percentage of empty slatted area per pen was negatively correlated to average avoidance distance (r= -0.62, P=0.003), while average percentage of empty litter area was positively correlated to average avoidance

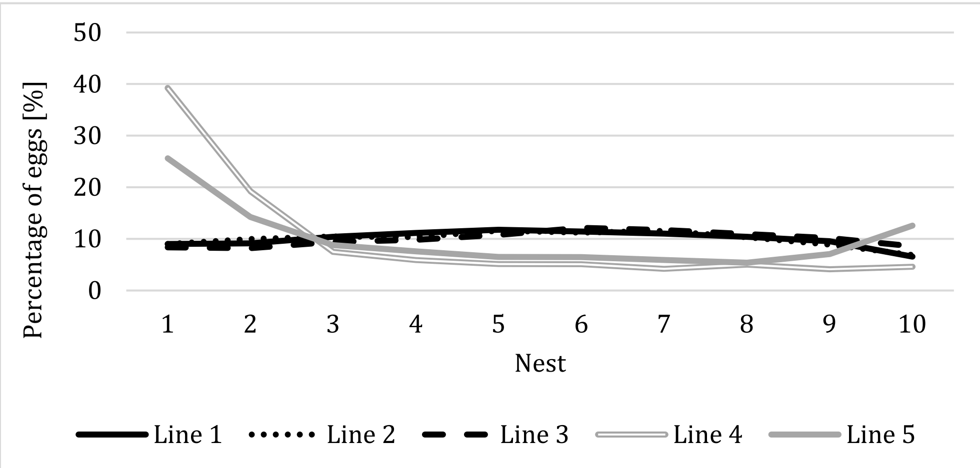

Figure 2. The distribution of eggs (%) over the 10 nests provided per pen, specified for each genetic line of broiler breeders.

distance (r= 0.49, P=0.026). No correlation between nest distribution and use of space was found.

Plumage condition and wounds

The mean plumage score was affected by the interaction between line and age ($F_{16,64}$ = 6.34, P<0.0001; see Figure 4A). At the age of 40 weeks lines 1 and 2 had more severe feather damage than line 4, while at the age of 60 weeks lines 4 and 5 had more severe feather damage than lines 1, 2 and 3. The prevalence of wounds was also affected by the interaction between line and age ($F_{16,64}$ = 2.49, P=0.0051; see Figure 4B). Lines 1, 2 and 3 had the highest prevalence of wounds at 32 weeks of age after which the prevalence decreased again, while for lines 4

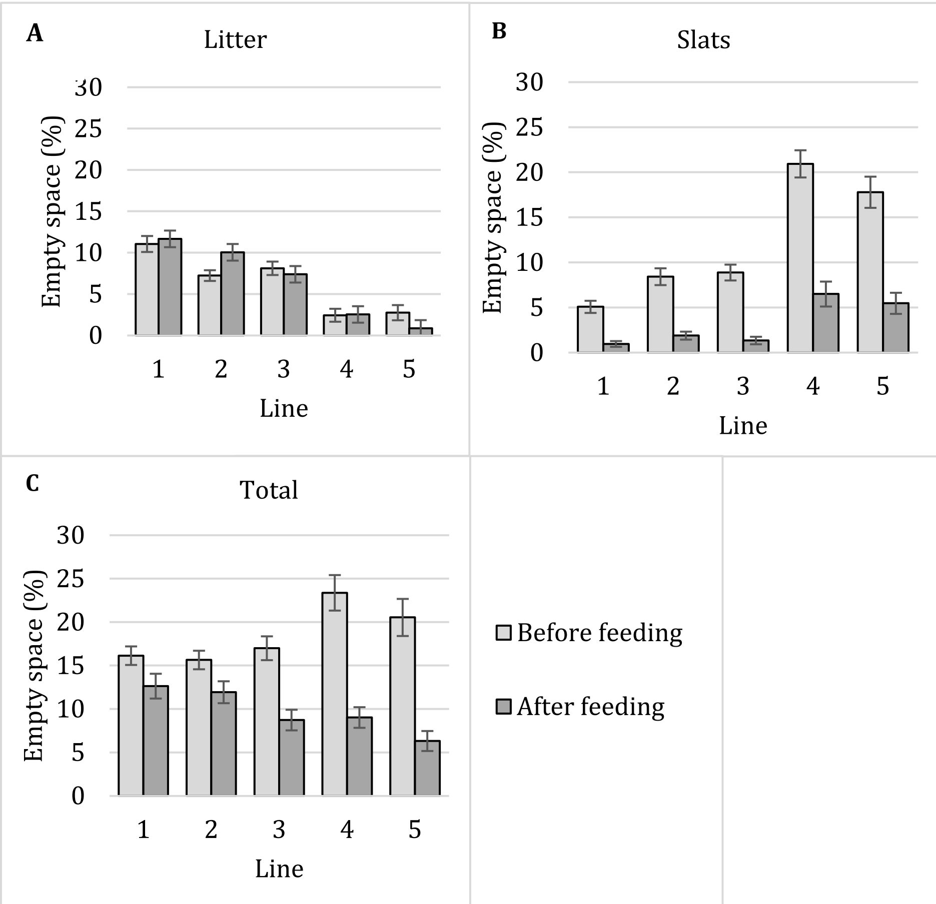

Figure 3. The percentage of empty space in the litter (A), on the slats (B) and for the total (C) area specified per broiler breeder line, comparing before and after feeding.

and 5 the prevalence increased from the age of 40 weeks onwards. Within individuals (across lines), the prevalence of wounds was positively correlated with severity of feather damage (r=0.31, P<0.0001). No correlation between distribution of eggs over nests and plumage condition or prevalence of wounds was found.

Fearfulness

The mean human avoidance distance differed per age ($F_{2,30}$ = 7.99, P=0.0017) and per line ($F_{4,16}$ = 4.83, P=0.0096). The mean avoidance distance was higher at the age of 22 weeks with 85.6 ± 1.8 cm than at ages 40 and 52 weeks with 61.8 ± 1.3 cm and 65.0 ± 1.2 cm respectively. Line 2 had a higher avoidance distance (81.6 ± 2.0 cm) than line 4 (50.8 ± 2.0 cm). Lines 1, 3 and 5 had intermediate avoidance distances (72.6 ± 1.5 cm, 70.1 ± 1.9 cm and 59.4 ± 1.9 cm respectively).

The responses to the novel object at 22 weeks of age did not differ between the lines, for both the latency of 7 hens to approach (overall average 43.4 ± 11.4 s) and the maximum number of hens approaching the object (overall average 12.3 ± 1.7). The latency of 7 hens to approach the novel object was negatively correlated to the maximum number of hens to approach per pen (r= -0.75,

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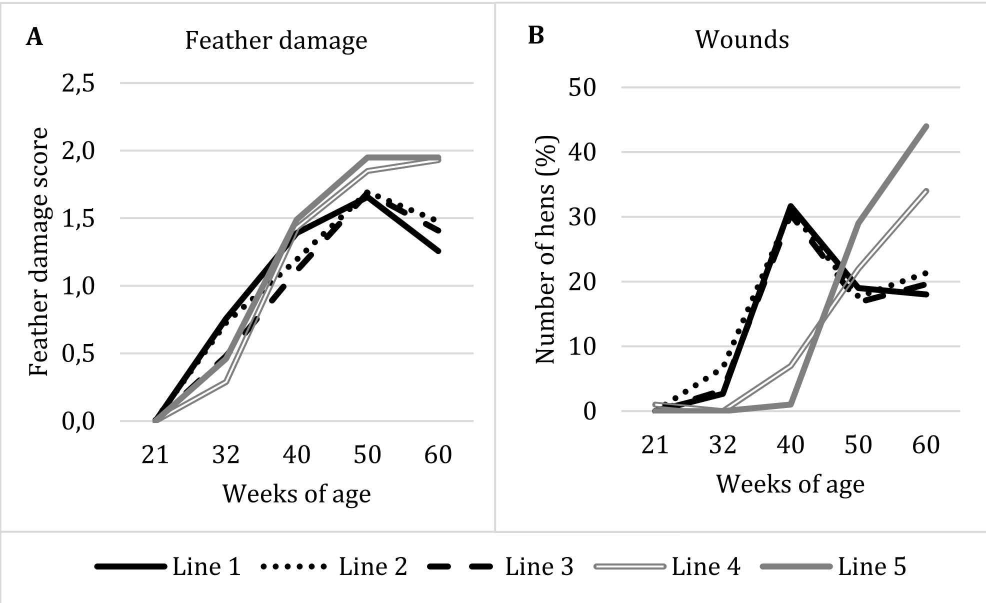

Figure 4. The development of feather damage score (A) and wound prevalence (B) over time per broiler breeder line.

P<0.0001). Responses to the novel object test were not correlated to avoidance distance of humans. No correlation between nest distribution and any of the fear test responses was found.

Production

Egg production percentage increased rapidly after the onset of lay and then declined again after the age of 30 weeks, while no clear pattern could be distinguished in the development of floor eggs. For specific results, see Chapter 3. Most of the floor eggs were found in the litter area. The percentage of litter eggs (expressed as a percentage of floor eggs) increased significantly from 78.3 ± 2.0% of the total number of floor eggs at 26 weeks of age to 88.9 ± 1.1% at 30 weeks of age ($F_{20,2840}$ = 15.0, P<0.0001) after which it did not increase anymore. The lines did not significantly differ in percentage of litter eggs. Average floor egg percentage was positively correlated to uneven nest distribution during the period when nest distribution was measured, e.g. 26-31 weeks of age (r_s=0.28, P=0.002). Floor egg percentage had a tendency for negative correlation to average prevalence of wounds per pen (r= -0.40, P=0.07). No correlation between distribution of eggs over nests and percentage of broken eggs was found.

4 Discussion

Gregarious nesting behaviour and use of space

The genetic lines of broiler breeders in this study differed in the distribution of eggs over the nests, which is most likely reflecting how much gregarious nesting behaviour was shown. However, it is possible that the moment of laying could have been spread over time and thereby reducing the relationship between the number of eggs and gregarious nesting behaviour. While line 4 had a very uneven distribution and line 5 also had an uneven distribution, the other lines had a very even distribution. The uneven distribution for lines 4 and 5 was caused by a higher use of the nests in the corner of the pen over the nests in the middle. This preference for nests in the corner or at the end of a row has been described previously for laying hens (Riber, 2010; Clausen and Riber, 2012; Ringgenberg et al., 2015b). Since the hens is our study were all kept in the same housing and management conditions, the difference is nest distribution can probably be attributed to genetic predisposition for a trait underlying this behaviour. Genetic selection against this behaviour should be possible and increasing the evenness of bird distribution over nests by genetic selection is expected to improve both broiler breeder welfare and performance. It should however be noted that possible negative genetic correlations of this selection are unknown and should be investigated in selection experiments.

Our study found a more uneven distribution over the nests at the age of 26 weeks compared to later weeks, independent of genetic line. Riber (2010) also found more gregarious nesting at the start of lay compared to 6 weeks later in laying hens. She suggested that with time hens will choose their own preferred nest rather than following the more experienced hens, although this theory has yet to be confirmed. In order to investigate whether lines 4 and 5 were generally more gregarious in their behaviour, we studied the use of space in the rest of the pen during the day. During the morning, when egg laying takes place, we did not find differences between the lines in terms of spatial clustering. The gregarious nesting behaviour was also not correlated to general spatial clustering, so this may be motivated by something else than preferring to be close to pen mates.

There was however a clear difference in proportional occupation of the litter and slatted area between the lines. Based on the percentage of areas left empty, lines 4 and 5 used the slatted area less and the litter area more than lines 1, 2 and 3. In the afternoon the slatted area was used more by all lines, possibly caused by fewer birds in the nest, although there was still a difference between the lines. A possible explanation for this difference in use of space can be found in the correlation between incidence of wounds and a reduction in use of the litter area at ages of 32 and 40 weeks. Male broiler breeders are known to be more aggressive in their mating behaviour compared to layer breeders, which can result in severe wounds on the back and flanks of the female birds (Millman et al., 2000; de Jong and Guémené, 2011). Male broiler breeders tend to spend most of their time in the litter area, only going to the slatted area to drink. It seems likely that the hens would avoid spending time in the litter area where the males are, since this increases the chance of aggressive mating (de Jong and Guémené, 2011). Genetic strains of broiler breeders are known to differ in their mating behaviour (McGary et al., 2003), which may have caused the different distributions of the genetic lines in our study. Future research should try to confirm this proposed relation between mating behaviour and spatial distribution, so it can be decided whether genetic selection could help improve optimal use of space in broiler breeders.

The results from the avoidance distance tests provide another explanation for a different use of space between lines. Pens, independent of genetic line, with a larger avoidance distance used the litter area less and the slatted area more. The house was set up in a way that the passages used by caretakers or researchers were between the litter areas of pens and the caretakers would enter the pens in the litter area as well. A larger avoidance distance is a sign of more fear of humans, which would explain the avoidance of the litter area where humans pass nearby and enter the pens. However, it could also be the case that an approaching human may elicit a similar response as an approaching male broiler breeder. The fear measured with this test could therefore reflect the fear of aggressive males rather than fear of humans.

Plumage condition and wounds

Feather damage increased with age, but with differences between the lines. Lines 1, 2 and 3 had more severe feather damage at the early age of 40 weeks, while lines 4 and 5 had more severe feather damage at 60 weeks of age. In chickens feather coverage is known to be influenced by genetics, feather pecking behaviour, feed and metabolism (Leeson and Walsh, 2010; Moyle et al., 2010). However, for broiler breeders the mating activity also strongly influences the plumage condition. In our study feather ruffling and loss were combined within one score, which does not allow us to differentiate between the genetic predisposition for feather loss and the ruffling or loss of feathers caused by the mounting of males. Interestingly lines 1, 2 and 3 improved their plumage condition from 50 to 60 weeks of age, while lines 4 and 5 did not. This could be a sign of genetic differences in capacity of feather regeneration or sexual activity (McGary et al., 2003; Lin et al., 2013). Genetic strains differ in their mating behaviour, where some strains stay continuously active in their sexual behaviour while other strains decrease sexual activity from 40 weeks of age onwards (McGary et al., 2003). It is unknown whether this was also the case in our study or that the difference in wound incidence at later ages was caused by a difference in feather coverage. When feather coverage declines, the skin of the females will be wounded more easily. This is reflected in the correlation between feather damage and the incidence of wounds at the age of 60 weeks.

Fearfulness

The avoidance distance was affected by both age and genetic line. This test is used to measure fear of humans, so it is expected that the distance will decrease with age as the birds get used to presence of humans. Line 2 was most fearful and line 4 least fearful, while the other lines had intermediate levels of fearfulness. It is known that fear of humans is a heritable trait in chickens, which could be the reason behind the differences between the genetic lines at the start of the experiment (Agnvall et al., 2014). As discussed before, fear of humans was related to decreased use of the litter area, but it does not seem to affect the distribution of hens over nests.

The genetic lines did not show any differences in response to the novel object test in terms of latency to approach the object or the maximum number of hens approaching the object. These two read out variables were positively correlated to each other, meaning that pens which would approach the object sooner would also approach with more individuals. Both of these variables are signs of reduced general fearfulness, which was not related to fear of humans in our study. Another study on different lines of laying hens found that fear of humans loaded on a different factor in the principal component analysis than the novel object test results (de Haas et al., 2014). This suggests that fear of novelty has a different origin than fear of humans, although more research is needed on this subject to gain a better understanding. Another explanation could be that a novel object test is not suitable to measure general fearfulness in broiler breeders, as they seemed to show very little interest. Earlier research has shown that broilers have less marked responses compared to laying hens (Keer-Keer et al., 1996), which might make it difficult to interpret their behaviour.

Production

The percentage of floor eggs was correlated to two of the studied parameters and not dependent on genetic line. A more uneven nest distribution was positively correlated to a higher percentage of floor eggs. This relation between gregariousness and floor eggs has previously been found in a study on broiler breeders (Perry et al., 1971 as cited by Riber, 2010). This is most likely due to overcrowding of the corner nests, causing hens to lay their eggs on the floor. An increased incidence of wounds was found to be correlated to a decreased percentage of floor eggs. The previously described relation between the incidence of wounds and avoidance of the litter area due to aggressive mating behaviour of the males seems to be involved in decreasing the number of floor eggs. A previous study with laying hens also concluded that the presence of roosters decreased the percentage of floor eggs (Rietveld-Piepers et al., 1985). Most floor eggs were laid in the litter area and not in the slatted area, so once the hens are on the slats, the likelihood of laying an egg outside the nest decreases.

No correlation was found between the percentage of broken eggs and gregariousness of nesting behaviour, which is contrary to our expectations. When the number of eggs exceeds the capacity of the egg belt, the eggs tend to pile on top of each other and this causes the eggs to break. This has also been reported in a previous study on gregarious nesting behaviour of laying hens (Appleby and McRae, 1986). The unevenness of egg distribution in our study was apparently not severe enough to affect egg quality.

Conclusion

The genetic lines of broiler breeders used in this study differed in the occurrence of gregarious nesting behaviour, which correlated to percentage of floor eggs. Genetic selection against gregarious nesting behaviour could therefore improve bird welfare and performance. The genetic lines also differed in use of space, although this was not related to gregarious nesting or floor laying behaviour, but was perhaps caused by differences in mating behaviour. Fear of humans at an early age was related to a decreased use of litter space, although fearfulness was not related to the distribution over nests or floor egg percentage. Percentage of wounded hens, possibly due to aggressive mating behaviour, was related to a decreased use of litter space and a decreased percentage of floor eggs. Most studies looking into floor eggs in broiler breeders focus on housing and management. These findings suggest that future research should focus on the effect of males on nesting behaviour and methods that help to reduce gregarious nesting behaviour.

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Supplementary Tables

P-values of pair-wise comparisons between lines for all measurements (with Tukey corrections).

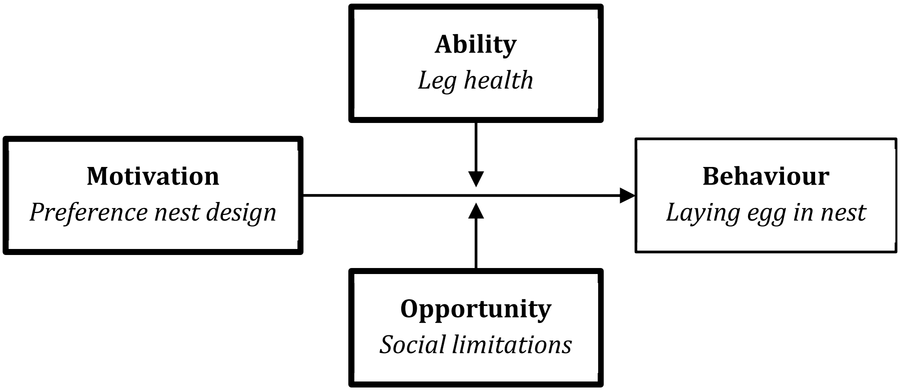
Pair-wise comparison		Distribution index	Empty litter area	Empty slats area	Empty total area	Wounds
Line 1	vs. 2	0.962	0.588	0.305	0.999	0.999
	vs. 3	0.987	0.369	0.366	0.452	0.990
	vs. 4	<.0001	0.025	<.0001	0.574	0.870
	vs. 5	0.0004	0.015	0.0003	0.098	1.000
Line 2	vs. 3	1.000	0.990	1.000	0.363	1.000
	vs. 4	<.0001	0.175	0.0003	0.648	0.781
	vs. 5	0.0001	0.111	0.004	0.122	0.999
Line 3	vs. 4	<.0001	0.320	0.0003	0.096	0.712
	vs. 5	0.0002	0.217	0.005	0.010	0.995
Line 4	vs. 5	0.006	1.000	0.784	0.861	0.941

4

Pair-wise comparison		Plumage condition	Avoidance distance	Latency NO	Maximum NO	Floor eggs
Line 1	vs. 2	0.998	0.999	0.572	0.978	0.506
	vs. 3	1.000	1.000	1.000	0.849	0.474
	vs. 4	0.998	1.000	0.874	0.977	0.958
	vs. 5	1.000	1.000	0.989	0.926	0.734
Line 2	vs. 3	0.996	0.998	0.547	0.548	1.000
	vs. 4	0.985	0.998	1.000	1.000	0.989
	vs. 5	1.000	1.000	0.553	0.994	1.000
Line 3	vs. 4	0.999	1.000	0.849	0.700	0.980
	vs. 5	1.000	1.000	0.995	0.569	1.000
Line 4	vs. 5	0.997	1.000	0.772	1.000	0.991

Chapter 5

Influence of a raised slatted area in front of the nest on leg health, mating behaviour and floor eggs in broiler breeders



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Abstract

European farms for broiler breeders often have raised slatted areas in front of the nests, but in other regions of the world no raised slatted areas are provided. This study aimed to investigate the effects of a raised slatted area on leg health, mating behaviour and floor laying behaviour. Ten groups of 33 broiler breeder hens and 3 males were housed in two pen types: with or without a raised slatted area in front of the nests. Each pen had one plastic and one wooden nest. Between 25-31 weeks of age, ten marked hens per pen were weighed and assessed weekly on foot pad dermatitis, hock burn and wounds. At the end of week 31 animals were euthanized and bone strength of the tibia and humerus of these individuals was assessed. At 24, 27 and 30 weeks of age, mating behaviour was observed for an hour per pen, noting both numbers of successful and unsuccessful copulations. The number of eggs laid in the nests and on the floor was recorded daily between 20-31 weeks of age. Foot pad dermatitis scores were affected by age, but not by pen type. Generally, there were only minor issues with foot pad dermatitis (scores <11 on a 0-100 scale), probably due to the young age of the hens. Body weight was not affected by pen type, while the prevalence of hock burns was too low to analyse and no difference in bone strength was found for the tibia and the humerus. Overall, mating behaviour was less frequent in pens with raised slats than in pens without raised slats (29±2 vs. 35±3 times/hour) and more frequent at 27 weeks of age than at 24 and 30 weeks of age (38±1 vs. 31±4 and 27±2 times/hour). The pens with raised slats had a lower percentage of floor eggs than pens without raised slats (11.2±0.4 vs. 19.3±0.5%). The wooden nest was preferred over the plastic nest as on average 63% of the eggs were laid in the wooden nest. This study shows that providing raised slats decreases mating behaviour and percentage of floor eggs, although its effects on leg health remain inconclusive.

1 Introduction

The importance of housing characteristics on the welfare of broilers and laying hens has received more attention in recent years (Dawkins et al., 2004; Lay et al., 2011), but housing for broiler breeders remains a less studied topic. However, major welfare issues including aggressive mating behaviour and poor foot pad health in the broiler breeder industry have been identified (de Jong and Guémené, 2011; Kaukonen et al., 2016), which could be improved by providing the right housing. Slatted areas that are raised from the litter area and usually cover 30-50% of the house are common within European broiler breeder farms. However, in other regions of the world like the Middle East or North Africa, commercial farms often do not have large slatted areas (F. Leijten, personal communication, 13 February 2019). The presence of a raised slatted area could affect, amongst others, leg health, mating behaviour and floor laying behaviour.

Providing raised slatted areas could be beneficial for leg health by reducing the development of contact dermatitis and by increased bone loading. Foot pad dermatitis affects up to 93% of broiler breeders in a flock at slaughter age of 55-64 weeks (Kaukonen et al., 2016; Chapter 3). Contact dermatitis is mainly caused by contact with (moist) litter, and slatted areas give the birds an opportunity to limit the amount of time that the feet come in contact with the litter. Providing slats has therefore been suggested to benefit foot cleanliness and health (Brake, 1998), although another study found that a larger slatted area was related to poorer foot pad condition (Kaukonen et al., 2016). Both studies only investigated provision of different proportions of slatted areas, and did not include the situation with no slatted area. Besides the effect on contact dermatitis, providing raised slats is also expected to increase bone strength due to loading through jumping on the raised slatted areas from the litter area and vice versa. Physical stimulation and increased load on bones have been found to be beneficial for bone strength in laying hens (Rath et al., 2000), but this relationship has not yet been investigated in broiler breeders.

Besides leg health, mating behaviour is also expected to be affected by the presence of slatted areas. Male broiler breeders show virtually no courtship behaviour and their aggressive mating behaviour frequently leads to feather loss and wounding of the females (Millman et al., 2000; McGary et al., 2003). When raised slatted areas are provided, females often use these areas for resting, as they are rarely used by the males (de Jong and Guémené, 2011). Broiler breeder groups with a larger proportion of wounded females were found to use the slatted areas more (Chapter 4), suggesting that raised slatted

areas might serve as a place for wounded females to avoid aggressive mating. To evaluate whether the absence of slatted areas can be considered a welfare risk, more information is needed on mating activity in housing systems with and without slatted areas.
Although raised slatted areas have the potential of improving health and welfare, it could negatively affect the number of floor eggs. Floor eggs are eggs laid outside the provided nests and are therefore often dirty and broken. This lowers their saleability and hatchability, while also requiring extra labour in the form of manual collection (van den Brand et al., 2016). Furthermore, bacterial contamination of these eggs has been identified as a critical point in salmonella infection of broiler chicks, which negatively affects their health and, moreover, also forms a public health risk (Cox et al., 2000). Hens are motivated to lay their egg in the nest that provides seclusion (Stämpfli et al., 2012) and large numbers of floor eggs could indicate problematic housing and reduced welfare. Floor laying behaviour could be caused by difficulty reaching the nest, which might be the case when the nests are only accessible via the raised slatted areas. Due to the high body weight of the hens, accessing the raised slatted areas might be difficult and thereby cause more floor eggs.

This experiment aimed to study the effects of providing raised slatted areas in front of the nests on leg health, mating behaviour and floor eggs of broiler breeders by comparing two pen types: with or without a raised slatted area. Each pen was fitted with one plastic and one wooden nest to also measure the preference for nest wall material (Chapter 2). The prevalence of contact dermatitis, body weight and bone strength were compared between pen types, as well as the frequency of mating behaviour, wounding of females and the number of floor eggs. Birds in pens with a raised slatted area were hypothesized to have less contact dermatitis, a lower body weight and stronger bones compared to birds in pens without a raised slatted area. Furthermore, mating behaviour was expected to be less frequent in pens with compared to in pens without a raised slatted area resulting in fewer wounded females and we anticipated a higher percentage of floor eggs in pens with compared to pens without a slatted area. Lastly, the birds were expected to have a preference for wooden nests, expressed by a higher proportion of eggs laid in this nest than in the plastic nest.

2 Material and methods

Animals and housing

The study was conducted during the summer of 2019 with Ross 308 broiler breeders and was approved by the Kantonal office of Bern, Switzerland (registration number BE9/19 - 31068). A total of 330 females and 30 males, all non-beak trimmed, were reared with raised platforms and perches from 0 to 20 weeks of age. The birds were then relocated to 10 pens in a different room of the same barn in groups of 33 females and 3 males per pen balanced for body weight. The pens were identical in size (4.3 x 2.3 x 2.0 m, length x width x height) and were placed in two rows. The pens had closed walls preventing visual contact. The litter area was covered with wood shavings and provided access to two feeding lines, which were partially covered with grids to create separate female and male feeding areas (20 cm per bird). The birds were given pre-lay feed (FORS Masteltern Prelay, FORS-Futter, Switzerland) for the first three weeks and lay feed (FORS Masteltern 1. Phase, FORS-Futter, Switzerland) for the remainder of the experiment. In an attempt to decrease the incidence of tail feather pecking, the feed was diluted with wheat flour pellets (Chicken-Bed,

Figure 1. Photos of the pen types for housing the broiler breeders. A) Pen with nests placed on the raised slatted area. B) Pen with nests placed in the litter with a short ramp for nest access.

Gebr. Herzog Hornussen, Switzerland). During the last four weeks of the rearing phase and the first four weeks of the laying phase, 10% wheat flour pellets were added, and thereafter this was 5%. The litter area also provided four perches that were raised 55 and 75 cm above the litter. Half of the pens had a slatted area (1.15 m wide and 0.5 m high) from which access to five drinking nipples and two nests was given (Figure 1A). The other half of the pens had drinkers situated in the litter area and a short ramp (0.4 m wide) that provided access to the two nests placed on the floor (Figure 1B). The two pen types were placed alternatingly in the house to minimise location effects.

The group nests were of a rollaway type, based on commercially available nests (1.15 x 0.50 x 0.50 m, width x depth x height). All nests had a green rubber nest floor slanting towards the back and red nest curtains with an opening of 20 x 23 cm in the middle. Each pen had a plastic and a wooden nest, which were randomised in location across pens. The plastic nest had a dark grey back wall and black plastic side walls, while the wooden nest had a brown hardboard back wall and dark brown epoxy coated birch plywood side walls.

The house was lit with artificial LED-lighting with a photoperiod schedule according to commercial practice. At 20 weeks of age, the animals had 8 h of light (9:00 to 17:00 h) with a light intensity of 10 lx measured at bird height. This gradually increased with increasing age and egg productivity to 14 h of light (3:00 to 17:00 h) with a light intensity of 19 lx at bird height at 24 weeks of age. The temperature was targeted at 19 ± 2 ^{0}C, although the temperature rose to a maximum of 30 ^{0}C on warm days despite the cooling efforts of a mist ventilator. Feed was provided at lights-on and given in a restricted amount according to the guidelines of the breeding company (Aviagen, 2018). At 20 weeks of age the animals received 98 g per individual per day, which was gradually increased to 152 g per individual per day with age and egg productivity. Random samples of five birds per pen were weighed weekly to ensure optimal body condition and flock uniformity. Water was provided *ad libitum*. The nests were available to the hens from 15 min before lights-on until 15 min before lights-off, from the day after the first egg was found (23 weeks of age) until the end of the experiment. The experiment was terminated when the birds were 32 weeks of age after which the birds were re-used in a second, unrelated study.

Data collection

In each pen 10 hens were marked with a backpack for individual recognition. Starting at 25 weeks of age until the end of the experiment, health assessments were performed weekly on all focal birds. The focal birds were weighed and scored for: foot pad dermatitis (left and right leg separately), hock burns (left and right leg separately), and wounds on the back and rump using visual analogue scales ranging from 0 to 100 based on a combination of the Welfare Quality® protocol for poultry (Welfare Quality®, 2009) and the MTool© (Keppler and Knierim, 2017). The visual analogue scales are included as supplementary materials S1-3. Scoring was done by two observers and 20 hens were scored by both observers to assess interobserver reliability. An intraclass correlation coefficient was calculated using a two-way mixed model based on consistency and average measures (Koo and Li, 2016). We found a good agreement for wounds (0.769; 95% CI 0.564-0.878; $F_{39,39}$=4.336, p<0.0001) and the right foot pad dermatitis score (0.748; 95 CI 0.524-0.867; $F_{39,39}$=3.969, p<0.0001), while the agreement was excellent for the left foot pad dermatitis score (0.927; 95% CI 0.862-0.962; $F_{39,39}$=13.744, P<0.0001). Hock burns were not observed in the test hens. The focal birds were euthanized to collect the tibia and humerus at the end of the experiment. The strength of these bones was then measured at 15°C using the three-point bending test as described in the ANSI/ASAE S459 MAR1992 (R2007) standard with some modifications as published by Gebhardt-Henrich et al. (2017a) using a Zwick and Roell universal testing machine with a 2.5 kN load cell.

Live observations on mating behaviour of the males were done at 24/25, 27/28 and 30/31 weeks of age, observing one pen with raised slats and one pen without raised slats per day between 14:00-16:00 h and thus observing all 10 pens in one week. Pen types were observed alternatingly for 2 x 30 min per pen with two observers, each observing 1 or 2 males to record the behaviour of all three males per pen. Frequencies of behaviours as listed in Table 1 were recorded continuously. Interobserver reliability was evaluated by doing two trial sessions of 20 minutes previous to the official observations, which resulted in full agreement on the frequency of the scored behaviours.

Eggs were collected separately from each nest and from other areas of the pen with the latter noted as floor eggs. Eggs were collected three times a day, seven days a week between 7:30 and 16:30 h. Egg collection started with the first egg at the age of 23 weeks and continued until the experiment was terminated at 31 weeks of age.

Table 1. Ethogram of mating behaviours recorded as frequencies during continuous observations of broiler breeder males per 2 x 30 min per pen per age (24/25, 27/28 and 30/31 weeks of age).

Behaviour	Description
Mount attempt	The male approaches a female and places one or both feet on her back. The female avoids the male, and no further elements of the copulatory sequence are observed.
Copulation	The male mounts, grips, and treads a female and appears to achieve cloacal contact. The female ruffles her feathers following the male's dismount.
Chasing	The male runs at a female, with or without wings raised.

Statistical analysis

The maximum foot pad score of either the left or the right leg was used for analysis. Hock burns scores and wounds scores were not analysed, as pen type averages at all ages were lower than 1 (on a 0-100 scale). Mating behaviour observations of 2 x 30 min were summed as frequencies per hour. Chasing behaviour was not analysed due to low incidence. Frequencies of mounting attempts and copulations were summed to calculate total mating activity, while mating success was calculated by dividing the number of copulations by the total mating activity. The percentage of eggs laid in the wooden nest was calculated by dividing the number of eggs laid in the wooden nest by the total number of eggs laid in both nests. The production percentage per pen was calculated by dividing the total number of eggs laid by the number of hens present in the pen. Production percentage was averaged per week for analysis. The floor egg percentage per pen was calculated by dividing the number of floor eggs by the total number of eggs.

All statistical analyses were performed with SAS (version 9.4). P-values below 0.05 were considered significant and the pairwise comparisons following significant results were performed with the Tukey method. To test for a preference for nest design, the proportion of eggs in wooden nests was analysed using the GENMOD procedure to perform a logistic regression model, which included pen as a repeated subject in which the autoregressive covariance structure AR(10) fitted best. To test for an effect of pen type, age and their interaction, the mating behaviour (number of attempts and copulations) was analysed using the GENMOD procedure to perform a negative binomial regression model, including the Wald test for type 3 effects and pen as a repeated subject with an exchangeable correlation structure. The success of mating was analysed with GENMOD with a binary distribution. The percentage of floor eggs (from the total of eggs) was analysed using logistic regression with height, age and their interaction in the model (PROC GENMOD). The egg production, body weight and bone strength were analysed using the MIXED procedure to perform general linear mixed models. As fixed effects pen type, week of age and its interaction were included, while age was included as a repeated effect with pen as subject. Since the relative bone strength was only measured at one time point, age was not included as a fixed or repeated effect. Foot pad dermatitis was analysed using the GLIMMIX procedure to perform a generalized linear mixed model with a multinomial distribution and cumulative logit link function. As fixed effects pen type, week of age and its interaction were included, while pen within pen type was included as a random effect. The assumptions of homogeneity of variance and normally distributed errors were examined visually using the conditional studentized residuals plots. The CORR procedure was used to calculate Pearson's correlations between frequency of mating behaviour and percentage of floor eggs. Results are shown as non-transformed means with the corresponding standard error of means.

3 Results

Foot pad dermatitis scores were low during the experiment, as the highest average score per pen type was less than 11 on a scale from 0 to 100. The foot pad dermatitis score had a tendency to be affected by the interaction between pen type and age ($F_{6,635}$=1.7, p=0.085) and was significantly affected by age ($F_{6,635}$ = 10.5, p<0.0001), see Figure 2. At 27 weeks of age, the hens had a higher foot pad dermatitis score compared to 25 and 29-31 weeks of age, while at the ages of 26 and 28 weeks the hens had an intermediate foot pad dermatitis score. No difference was seen between pen types during all ages. Body weight of the hens steadily increased with age ($F_{6,580}$=71.4, p<0.0001) from 3 245 ± 23 g at 25 weeks of age to 3 659 ± 23 g at 31 weeks of age with no differences between the pen types. Hock burns were barely observed, resulting in average scores per pen type of less than 1 (on a 0-100 scale) at all ages. The bone strength of both the tibia and humerus did not differ between the pen types. Tibia strength was on average 199.1 ± 8.6 N for the pens with raised slats and 195.9 ± 8.4 N for the pens without raised slats at the point of failure. Humerus strength was on average 386.4 ± 18.6 N for the pens with raised slats and 387.8 ± 19.5 N for the pens without raised slats at the point of failure.

The results regarding the mating behaviour are depicted in Figure 3. Total mating activity (copulations + mating attempts) was higher at 27 weeks of age compared to 24 and 30 weeks of age (p<0.0001), while the pens without raised slats had more mating activity than pens with raised slats (p=0.0414). For the number of mating attempts the interaction between age and pen type was significant (p=0.0218). At 24 weeks of age the males in the pens without raised slats had almost twice as many attempts compared to the males in pens with raised slats (20.0 vs. 10.6 attempts per hour). The frequency of mating attempts did not differ between the pen types at the later ages. The number of copulations was affected by age (p<0.0001), but not by pen type. At 27 weeks of age three males copulated on average 23.0 times per hour, compared to 15.5 and 16.4 copulations per hour at 24 and 32 weeks of age. Males had lower mating success at 24 weeks of age with 52.2 ± 2.6% compared to 27 and 30 weeks of age with respectively 61.1 ± 3.3% and 59.8 ± 2.6% (p=0.015) with no differences between the pen types. Hardly any wounds were observed on the hens, resulting in average wound scores of less than 1 (on a 0-100 scale) for each pen type.

During the experiment 10 574 eggs were laid in the nests and 1 893 eggs were laid on the floor (15.2% floor eggs). Egg production increased with age ($F_{9,64}$=1016.4, p<0.0001) without any differences between pen types. Floor egg

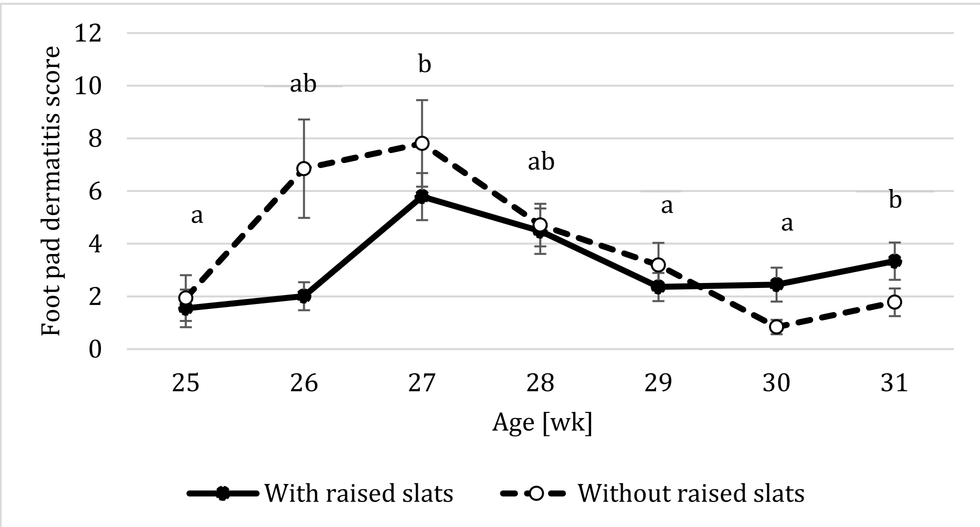

Figure 2. Foot pad dermatitis score per week of age based on 10 broiler breeder hens per pen, specified for pens with (n=5) and without raised slats (n=5). Error bars depict standard error of means, letters indicate significant differences between weeks of age (P<0.05).

percentage was, however, affected by pen type (p<0.0001) with fewer floor eggs in the pens with raised slats (11.2 ± 0.4%) than in pens without raised slats (19.3 ± 0.5%). The percentage of floor eggs was not correlated to the frequency of mating attempts, copulations, total mating activity or mating success.

The percentage of eggs laid in the wooden nest (63.1 ± 0.5%) was higher than that laid in the plastic nests (36.9%). Corrected for the random pen effect, the probability that eggs are laid in a wooden nest is 63.4% (95% CI: 53.0-72.6) and thus significantly higher than 50% expected if the birds would have no preference (p=0.0118). The pens, however, varied highly in the percentage of eggs laid in the wooden nests, with two pens laying fewer eggs in the wooden

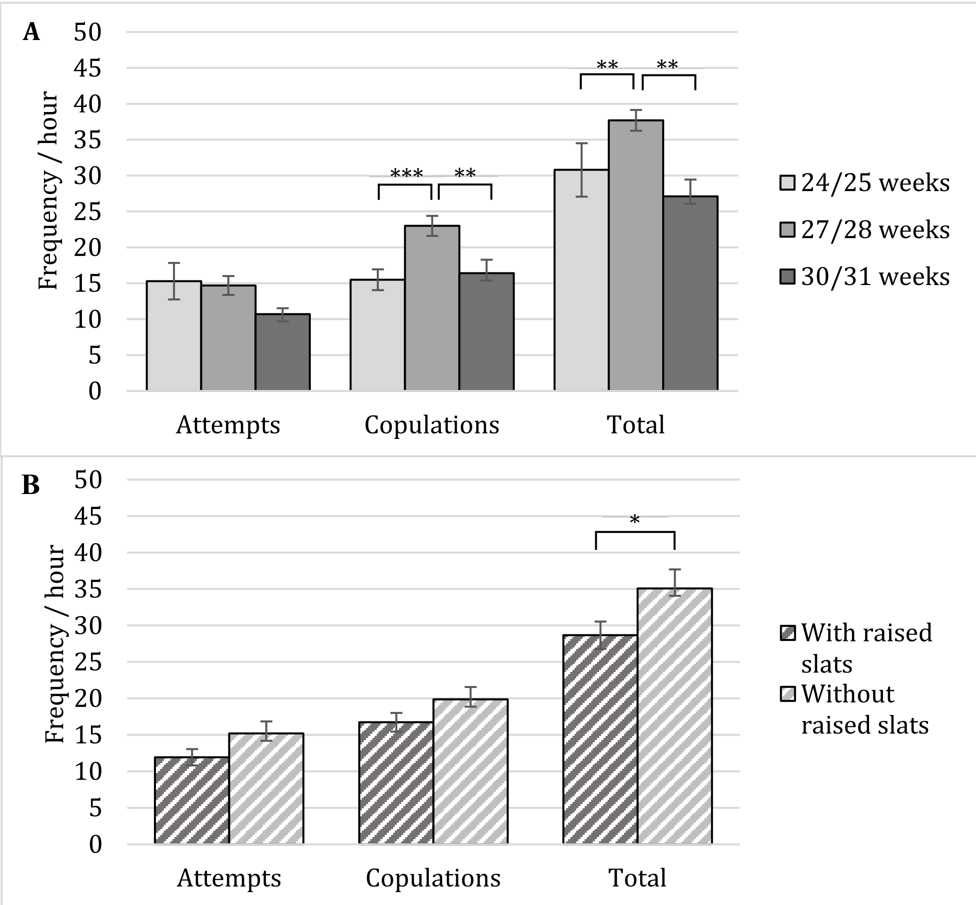

Figure 3. Average frequencies of mating attempts, copulations and total mating activity performed by 3 broiler breeder males per pen specified per age (A) and pen type (B). Effects of age are based on 10 pens and each pen type was replicated 5 times. Asterisks indicate significant differences (=P<0.05; **=P<0.01; ***=P<0.001).*

nest than in the plastic nest, and one pen laying exactly 50% of their eggs in each of the nests (Figure 4). The percentage of eggs laid in the wooden nests was not affected by age or pen type.

4 Discussion

Leg health

Contact dermatitis on both foot pads and hocks was hardly observed and no differences were found between average scores of foot pad dermatitis in hens kept in pens with a slatted area compared to hens kept in pens without slatted areas. The provision of a slatted area was expected to be beneficial for foot pad health, since contact with (moist) litter is the main cause for developing foot pad dermatitis (Martland, 1985) and providing slatted areas gives hens an opportunity to limit their contact with the litter. The effect of the slatted area on contact dermatitis, as well as the later discussed of body weight and bone strength, could have been diminished by the provision of perches in all pens. Although no regular and objective observations were performed, a large proportion of the hens used the perches, which is in line with earlier studies on perch use in broiler breeders (Gebhardt-Henrich et al., 2017). Perches are still uncommon in commercial housing for broiler breeders, although an increasing number of European countries have included perches as a minimum housing requirement for broilers breeders. Another possible reason for not finding

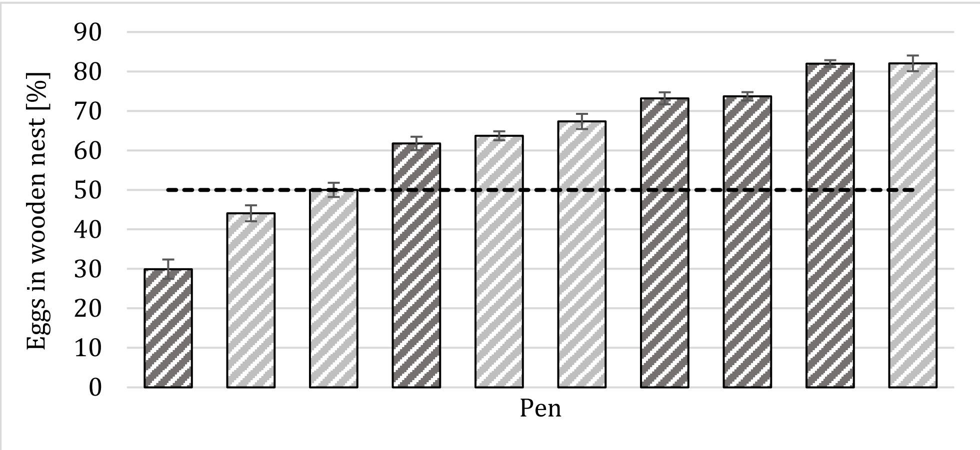

Figure 4. The percentage of eggs laid in the wooden nests by the broiler breeder hens, specified per pen based on daily measurements of number of eggs laid in the wooden and plastic nest provided in each pen (total 59 days). The dark bars depict pens with raised slats (n=5), the light bars depict pens without raised slats (n=5). The dotted line depicts 50% of eggs.

consistent beneficial effects of a raised slatted area on contact dermatitis is that the hens were too young to develop foot pad problems. Foot pad dermatitis generally increases with age, so we might have found more differences in foot pad health if the experiment was terminated at a later age (Chapter 3; Kaukonen et al., 2016). Hock burns were observed with such rarity that they could not be analysed. This low occurrence of hock burns is probably also partially due to the young age of the hens, but hock burns seem to have a low prevalence in broiler breeders at all ages with less than 1% of the hens affected by severe hock burns (Chapter 3; Kaukonen et al., 2016).

Also against expectations, we did not find an effect of providing raised slats on the bone strength of both the tibia and humerus. Our hypothesis was based on previous studies with laying hens, which show that more complex housing systems, including multi-tier aviaries or extra perches, are beneficial for the bone strength at the end of the production period (Fleming et al., 1994; Wilkins et al., 2011). However, in a previous study with broilers, placing barriers between the feeder and drinker did not have an effect on bone strength at the slaughter age of 42 days (Bizeray et al., 2002). The lack of an effect of raised structures on bone strength in the broiler study as well as our current study could be explained by a shorter exposure time. It could also be due to a lower responsiveness of bones to mechanical loading in broiler breeders, as has been established in broilers compared to laying hens (Pitsillides et al., 1999).

The body weight of the hens increased steadily with age, but without any differences between pen types. This is not in line with our expectations, as we hypothesized that the raised slatted areas would result in a lower body weight due to the extra energy expenditure of jumping on and off the raised slatted area, while the hens were not able to increase their feed intake due to feed restriction. The birds housed with raised slatted areas could have compensated for the jumping by having a lower activity level during the rest of the day and therefore not increasing their total energy expenditure.

Mating behaviour

As expected, more mating behaviour was observed in the pens without a slatted area than in the pens with a slatted area. Mating behaviour generally takes place in the litter (de Jong and Guémené, 2011), so a larger litter area allows for more mating behaviour. The mating behaviour was also affected by age, as the males were most active at 27 weeks of age and less active at 24 and 30 weeks of age. At 24 weeks of age the males are still inexperienced and a large number of females are not mature enough yet, explaining the low mating activity and low mating success (= successful copulations divided by total mating activity). Furthermore, it is known that the mating activity of broiler breeder males decreases with age (Duncan et al., 1990; McGary et al., 2003) and an earlier study described the peak of mating behaviour to be at 28 weeks of age in two strains of broiler breeders (Moyle et al., 2010) which is supported by our findings.

Although the mating activity differed between pen types, the frequency of successful copulations was not lower in pens with a raised slatted area compared to pens without this area. This absence of a difference in copulation frequency suggests that the fertilisation of the eggs is probably not influenced by the provision of a raised slatted area. The slatted area can therefore increase hen welfare by lowering the general mating activity and providing an opportunity for hens to avoid mating, likely without compromising the fertility rate that forms the basis of the farmers' income. As a measure of the effect of mating on the hens' welfare, the prevalence of wounds was monitored during the course of the experiment. Wounds were observed so little on the hens that it could not be analysed. A previous study on broiler breeders showed that the majority of wounding in females happens later in the production cycle, which was at least partially due to a poor feather coverage (Chapter 4). So while we did find a difference in mating frequency between the pen types, it remains unsure whether this affects the prevalence of wounds at a later age.

Floor eggs

The percentage of floor eggs was much higher during this experiment (7-26%) compared to the 6% found in our previous experiment on nest design preference (Chapter 2), which can be explained by a number of factors. First, the pens in the current experiment were half as wide as the pens in the previous experiment. Smaller sized pens appear to be more inviting for floor laying behaviour, since there are relatively more sheltered areas against walls and fewer open spaces than in wider pens. Chickens tend to look for a sheltered space to lay their egg as this provides a sense of safety and less chances for disturbance (Duncan and Kite, 1989). Second, the light intensity was kept at 19 lx during the experiment to prevent further development of gentle feather pecking behaviour directed at the tails that had started in the rearing phase. The light intensity was chosen so that the behaviour did not worsen (Kjaer and Vestergaard, 1999) while also providing more than 10 lx of photostimulation needed for normal egg production (Lewis et al., 2008). Although the chosen light intensity was successful in terms of these two goals, it is lower than the recommended 30-60 lx to prevent creating dark areas that are preferred for floor laying (Aviagen, 2018). A third explanation can be found in the group size. Small groups of chickens allow for individual recognition and the establishment of a dominance hierarchy, while in larger groups a system of social tolerance is maintained (D'Eath and Keeling, 2003). The previous experiment had groups of 100 hens, which is considered large for chickens (Nicol et al., 1999) while the groups of 33 hens in this experiment could be problematic. When comparing groups of 15, 30, 60 and 120 laying hens, the groups of 30 had a lower body weight and egg production than the smaller or larger groups (Keeling et al., 2003). It was proposed that this 'intermediate' group size creates social disruption around key resources like the nest area which could increase the number of floor eggs. Furthermore, the willingness of a hen to defend or compete for a nest is thought to be higher in smaller groups compared to larger groups, which is also expected to affect the percentage of floor eggs (Estevez et al., 2007).

Surprisingly, the pens with raised slatted areas had a lower percentage of floor eggs than the pens with the nest directly placed on the litter. The fact that hens needed to jump onto the raised slatted area to reach the nest was apparently not a limiting factor for laying the eggs in the nest. It is possible that the hens were simply too young, and therefore mobile, to be burdened by the jump. It is also possible that the motivation to reach the nest is larger than the effort of jumping. A previous study of broiler breeders housed with raised slatted areas found that deteriorating leg health with age was not related to an increase in floor eggs, suggesting that mobility is not the most important factor involved in floor laying behaviour (Chapter 3). Most floor eggs are laid in the litter and not on structures such as slatted areas. This could explain why the pens without slats, and therefore with a larger litter area, had a higher percentage of floor eggs. Furthermore, as mentioned before, males tend to avoid spending time on the slatted area, which means that the males probably spend less time near the nests in the pens with a raised slatted area. Hens in the nest likely experience less disturbance of males in front of the nest, which could also increase the number of eggs laid in the nest.

Preference nest design

The preference for wooden nests found in this study is in agreement with the findings of our previous preference test on nest design (Chapter 2). However, the proportion of eggs laid in the wooden nest was slightly lower with 63% in this study compared to 69% in our previous study. The current study also showed more variation between pens regarding their preference for nest design. In two out of ten pens fewer eggs were laid in the wooden nest than in the plastic one, and in one pen the eggs were divided equally over each of the nests. This slightly lower and less consistent preference for the wooden nest compared to the previous study could be explained by some design differences, namely shape, material and colour. The nests in the current experiment had a curved back wall instead of straight, as this was standard for the commercial nest used in this study. The material used for the walls in the previous experiment could not be curved and was therefore replaced by a softer type of wood. This type of wood has no coating, making the surface more rough and perhaps slightly less attractive for the hens. The plastic nests in the current experiment had dark grey coloured walls, while these were black in the previous experiment. As the reasons behind the preference for wooden materials remains unknown, it cannot be ruled out that shape, type of wood or colour influence nest design preference.

In conclusion, this study shows that providing raised slatted areas to broiler breeders positively affects their behaviour, but the consequences for their leg health remain unclear. The frequency of mating behaviour was lower in groups with a raised slatted area, which suggests that this is beneficial for the welfare of the hens as mating is known to be aggressive in broiler breeders. The percentage of floor eggs was lower as well in groups with raised slats, meaning more hens laid their eggs in a secluded nest as they are intrinsically motivated to do. We did not find the expected beneficial effects of a raised slatted area on leg health, but this was likely due to their young age and should be investigated in a longer running experiment in the future.

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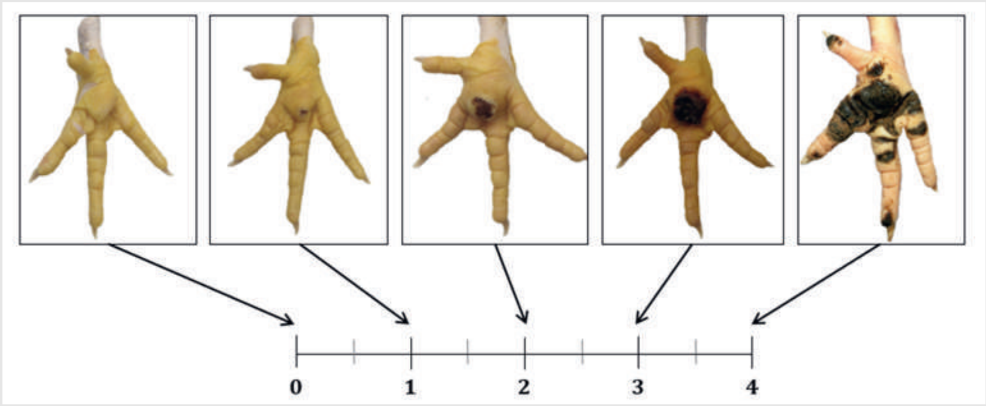
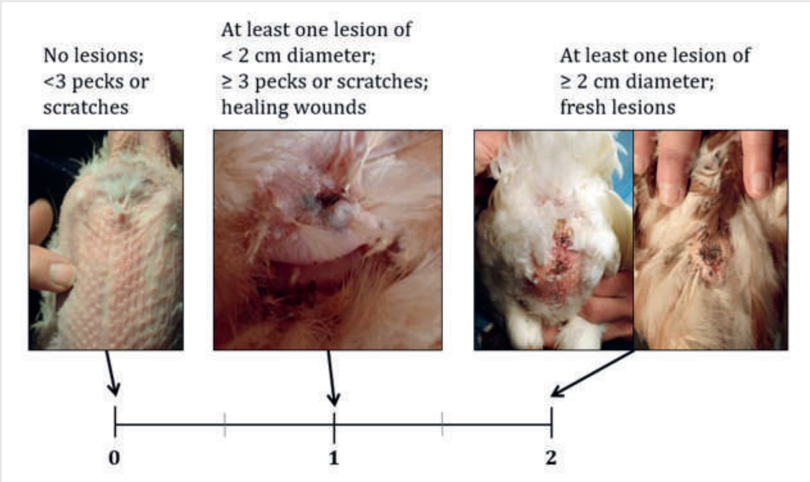
Supplementary materials

S1 Visual analogue scale for foot pad dermatitis (adapted from Welfare Quality®, 2009).



S2 Visual analogue scale for hock burn (adapted from Welfare Quality®, 2009).

S3 Visual analogue scale for wounds (adapted from Keppler and Knierim, 2017).



Chapter 6

General discussion

Introduction

This thesis discusses nesting behaviour in broiler breeders, the parents of broilers. Broiler breeders are expected to have a high egg production, while the genetic ability for fast growth needs to be passed on to their offspring. The reproductive system is known to be negatively affected by high body weight and the selection for fast growth, which forms the basis of the broiler breeder paradox (Decuypere et al., 2006). This negative relationship between growth and reproduction is apparent in a later on-set of egg production, more abnormal eggs and decreased hatchability of the eggs (Dunnington and Siegel, 1996; Jambui et al., 2017). The reproductive behaviour also seems affected by the selection for growth, since broiler breeder hens have been observed to lay more floor eggs (i.e. eggs laid outside the nests) compared to laying hens (Sheppard and Duncan, 2011) and to perform excessive gregarious nesting behaviour. Floor eggs can be a sign of reduced welfare, since the hens apparently find the nests not suitable for egg laying and are thus not meeting their behavioural needs. Furthermore, manual collection of floor eggs is time consuming and floor eggs are often dirty and broken, which reduces their saleability and hatchability (van den Brand et al., 2016). Excessive gregarious nesting can lead to overcrowding of the nests, which is a welfare risk (Riber, 2010) and moreover can increase the chances of floor eggs and broken eggs. Research on nesting behaviour in broiler breeders is very limited and often dated, with many studies performed between 1960-1990.

This thesis aimed to expand and update the knowledge on nesting behaviour in broiler breeders. Three experiments were performed to gain more insight into factors affecting nesting behaviour, structured according to the Motivation-Ability-Opportunity (MAO) model (Maclnnis and Jaworski, 1989). Motivation to lay an egg in the nest was studied by offering different nest designs in relative preference tests. Ability was interpreted as the physical ability to reach the nest, for which the occurrence and severity of contact dermatitis, gait deviations and body weight were recorded over the course of an entire production period. The opportunity of hens to get in the nest could be limited if nests are already occupied by other hens, so the occurrence of gregarious nesting behaviour was studied. Furthermore, the possible interference by the presence and behaviour of males in the opportunity to perform nesting behaviour was investigated.

In this chapter, the findings of the previous chapters will be discussed and integrated according to the three terms of the MAO model, while placing them into a larger framework of existing literature. The chapter starts with the motivation of the breeder hen to lay their egg inside the nest, then ability is discussed and lastly opportunity. The suitability of the MAO model in this research and the relative importance of each term of this model is then considered. This chapter closes with the conclusions that can be drawn from this thesis and recommendations for future research to improve our understanding of nesting behaviour of broiler breeders and improve their management.

Motivation: nest design preference

What nest design do the hens prefer?

In Chapter 2, we provided groups of broiler breeder hens a choice of four different nest designs: a plastic control nest, a plastic partition nest with a low wall in the middle of the nest floor dividing the nest in two areas, a plastic ventilator nest with a ventilator underneath the nest creating an air flow inside the nest and a wooden nest with wooden instead of plastic walls. The hens had a clear preference for the wooden nests with 69% of the eggs laid in these nests. This preference was confirmed in another preference test with plastic and wooden nests described in Chapter 5, where 63% of the eggs were laid in the wooden nests. The behaviour in the wooden nest could be described as settled, when looking at the proportion of time spent sitting compared to walking and the relative low numbers of nest inspections and visits per egg (Freire et al., 1996; Cronin et al., 2012). Due to the strong preference for the wooden nest, these nests became crowded leading to high frequencies of piling, aggression, nest displacement and head shaking. Despite this, the hens continued to return to the wooden nest and were thus willing to pay this price to visit the wooden nests. This further underlines the strength of preference for the wooden nest. The nest with a ventilator underneath, creating a constant air flow inside the nest, was least preferred both in terms of number of eggs and behaviour in the nest. This emphasizes the value of measuring the microclimate inside the nest, which is the result of the climate in the poultry house and nest design. Especially in countries with colder climates, an air flow in the nest will create wind chill (Osczevski, 1995) and this makes hens avoid the nest and thus increases the chance of floor eggs.

The reasons for the preference for wooden walls over plastic walls remain unclear, although we included some measurements to characterise the differences between these materials. The wooden nest had a higher air temperature inside the nest and lower electrostatic fields compared to the plastic nests, although their importance remain unknown. Our current broiler breeder could prefer wooden materials as a remnant of the original forest habitat of the ancestor of the domestic hen (Collias and Collias, 1967), but it is not yet known which physical characteristics of the wooden nest are responsible for this preference. The wooden nests still had a distinct 'wood' smell, even though the material was epoxy coated for biosecurity reasons. Domestic hens can detect a large variety of odours, which plays a role in fear responses, feeding and drinking, recognition of familiar items and avoidance of harmful situations (Jones and Roper, 1997). Several bird species use olfactory cues in relocating their nest (Balthazart and Taziaux, 2009), while blue tits and starlings have been observed to purposefully create a certain olfactory atmosphere in the nest (Gwinner and Berger, 2008). The role of olfaction in nest site selection is not yet studied in chickens and should receive attention in the future. Acoustics is another factor that could possibly influence nest site selection, since domestic hens vocalise often during the pre-laying period in the nest (Zimmerman et al., 2000). The nest wall material influences the reflection and absorption of sound and could thus create a different acoustic environment around egg laying. Moreover, the plastic material reflected more light than the wooden walls, which might create a hectic sensation. However, visual cues are expected to be of less importance, since the light intensity is low inside the nests. Further research is needed to fully understand why wooden nests are preferred.

Interpreting preference tests

The preference tests performed for this thesis provided multiple nest designs to groups of hens to gain insight into what nest design was most preferred (Chapters 2 and 5). There are three limitations to preference tests to discuss. The first limitation of preference tests is that they only measure relative preference (Duncan, 1991). Other designs that are not included in the test might be more preferred than the options provided, so no conclusion can be drawn on an optimal design, only on an improved design. The designs chosen in this study were based on observations from the field as well as previous scientific studies, focussing on commercially feasible designs. Secondly, a design can be relatively preferred when offered next to other designs, but this might not increase the motivation to visit the nest when only this design is offered and the contrast with other designs is gone (Williams, 1992). To test for this, groups of broiler breeders should be housed with either wooden or plastic nests to compare nesting behaviour and the percentage of floor eggs. Lastly, the results of preference tests are only valid for the context they were tested in and the type of birds (hybrids) used. This thesis has only described preference tests with Ross 308 broiler breeders, while other hybrids might have different preferences.

In social animals such as broiler breeders, it is difficult to distinguish individual preference for a nest design from local enhancement (Rendell et al., 2011). The first hens to start laying eggs in a group will have to make their own decision on nesting location, but hens that start egg production later could simply follow the choice made by these first hens. Since broiler breeders are known to nest gregariously (discussed more extensively in a later paragraph), local enhancement is expected to be of importance in nest site selection. However, there will always be hens that have a preference for a different nest design than the majority of the group. Even though domestic chickens are genetically very similar, individuals still have different personalities (Cockrem, 2007; de Haas et al., 2017). Personalities can be reflected in sociability, which in term might affect the preference for nest size and thus space for other hens, or perception of cues such as acoustics or smell. The question remains whether hens should only be provided one nest design in commercial housing or rather a variety of nest designs to cater for the different preferences that exist within a flock.

Effect of age on nest site selection

After the preference for wooden nests was stable for six weeks in our experiment described in Chapter 2, these nests were closed at 32 weeks of age. The hens chose a new nest to lay their eggs as the percentage of floor eggs did not increase and this choice was dependent on both nest design and location. Before closing the wooden nests, the preference for the other designs in order from most to least preferred was control, partition and ventilator. This order remained intact after closing the wooden nests, but the nests directly next to the wooden nests were more preferred than the nests further away. These results confirm that broiler breeders are conservative in their nesting location, which was previously also found for laying hens (Riber, 2010; Riber and Nielsen, 2013). Hens were also found to make fewer nest inspections before a nest visit as they aged (Chapter 2), which is another sign that nest site location becomes consistent and does not require as much exploration behaviour compared to a younger age.

The first weeks of egg production are thus influential in nest site selection for broiler breeders, not only on which nest to lay eggs in, but also if a hen chooses to lay eggs outside the nests provided. Data on daily egg production and number of floor eggs (age 20-60 weeks) were collected from six commercial broiler breeder farms in the Netherlands between October 2014 and December 2019. The sample population consisted of 102 flocks of Ross 308 broiler breeders, all non-beak trimmed. Bird density was 7-8 birds per m^2 at all farms with flock sizes varying from 6170 to 13170 hens and 590 to 1190 roosters per house. All houses had similar lay-outs with nests provided on raised slatted areas, which gave access to drinking nipples or bell drinkers, and a litter area with feeding lines. Mean floor egg percentages for the period until 27 weeks of age and for the period from 27 weeks of age onwards were calculated per flock. Mean floor egg percentage until 27 weeks of age was positively correlated to mean floor egg percentage from 27 weeks of age onwards ($r_s = 0.320$, P=0.001), see Figure 1. This means that if many hens lay floor eggs during early age, the risk of a high floor egg percentage for the entire production period is higher. Floor eggs should thus get extra attention during the start of the egg production. The most effective action is the frequent removal of floor eggs to stop other hens from laying their eggs near the floor eggs already present (Aviagen, 2018). Attention should also be given to a uniform light intensity throughout the house as well as defective or dirty nests.

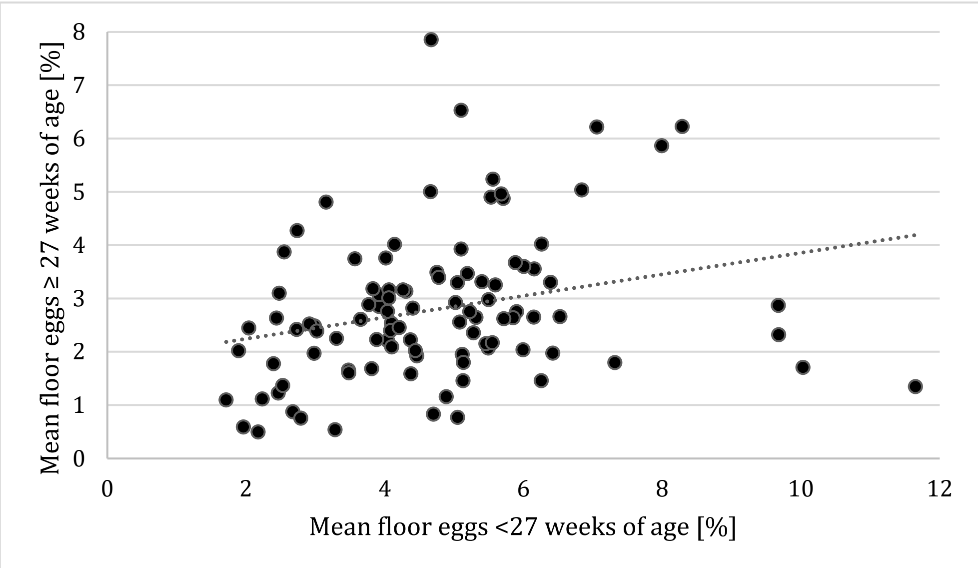


Figure 1. Mean percentage of floor eggs < 27 weeks of age plotted against mean percentage of floor eggs ≥ 27 weeks of age of 102 commercial broiler breeder flocks.

Ability: leg health

Are hens physically able to reach the nest?

If the hens are motivated to lay their eggs in the nest, they need to be physically able to reach the nest. Leg health problems, such as contact dermatitis and leg weakness are likely painful for the affected birds and could thus impede the movement of birds to the nest for egg laying. When broilers are offered feed with and without analgesic drugs, lame broilers select more drugged feed than unaffected broilers (Danbury et al., 2000). Although no direct studies have been performed on the pain related with leg health problems in broiler breeders, it seems highly likely that lameness also results in pain in broiler breeders. As described in Chapter 3, foot pad dermatitis is a major issue for broiler breeders housed in commercial floor systems with a partially slatted area. The incidence of foot pad dermatitis ranged from 78-95% of the birds and 8-35% had severe foot pad dermatitis between the ages of 32-60 weeks without any difference between the genetic lines included in this study. The incidence of foot pad dermatitis in broiler breeders seems to be similar to fast-growing broilers, where up to 65% of the birds is affected at the slaughter age of 6 weeks (Haslam et al., 2007; de Jong et al., 2012; Bassler et al., 2013). Other leg health parameters included in this study, namely hock burn and gait deviations, were observed much less. Maximum 0.5% of broiler breeders had severe hock burns and maximum 2.7% had severe gait deviations. Both these issues are much more common in broilers, where severe hock burns were observed in up to 41% of the birds and leg weakness or lameness in 14-30% of the flock (Sanotra et al., 2003; Bassler et al., 2013). Broilers are likely more affected by these conditions due to a faster growth pattern compared to broiler breeders.

Against expectations, no correlation was found between foot pad dermatitis, hock burn or gait with egg production or floor egg percentage in Chapter 3. Leg health problems thus seem not to be limiting the broiler breeder hens in their egg production or movement towards the nest or the motivation to lay an egg in the nest overrules these health problems. Also, we found no correlation between floor egg percentage and body weight in Chapters 3 and 5. Nests are provided on slatted areas, which are raised at 40-50 cm, in most regions of the world. This requires the broiler breeder hens to jump up before being able to lay an egg in the nest, which is expected to be more difficult with a higher body weight. However, pens with nests on a raised slatted area had a lower percentage of floor eggs in compared to pens with nests directly accessible from the litter (Chapter 5). This lower floor egg percentage might be caused by a smaller litter area, which is the preferred area for floor eggs. On the basis of our results, it seems unlikely that floor laying behaviour is caused by leg health or excessive body weight problems. Even so, lowered slatted areas for which a manure pit has to be build, are increasingly popular amongst farmers. These investments are made based on the positive experiences of fellow farmers (van Loon, 2020a), contradictory with our results. This discrepancy could be caused by the differences between small-scale experimental studies and commercial farms or because data collection outside of scientific studies tends to be less objective. Thus, further research on the relation between height of the slatted area and floor egg percentage is needed, ideally in commercial production environments.

Housing and management measures against foot pad dermatitis

While leg health problems seem not to affect the ability of broiler breeders to reach the nest, the high occurrence and severity of foot pad dermatitis is still a welfare concern against which measures should be taken. Foot pad dermatitis in broiler and laying hens is known to be influenced by the litter quality, because ammonia is released more easily from litter with a high moisture level and this irritates the foot pads (Greene et al., 1985; Shepherd and Fairchild, 2010). The correlation between litter quality and foot pad dermatitis is also confirmed for the broiler breeders in this study (Chapter 3). This paragraph describes practical solutions to improve foot pad conditions by maintaining a good litter quality through ventilation and water management, which were identified as the most important management risk factors for wet litter (Hermans et al., 2006). Subsequently, we look at opportunities for birds to limit the time their feet come into contact with the litter through the provision of perches or slats.

Ventilation rate is generally controlled based on the temperature inside the poultry house, which means that these rates become very low with cold weather to avoid heat loss. Low ventilation rates lead to a high relative humidity of the air in the house, which in turn increases the humidity of the litter (Weaver and Meijerhof, 1991). When a poultry house is equipped with a heating system, minimum ventilation rates can be increased and thus ensure a better litter quality. Depending on the type of heating system, this can be a considerable investment with continuing energy consumption costs. In the Netherlands and Belgium, many poultry farmers install a heat exchanger to abide by the air pollution laws, which has the additional benefit of heating the incoming air and

thus also functions as a heating system. This improves the litter quality and thus the foot pad health of the birds.
Another risk factor for wet litter is overconsumption of water, which is often observed in broiler breeders and is thought to be related to hunger or boredom due to feed restriction (Mench, 2002). Water restriction during both the rearing and production phase is a common practice to maintain good litter quality. Although access to water is generally considered a basic requirement for all animals, limiting access to water had little to no effect on behavioural and physiological welfare indicators in broiler breeders (Hocking et al., 1993). The Dutch law is vague about minimal requirements for water access for poultry and thus water restriction is permitted, but this practice is increasingly questioned by welfare researchers as well as politicians. Unlimited water access does not necessarily lead to excessive drinking in broiler breeders as is apparent from countries that do not restrict water (de Jong et al., 2016). Combining unlimited water access with measures that decrease the feeling of hunger and boredom, such as twice a day feeding or offering diluted feed, have the highest chance of preventing overconsumption of water. Furthermore, overconsumption of water is not observed in slow growing breeds that do not require feed restriction (Heck et al., 2004).
Interestingly, during a second experiment with the same breed of broiler breeders, we found a very low incidence of foot pad dermatitis until 32 weeks of age (Chapter 5). The birds in this experiment were housed with perches, which are still uncommon in commercial housing. Under experimental conditions, 20-50% of broiler breeders use perches depending on age and the amount of perch space provided (Gebhardt-Henrich et al., 2017b). Perches provide an opportunity besides the slatted area for birds to rest without contact with litter and could thus be beneficial to prevent foot pad dermatitis. An increasing number of European countries include perches as a minimum requirement for broiler breeders, in a similar manner as for laying hens. While research suggests that broiler breeders prefer perches over raised slatted areas for roosting, the provision of perches significantly increased the number of keel bone fractures (Gebhardt-Henrich et al., 2016 as cited by Riber et al., 2017). To avoid moving from one problem to the next, optimal perch design for broiler breeders should be studied to avoid an increase the incidence of keel bone fractures.
Although slatted areas are usually incorporated into housing for broiler breeders to make manure management easier (Brake, 1998), it also helps to reduce the time that the birds' feet come into contact with the litter. In an

experimental study comparing 20% and 50% slatted area, the feet lesion score (damages, swelling, wounds) of broiler breeder males lowered by 1.5 point on a 10-point scale with 50% slatted area (van der Haar, 1995). Whether these beneficial effects were caused by the birds spending less time on the litter or because in their study the litter moisture was approximately 5% lower in the pens with 50% slats remains unknown. However, Kaukonen et al. (2016) found contrasting results. Commercial broiler breeder flocks housed with 29-48% of slatted area were scored for foot pad dermatitis and flocks housed with a larger proportion of slats had a poorer foot pad condition. The initial cleanliness, quality and material of slats was not determined in this study and these factors might influence the beneficial effects of the slats. Although the presence or absence of a slatted area in our experiment described in Chapter 5 did not make a difference on the incidence of foot pad dermatitis, it would be useful to repeat this experimental set-up in a longer lasting experiment without perches.

Investing in the leg health of broiler breeders

The occurrence of foot pad dermatitis could thus be lowered through housing and management measures. Implementing these measures requires an investment and farmers can be motivated to make these investments through financial incentives. While most farmers care for the wellbeing of their animals, their income depends on the productivity of the animals. Foot pad dermatitis and hock burn are associated with other health and performance problems in broilers (Ekstrand et al., 1998; de Jong et al., 2012), which is at least partially due to a decreased feed intake and a higher chance of infection with microorganisms such as Staphylococcus aureus (Martland, 1985; Hester, 1994). This correlation between leg health problems and productivity may be absent in broiler breeders, since no correlation was found between foot pad dermatitis, hock burn or gait with egg production or floor egg percentage (Chapter 3). This means that even though the welfare of the broiler breeder hens is likely reduced due to these leg health problems, the performance is unaffected and therefore this does not provide a financial incentive for farmers to try to prevent this condition.

Implementing regulations on a (inter)national level can also motivate farmers to pay attention to certain issues. In the Netherlands, broiler farmers can be forced to reduce their bird density if the proportion of broilers with foot pad dermatitis is deemed too high at the slaughterhouse (Rijkdienst voor Ondernemend Nederland, 2014). Other European countries including Germany decrease the price given for the broilers if the incidence of foot pad dermatitis is too high, which will possibly be implemented in the future in the Netherlands as well (van Loon, 2020b). Unlike the regulation for broiler farmers, the incidence of foot pad dermatitis is not routinely checked when slaughtering broiler breeders and thus farmers are not penalised on the basis of this condition. If the regulations are found to be effective in reducing foot pad dermatitis in broilers, it should be considered whether expanding these regulations towards broiler breeders is an option.

Burden of a high body weight

Body weight was not correlated to leg health problems in this study, but the high body weights of broiler breeders seem to have other disadvantageous consequences for health and production. Fast growing broiler breeders weigh just over 2 kg at the start of the production phase and end up between 3.5-4.5 kg at slaughter age. This growth profile is achieved with feed restriction in order to keep egg production at an optimal rate (Decuypere et al., 2010). While genetic lines differ in their advised body weight curves during lay and maximum egg production, we found that even within a genetic line the negative correlation between body weight and egg production is present (Chapter 3). The fact that a higher body weight was correlated to a higher mortality rate in our experiment inflates the negative effects of body weight on egg production of the flock. Although the cause of death was unknown, feed restricted broiler breeders are known to have less bone and joint problems, while antibody responses and disease resistance are higher than in ad libitum fed birds (Mench, 2002). Furthermore, broiler breeders in warmer climates are often kept below the body weight goals advised by breeding companies to avoid heat stress (I. Karmon, personal communication, 14 September 2018). High body weights are thus a risk for the health of broiler breeders and should be controlled.

An increasing proportion of broilers worldwide is of a slow growing breed, which has been stimulated from an animal welfare point of view by researchers and consumers. This proportion is estimated at 40% for the Netherlands, 25% in France and 11% in the UK, while outside of Europe this market is only a few percent of the total broiler industry (Davies, 2019). To supply these slow growing broilers, broiler breeders of slow growing (also called 'label') breeds are gaining interest. Slow growing broiler breeders do not need feed restriction, since their natural feed intake is much lower than conventional breeds and this results in body weights of less than 2.5 kg at 40 weeks of age (Heck et al., 2004). Mortality of slow growing broiler breeders is 2% lower compared to fast growing birds, while egg production and the percentage of settable eggs is similar to restrictively fed fast-growing breeds. Besides health benefits, slow growing broiler breeders spend less time on performing stereotypical oral behaviour in the form of pecking at feeders or litter during both rearing and laying (Puterflam et al., 2006). However, farmers report a higher percentage of floor eggs and a higher tendency of clustering in slow growing broiler breeders (Graumans, 2020). Future research and experience with slow growing broiler breeders should lead to management and housing recommendations for these hybrids.

Opportunity: social constraints

Factors associated with gregarious nesting

Since reduced leg health does not seem to limit ability of a broiler breeder hen to reach the nest, it is important to see whether there are other constraints to entering the nest. In this thesis we studied broiler breeders housed with family nests as these are most commonly used in Europe, while other regions of the world mainly use single nests. Family nests give hens the option to nest gregariously, which means that a hen prefers a nest with other hens present over an empty nest. If too many hens in a flock perform gregarious nesting, this can lead to insufficient nest space. Furthermore, bird welfare is at risk when hens use excessive energy to enter an overcrowded nest (Appleby, 1986), which often leads to scratches (Clausen and Riber, 2012) or in rare cases to suffocation (Lentfer et al., 2011). Even though the ancestor of the domestic hen nested solitary, laying hens have been reported to nest gregariously (Riber, 2010; Clausen and Riber, 2012; Ringgenberg et al., 2015b) and Chapter 4 describes this behaviour in broiler breeders. We found that pens with a higher occurrence of gregarious nesting had a higher percentage of floor eggs, which shows that the unavailability of preferred nests contributes to floor laying behaviour. It should be noted that there was a striking effect of genetic lines on the occurrence of gregarious nesting with some lines distributing themselves evenly over the nests available, while other lines had a clear preference for nests in the corner of the pen. This preference for nests at the end of a row or in a corner has also been described in laying hens (Riber, 2010; Clausen and Riber, 2012; Ringgenberg et al., 2015b) and genetic effects were also reported in laying hens, observing more gregarious nest visits in ISA Brown hens compared to Dekalb White hens (Schakel, 2015).

The reasons behind this preference for nests with hens already present is not entirely clear, but may relate to social learning and coherence, fear of predators or shared preferences for nest features. First, having limited experience with egg laying might stimulate hens to look for company or a demonstrator (Rendell et al., 2011). This is supported by the finding that gregarious nesting was more pronounced at earlier ages in both laying hens (Riber, 2010) and broiler breeders (Chapter 4). Second, preferring to be close to hens when nesting might be a reflection of general social coherence, where birds prefer to stay close to conspecifics (Väisänen and Jensen, 2003). However, the tendency for clustering in the rest of the pen was unrelated to the distribution over the nests in our study, so social coherence might only be important during egg laying or just does not play a role in gregarious nesting. Third, gregarious nesting may be an anti-predator response. In the presence of a model of an egg eating predator (hooded crow), laying hens preferred to nest socially rather than solitary (Riber, 2012). Since broiler breeders are usually kept in closed houses, the only predator that the hens come into contact with is humans. However, we did not find a correlation between fear of humans and the amount of gregarious nesting (Chapter 4).

Lastly, gregarious nesting may occur simply because hens prefer the same nest features. It has been suggested that nests at the end of rows or in corners are more preferred and therefore at higher risk for excessive gregarious nesting, because of higher recognisability. However, offering three different appearing nests to groups of laying hens did not reduce the occurrence of gregarious nesting compared to groups with access to only one nest design (Clausen and Riber, 2012). Perhaps we should not try reduce the number of hens in nests at the end of a row, but attempt to create more ends in a row to provide more nests that are preferred. In certain regions of the world, nests are provided in blocks of two or three nests with open spaces in between them (Figure 2). This seems to help to distribute hens more evenly over the available nests and thus reduce the negative welfare and economic consequences of excessive gregarious nesting. It should be noted that having open spaces between nests lowers the total nest surface area and thus the maximum bird density, which affects the return of initial investment in housing for the farmer. This solution might thus only be feasible within a welfare focussed label for broiler meat production.

We also studied general fearfulness as a possible explanation for the occurrence of gregarious nesting behaviour (Chapter 4), but the novel object test that was used to measure this seems to be unsuitable for broiler breeders. Novel object tests are commonly used in poultry and found to be repeatable over time in laying hens (Forkman et al., 2007). In our experiment, the novel object test was repeated with an interval of 10 weeks and a slightly different object to maintain

Figure 2. Housing lay-out for broiler breeders with blocks of nests instead of long rows.

interest. From the second test onwards, the birds initially moved away from the novel object, but later showed no response by approaching or avoiding the object. This low responsiveness or willingness to approach the novel object has also been described in other studies with adult broiler breeders and pullets (Lindholm et al., 2018; Tahamtani and Riber, 2020). Broilers have likewise been found to have a 'docile' response in fear tests in comparison with white laying hens that have a more 'flighty' response (Keer-Keer et al., 1996). This does not necessarily reflect their fearfulness as birds in similar states of fear can respond to this fear according to different behavioural strategies, where proactive birds are more bold and approach novelty quickly and reactive birds are more shy and explore slow and thoroughly (Cockrem, 2007). Including physiological measurements of fear responses during fear tests such as heart rate or corticosterone could help elucidate the suitability of these tests for broiler breeders. For now, no conclusions can thus be made on the role of general fearfulness in gregarious nesting behaviour in broiler breeders.

Influence of males on nest usage

Apart from the influence other hens can have on nest site selection of broiler breeder hens, the behaviour and presence of males can affect nesting behaviour as well. Broiler breeders hens are housed with on average 7-10% males to

ensure optimal fertilisation of the eggs. These males are known to be aggressive in their mating behaviour as they peck, chase and forcefully mount hens, causing feather loss and wounds on hens (de Jong and Guémené, 2011). Mating mainly happens in the litter area (Gebhardt-Henrich et al., 2020) and in our experiment a high proportion of wounded hens was correlated with a lower use of the litter area (Chapter 4). This correlation suggests that wounded hens try to avoid further mating, although no observations of mating behaviour were included in this study. An increased proportion of wounded hens was also correlated to a lower percentage of floor eggs (Chapter 4). The lower use of litter area by these wounded hens could explain this correlation, since floor eggs are mostly laid in the litter area rather than the slatted area. A previous study found that placing mesh panels in the litter area resulted in fewer floor eggs, as this was thought to create more general activity in the litter area and thus disturb floor laying (Leone and Estévez, 2008). So the presence of males in the litter area could deter some hens from laying eggs in the litter area.
Since males spend less time on slatted areas (de Jong and Guémené, 2011), the effect of having a slatted area in front of a laying nest on nesting behaviour was studied in Chapter 5. Pens with nests directly on the litter had a higher floor egg percentage than nests with a raised slatted area in front of it. This could be caused by the larger litter area in pens without a slatted area, since most floor eggs are laid in the litter, or a preference of the hens to lay their eggs in raised nests. Another possibility is that hens might be deterred from visiting the nest, if males are more often near the nest, which is likely to happen if there is no slatted area in front of the nest. Due to camera malfunctions in our experiment, the behaviour near the nest could not be observed and thus the social constraints on nest visits caused by males should be studied in the future. It can be concluded that the presence and behaviour of males affect the spatial behaviour of hens and possibly deter hens from laying eggs in the litter area, but the direct effects on behaviour in the nest remain unknown.

How many males are needed?

A larger litter area was found to increase the frequency of general mating activity (i.e. successful copulations and unsuccessful attempts), although no increase was found in the number of copulations (Chapter 5). Unfortunately fertilisation measurements were not included, but it seems likely that copulations are most important for egg fertilisation and thus the size of the litter area probably has no effect on the percentage of fertilised eggs. The three males housed with 33 hens performed on average 40 copulations in the 1-3 hours before lights-off. This means that on average each hen was inseminated at least once a day, while chickens can store sperm for over 3 weeks (Brillard, 1993) and lay fertilised eggs for at least 10 days after insemination (Fontana et al., 1992). This raises the question whether the current 7-10% of males in most commercial flocks could be lowered without compromising fertilisation results. When comparing a new housing concept for broiler breeders where males are separated from the hens for part of the day or even every other day (Quality Time®) to standard housing with a constant presence of males, fertilisation rates did not differ (Van Emous, 2011; R. van Emous, personal communication, 19 October 2020).

An important aspect of the number of males required for optimal fertilisation is the variation in mating behaviour between individual males. In our study described in Chapter 5, the frequency of copulations was twice as high in some pens compared to other pens of the same lay-out with males of the same age and breed, which is comparable to differences found between individual males (Bilcik et al., 2005). Farmers attempt to remove the males that perform less or no mating behaviour, based on morphological characteristics including body weight, redness of legs and cloaca, leg health and fleshing (van Emous et al., 2020). The correlations between these characteristics and mating behaviour or fertility seem to be strain-specific and weak (McGary et al., 2003; Bilcik and Estevez, 2005). Therefore, better indicators of the frequency of mating behaviour on an individual level is needed. With this information, only a limited number of active males can be included in the flock and thus the stress and possible wounding of having too many males in a flock can be avoided.

Males can interfere with the mating activity of other males as part of male-male competition, which leads to relatively more failed mating attempts (Kratzer and Craig, 1980). When males are moved from a housing situation with two other males and 10 hens to a non-competitive situation without other males and the same number of hens, general mating activity increases as well as successful copulations (Bilcik and Estevez, 2005). Furthermore, the proportion of sired offspring differs greatly between males due to male-male competition and sperm competition (Bilcik et al., 2005). So, decreasing the number of males per flock might not decrease the frequency of copulations or the fertilisation of eggs. It is important to note that male-male competition depends on the dominance status of the males, where dominant males are more likely to interfere with other males compared to subordinate males (Pizzari and Birkhead, 2001). In order to translate this knowledge to practical advice for farmers, more research is needed on the effects of group size and dominance status on male mating behaviour and competition in commercial sized flocks.

Hens' welfare affected by mating

As mentioned previously, broiler breeder males are known to be more aggressive in their mating behaviour and hardly perform courtship behaviour in comparison to layer breeders (Millman et al., 2000). This leads to wounding of females and contributes to feather loss (de Jong and Guémené, 2011). During our study, over 30% of females had wounds at a certain point during the production period and the majority of females had severe feather damage from the age of 40 weeks onwards (Chapter 4). In three of our tested genetic lines, the peak of the number of wounded females was at 40 weeks of age, while for the two other lines females only became wounded after this age. Unfortunately our study did not include direct observations on mating behaviour to link these findings to differences in mating frequency or aggression involved in mating behaviour, but fast-growing strains of broiler breeders are known to differ in their mating behaviour which is partially caused by differences in body weight (McGary et al., 2003).

Not only the behaviour of males influences the damaging of hens, but also the hens' response to mating. A recent study compared mating behaviour of Ross 308 (fast-growing) and Sasso (slow-growing) broiler breeders (Gebhardt-Henrich et al., 2020). The frequency or success of male mating behaviour did not differ between breeds, but Sasso hens crouched less and struggled more during mating compared to Ross 308 hens. It is suggested that the large size difference between Sasso males and hens is causing this unwillingness of hens, although there might also be a genetic component in the docility or fearfulness of the hens. So while slow-growing breeds are beneficial for solving issues surrounding feed restriction, this difference in response to mating requires more attention in the future.

Hens with a poorer feather coverage or with wounds are likely less willing to mate, since mounting will be more painful. A bad feather condition was found to be correlated to a higher incidence of wounds in our study described in Chapter 4. In order to prevent wounding of females, male broiler breeders are often despurred and toe clipped (de Jong and Guémené, 2011). These mutilations are no longer practiced in some European countries, while other countries including the Netherlands are banning them on short term due to concerns for male welfare and respect for animal integrity. Studies with both layer breeders and broiler breeders show that feathering coverage is worse when males are left intact (Riber, 2017; Shi et al., 2019). Although finding alternatives to these mutilations proves to be difficult (Riber, 2017), a recent study which implemented abrasive strips in colony cages resulted in reduced length and sharpness of claws and an improved feather coverage of the back and rump of hens (Shi et al., 2019). Allowing birds to shorten their nails by natural scratching behaviour might thus be a solution, although this should also be studied in non-cage systems.

MAO model applied in this animal behaviour study

To our knowledge, this was the first time that the MAO model was used for animal behaviour studies. The MAO model is generally used for studies on consumers' processing of marketing and advertisement (Maclnnis and Jaworski, 1989). These study fields hardly seem related, but in the core both human and animal research deal with behaviour. Motivation is defined as the drive or urge to perform behaviour, both in our study and in human studies. For the term ability, the approach was different between these research fields, which is likely caused by the difference in the nature of the behaviour that is studied. Advertisement processing is a cognitive behaviour and thus these studies focus on the cognitive ability or skills needed, whereas we studied an innate behaviour that could mainly be limited by physical ability. Opportunity deals with situational constraints that could hinder the behaviour and for information processing in humans, this is focussed on distraction or time limitations. Again, situational constraints in this study are more focussed on physical constraints in the form of insufficient space in the nest or negative social interactions. The MAO model thus seems suitable as a structural approach to study animal behaviour, although the interpretation of each aspect is dependent on the studied species and type of behaviour.

The order and magnitude in which each aspect within the MAO model affects the performance of the behaviour is not clearly implied from the visualisation of the framework (Figure 3), but should be discussed to come to conclusions on which aspect is most important for the behaviour of laying an egg in the nest. Motivation to perform the behaviour is essential, without this drive the behaviour would not be performed. Even though we did not study the motivation for laying an egg in the nest compared to other nesting locations, it is expected that protecting future offspring by choosing a sheltered location for egg laying is an innate drive for broiler breeders. Offering wooden nests is likely to increase the motivation to lay in these nests, since wooden nests were

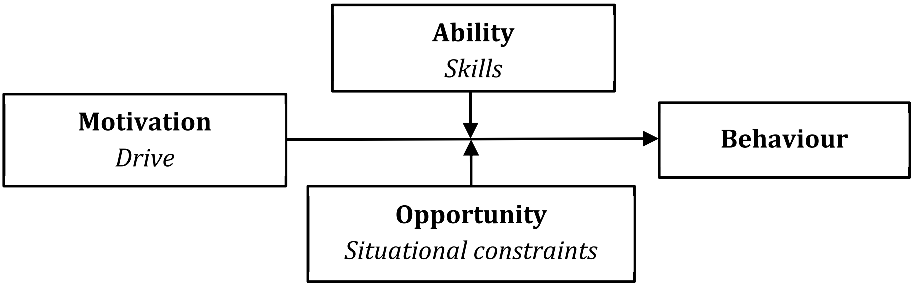


Figure 3. Visualisation of the MAO model, which shows the proposed relationship between motivation, ability, opportunity and behaviour.

clearly preferred over plastic nests. Ability and opportunity modify the process of turning this motivation into action, in which ability comes first. If the animal is not able to perform the behaviour, it does not matter whether they get the opportunity or not. From the results of our study, the ability in terms of leg health and mobility did not seem to limit the hens in reaching the nest, since there was no correlation between leg health problems, body weight and floor eggs. As there are no indications that ability limits the broiler breeder to visit the nest, we can discuss whether there are situational constraints that hamper the opportunity to visit the nest for egg laying. Excessive gregarious nesting was found to increase the percentage of floor eggs, with clear differences in the occurrence of gregarious nesting between the genetic lines. The influence of males on nest visits was not studied directly, but the positive correlation between proportion of wounded hens and floor egg percentage suggests that mating behaviour affects nest site location. To sum up, nest design influences the motivation of broiler breeders to lay an egg in the nest, but the opportunity to visit the nest might be constrained by excessive gregarious nesting and male behaviour. Leg health problems do not seem to limit the ability to reach the nest or are overruled by the high motivation to get to the nest.

Conclusions

Based on the findings described in this thesis, the following conclusions can be drawn:

- Broiler breeders have a clear preference for wooden nests compared to plastic nests, although the exact causes behind this preference remain unknown.

- Individuals chose their nest site location during the first weeks of egg production and are not likely to alter this location unless forced.
- Our study suggests that foot pad dermatitis in commercially housed broiler breeders is as common and severe as in broiler flocks, but hock burn and affected gait are observed less.
- Leg health problems did not correlate to an increased percentage of floor eggs in our study, showing that physical ability does not seem to hinder nest visits.
- Broiler breeders show excessive gregarious nesting behaviour in a similar manner to laying hens, although this is dependent on hybrid.
- Excessive gregarious nesting is observed mostly in the nests at the end of the row and is correlated to a higher percentage of floor eggs.
- The frequency of total mating behaviour, but not copulations, is dependent on the size of the litter area.
- Groups with more wounded hens, likely caused by males' aggressive mating behaviour, use the litter area less and have a lower percentage of floor eggs.

Recommendations

As discussed in this chapter, several recommendations can be made for future research to help understand nesting behaviour of broiler breeders and improve their management:

- Why broiler breeders prefer wooden nests remains unknown and should be studied further to understand what properties are attracting the birds. This would help nest designers to create a nest that has these exact properties together with other features that might be attractive for birds or be better for durability or maintaining hygiene.
- The first weeks of egg production are vital for nest site selection and thus farmers should invest time and effort to avoid floor eggs in this period to benefit from it during the rest of the productive life.
- Studies on the effectiveness of housing and management adjustments on foot pad dermatitis in broiler breeders are needed, including climate control, water access, perches or slat features. This can be translated into practical tools for farmers to increase attention for this problem and improve broiler breeder foot health and thus welfare. Whether routine controls at slaughter houses can contribute to reducing foot pad dermatitis in broiler breeders should be studied.

- Excessive gregarious nesting is still poorly understood, but seems to be at least partially caused by genetic background. Further research should focus on what repercussions selecting against this behaviour has on other behaviour and performance. In the meantime, practical solutions for this behaviour should be tested at commercial farms, including the suggested set up with blocks of nests rather than long lines of nests.
- The optimal proportion of males in a flock should be investigated to find the balance between optimal fertilisation of eggs and reducing the stress and damage of hens caused by mating behaviour. Collecting data on mating behaviour of individual males would be ideal, but gaining experience with different proportion of males or exposure time to males can also lead to new insights. Collaboration with breeding companies could enhance this research by focussing selection on males with a high number of sired offspring and less aggressive behaviour during mating.
- Differences between fast and slow-growing broiler breeders should be reviewed carefully to ensure that new problems do not arise from trying to solve others. Slow-growing broiler breeders likely have a better welfare because no feed restriction is required to keep the body weight low, which decreases the performance of oral stereotypies. However, these slow-growing strains have been observed to show increased struggling during mating and more floor laying behaviour, which are both signs of other possible welfare issues.
- Besides performing small-scale experiments with poultry, more research should be done on commercial sized flocks to translate new knowledge to practical advice. Cooperation of farmers is essential for this, since gathering reliable data from enough flocks to perform statistical analysis can be challenging.

References

Agnvall B., Ali A., Olby S. and Jensen P. 2014. Red Junglefowl (Gallus gallus) selected for low fear of humans are larger, more dominant and produce larger offspring. Animal 8, 1498–1505.

Appleby M.C. 1984. Factors affecting floor laying by domestic hens: a review. Worlds. Poult. Sci. J. 40, 241–249.

Appleby M.C. 1986. Hormones and husbandry: control of nesting behaviour in poultry production. Poult. Sci. 65, 2352–2354.

Appleby M.C. 1990. Behaviour of laying hens in cages with nest sites. Br. Poult. Sci. 31, 71–80.

Appleby M.C., Duncan I.J.H. and Mcrae H.E. 1988. Perching and floor laying by domestic hens: experimental results and their commercial application. Br. Poult. Sci. 29, 351–357.

Appleby M.C. and Hughes B.O. 1991. Welfare of laying hens in cages and alternative systems: environmental, physical and behavioural aspects. Worlds. Poult. Sci. J. 47, 109.

Appleby M.C. and Hughes B.O. 1995. The Edinburgh Modified Cage for laying hens. Br. Poult. Sci. 36, 707–718.

Appleby M.C., Maguire S.N. and McRae H.E. 1986. Nesting and floor laying by domestic hens in a commercial flock. Br. Poult. Sci. 27, 75–82.

Appleby M.C. and McRae H.E. 1986. The individual nest box as a super-stimulus for domestic hens. Appl. Anim. Behav. Sci. 15, 169–176.

Appleby M.C., McRae H.E. and Duncan I.J.H. 1984a. Nest-site selection by domestic hens. In Commission of the European Communities Farm Animal Welfare Program Evaluation Report 1979-1983 (ed. P.V. Tarrant). Commission of the European Communities, Brussels.

Appleby M.C., McRae H.E., Duncan I.J.H. and Bisazza A. 1984b. Choice of social conditions by laying hens. Br. Poult. Sci. 25, 111–117.

Appleby M.C., Smith S.F. and Hughes B.O. 1993. Nesting, dust bathing and perching by laying hens in cages: effects of design on behaviour and welfare. Br. Poult. Sci. 34, 835–847.

Ask B. 2010. Genetic variation of contact dermatitis in broilers. Poult. Sci. 89, 866–875.

Aviagen 2018. Ross PS Management Handbook 2018. Retrieved on 8 April 2020, from *http://en.aviagen.com/brands/ross/products/ross-308*.

Balthazart J. and Taziaux M. 2009. The underestimated role of olfaction in avian reproduction? Behav. Brain Res. 200, 248–259.

Bassler A.W., Arnould C., Butterworth A., Colin L., De Jong I.C., Ferrante V., Ferrari P., Haslam S., Wemelsfelder F. and Blokhuis H.J. 2013. Potential risk factors associated with contact dermatitis, lameness, negative emotional state, and fear of humans in broiler chicken flocks. Poult. Sci. 92, 2811–2826.

Beilharz R.G., Luxford B.G. and Wilkinson J.L. 1993. Quantitative genetics and evolution: Is our understanding of genetics sufficient to explain evolution? J. Anim. Breed. Genet. 110, 161–170.

Berrang M.E., Frank J.F., Buhr R.J., Bailey J.S., Cox N.A. and Mauldin J.M. 1997. Microbiology of sanitized broiler hatching eggs through the egg production period. J. Appl. Poult. Res. 6, 298–305.

Bilcik B. and Estevez I. 2005. Impact of male-male competition and morphological traits on mating strategies and reproductive success in broiler breeders. Appl. Anim. Behav. Sci. 92, 307–323.

Bilcik B., Estevez I. and Russek-Cohen E. 2005. Reproductive success of broiler breeders in natural mating systems: the effect of male-male competition, sperm quality, and morphological characteristics. Poult. Sci. 84, 1453–1462.

Bizeray D., Estevez I., Leterrier C. and Faure J.M. 2002. Influence of increased environmental complexity on leg condition, performance, and level of fearfulness in broilers. Poult. Sci. 81, 767–773.

Brake J. 1985. Comparison of two nesting materials for broiler breeders. Poult. Sci., 2263–2266.

Brake J. 1987. Influence of presence of perches during rearing on incidence of floor laying in broiler breeders. Poult. Sci. 66, 1587–1589.

Brake J. 1993. Influence of nest pad color on nest preference, percentage of floor eggs, and egg production of broiler breeder hens. Poult. Sci. 72, 1663.

Brake J. 1998. Equipment design for breeding flocks. Poult. Sci. 77, 1833–1841.

van den Brand H., Sosef M.P., Lourens A. and van Harn J. 2016. Effects of floor eggs on hatchability and later life performance in broiler chickens. Poult. Sci. 95, 1025–1032.

Brantsæter M., Nordgreen J., Rodenburg T.B., Tahamtani F.M., Popova A. and Janczak A.M. 2016. Exposure to increased environmental complexity during rearing reduces fearfulness and increases use of three-dimensional space in laying hens (Gallus gallus domesticus). Front. Vet. Sci. 3, 1–10.

Brillard J.P. 1993. Sperm storage and transport following natural mating and artificial insemination. Poult. Sci. 72, 923–928.

Buchwalder T. and Fröhlich E.K. 2011. Assessment of colony nests for laying hens in conjunction with the authorization procedure. Appl. Anim. Behav. Sci. 134, 64–71.

Burger J. 1982. An overview of proximate factors affecting reproductive success in colonial birds: concluding remarks and summary of panel discussion. Colon. Waterbirds 5, 58–65.

Campo J.L., Gil M.G. and Dávila S.G. 2007. Differences among white-, tinted-, and brown-egg laying hens for incidence of eggs laid on the floor and for oviposition time. Arch. fur Geflugelkd. 71, 105–109.

Clausen T. and Riber A.B. 2012. Effect of heterogeneity of nest boxes on occurrence of gregarious nesting in laying hens. Appl. Anim. Behav. Sci. 142, 168–175.

Cockrem J.F. 2007. Stress, corticosterone responses and avian personalities. J. Ornithol. 148, 169–178.

Collias N.E.. and Collias E.C. 1967. A field study of the red jungle fowl in North-Central India. Condor 69, 360–386.

Collias N. and Joos M. 1953. The spectrographic analysis of sound signals of the domestic fowl. Behaviour 5, 175–188.

Collias N. and Saichuae P. 1967. Ecology of the red jungle fowl in Thailand and Malaya with reference to the origin of domestication. Nat. Hist. Bull. Siam Soc 22, 189–209.

Cooper J.J. and Appleby M.C. 1995. Individual variation in the demand for a nest-box and the incidence of floor eggs. Appl. Anim. Behav. Sci. 44, 257–281.

Cooper J.J. and Appleby M.C. 1996. Demand for nest boxes in laying hens. Behav. Processes 36, 171–182.

Cooper J.J. and Appleby M.C. 2003. The value of environmental resources to domestic hens: A comparison of the work-rate for food and for nests as function of time. Anim. Welf. 12, 39–52.

Cox N.A., Berrang M.E. and Cason J.A. 2000. Salmonella penetration of egg shells and proliferation in broiler hatching eggs - a review. Poult. Sci. 79, 1571–1574.

Cronin G.M., Barnett the late J.L. and Hemsworth P.H. 2012. The importance of pre-laying behaviour and nest boxes for laying hen welfare: a review. Anim. Prod. Sci. 52, 398–405.

D'Eath R.B. and Keeling L.J. 2003. Social discrimination and aggression by laying hens in large groups: from peck orders to social tolerance. Appl. Anim. Behav. Sci. 84, 197–212.

Danbury T.C., Weeks C.A., Chambers J.P. and Kestin S.C. 2000. Self-selection of the analgesic drug carprofen by lame broiler chickens. Vet. Rec. 146, 307–311.

Davies J. 2019. Slow-growing birds are fast becoming mainstream. Poultry World. Retrieved on 16 December 2020, from *https://www.poultryworld.net/Meat/Articles/2019/7/Slow-growing-birds-are-fast-becoming-mainstream-454287E/*.

Dawkins M.S. 1983. Battery hens name their price: consumer demand theory and the measurement of ethological 'needs'. Anim. Behav. 31, 1195–1205.

Dawkins M.S., Donnelly C.A. and Jones T.A. 2004. Chicken welfare is influenced more by housing conditions than by stocking density. Nature 427, 342–344.

Dawkins M.S. and Layton R. 2012. Breeding for better welfare: Genetic goals for broiler chickens and their parents. Anim. Welf. 21, 147–155.

Decuypere E., Bruggeman V., Everaert N., Li Y., Boonen R., de Tavernier J., Janssens S. and Buys N. 2010. The broiler breeder paradox: ethical, genetic and physiological perspectives, and suggestions for solutions. Br. Poult. Sci. 51, 569–579.

Decuypere E., Hocking P.M., Tona K., Onagbesan O., Bruggeman V., Jones E.K.M., Cassy S., Rideau N., Metayer S., Jego Y., Putterflam J., Tesseraud S., Collin A., Duclos M., Trevidy J.J. and Williams J. 2006. Broiler breeder paradox: a project report. Worlds. Poult. Sci. J. 62, 443–453.

Dickens M. 1955. A statistical formula to quantify the “spread-of-participation” in group discussion. Speech Monogr. 22, 28–30.

Duncan I.J.H. 1991. Measuring preferences and the strength of preferences. Poult. Sci. 71, 658–663.

Duncan I.J.H., Hocking P.M. and Seawright E. 1990. Sexual behaviour and fertility in broiler breeder domestic fowl. Appl. Anim. Behav. Sci. 26, 201–213.

Duncan I. and Hughes B. 1988. The notion of ethological need, models of motivation and animal welfare. Anim. Behav. 36, 1696–1707.

Duncan I.J.H. and Kite V.G. 1989. Nest site selection and nest-building behaviour in domestic fowl. Anim. Behav. 37, 215–231.

Duncan I.J.H., Savory C.J. and Wood-Gush D.G.M. 1978. Observations on the reproductive behaviour of domestic fowl in the wild. Appl. Anim. Ethol. 4, 29–42.

Dunnington E.A. and Siegel P.B. 1985. Long-term selection for 8-week body weight in chickens - direct and correlated responses. Theor. Appl. Genet. 71, 305–313.

Dunnington E.A. and Siegel P.B. 1996. Long-term divergent selection for eight-week body weight in white Plymouth rock chickens. Poult. Sci. 75, 1168–79.

Ekstrand C., Carpenter T.E., Andersson I. and Algers B. 1998. Prevalence and control of foot-pad dermatitis in broilers in Sweden. Br. Poult. Sci. 39, 318–324.

Emmerson D. 2003. Breeding objectives and selection strategies for broiler production. In Poultry Genetics, Breeding, and Biotechnology (eds. W.M. Muir and S.E. Aggrey), pp. 113–126. CABI Publishing.

van Emous R.A. 2011. Innovatieve huisvesting voor vleeskuikenouderdieren: ‘Quality Time’ stal.

van Emous R.A., Holleman J. and van Schie T. 2020. Breeder signals. Roodbont Publishers B.V.

Estevez I., Andersen I.L. and Nævdal E. 2007. Group size, density and social dynamics in farm animals. Appl. Anim. Behav. Sci. 103, 185–204.

FAO 2020. Livestock Primary Data. Retrieved on 30 June 2020, from *http://www.fao.org/faostat/en/#data/QL*.

Fleming R.H., Whitehead C.C., Alvey D., Gregory N.G., Wilkins L.J., Whitehead C.C., Alvey D., Gregory N.G. and Wilkins L.J. 1994. Bone structure and breaking strength in laying hens housed in different husbandry systems. Br. Poult. Sci. 35, 651–662.

Fontana E.A., Weaver W.D. and Van Krey H.P. 1992. Intermittent periods of infertility identified in naturally mated broiler breeder hens. J. Appl. Poult. Res. 1, 190–193.

Forkman B., Boissy A., Meunier-Salaün M.C., Canali E. and Jones R.B. 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol. Behav. 92, 340–374.

Freire R., Appleby M.C. and Hughes B.O. 1996. Effects of nest quality and other cues for exploration on pre-laying behaviour. Appl. Anim. Behav. Sci. 48, 37–46.

Freire R., Appleby M. and Hughes B. 1997. Assessment of pre-laying motivation in the domestic hen using social interaction. Anim. Behav. 54, 313–319.

Freire R., Appleby M.C. and Hughes B.O. 1998. Effects of social interactions on pre-laying behaviour in hens. Appl. Anim. Behav. Sci. 56, 47–57.

Garner J.P., Falcone C., Wakenell P., Martin M. and Mench J.A. 2002. Reliability and validity of a modified gait scoring system and its use in assessing tibial dyschondroplasia in broilers. Br. Poult. Sci. 43, 355–363.

Gebhardt-Henrich S.G., Jordan A., Toscano M.J. and Wurbel H. 2020. The effect of perches and aviary tiers on the mating behaviour of two hybrids of broiler breeders. Appl. Anim. Behav. Sci.

Gebhardt-Henrich S.G., Pfulg A., Fröhlich E.K.F., Käppeli S., Guggisberg D., Liesegang A. and Stoffel M.H. 2017a. Limited associations between keel bone damage and bone properties measured with computer tomography, three-point bending test, and analysis of minerals in swiss laying hens. Front. Vet. Sci. 4.

Gebhardt-Henrich S.G., Toscano M.J. and Wurbel H. 2016. Perching behavior in broiler breeders. In Proceedings of the 50th Congress of the International Society of Applied Ethology.

Gebhardt-Henrich S.G., Toscano M.J. and Würbel H. 2017b. Perch use by broiler breeders and its implication on health and production. Poult. Sci. 96, 3539–3549.

Goodarzi M.O., Dumesic D.A., Chazenbalk G. and Azziz R. 2011. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nat. Rev. Endocrinol. 7, 219–231.

Graumans K. 2020. Meer grondeieren bij langzamergroeiend ouderdier. Boerderij. Retrieved on 10 July 2020, from *https://www.boerderij.nl/Pluimveehouderij/Nieuws/2020/7/Meer-grondeieren-bij-langzamergroeiend-ouderdier-609614E/*.

Greene J.A., Mccracken R.M. and Evans R.T. 1985. A contact dermatitis of broilers - clinical and pathological findings. Avian Pathol. 14, 23–38.

Gwinner H. and Berger S. 2008. Starling males select green nest material by olfaction using experience-independent and experience-dependent cues. Anim. Behav. 75, 971–976.

van der Haar J.W. 1995. Legnesttype en stalinrichting bij vleeskuikenouderdieren. Prakt. Puimveehouderij 29.

de Haas E.N., Bolhuis J.E., de Jong I.C., Kemp B., Janczak A.M. and Rodenburg T.B. 2014. Predicting feather damage in laying hens during the laying period. Is it the past or is it the present? Appl. Anim. Behav. Sci. 160, 75–85.

de Haas E.N., Lee C., Hernandez C.E., Naguib M. and Rodenburg T.B. 2017. Individual differences in personality in laying hens are related to learning a colour cue association. Behav. Processes 134, 37–42.

Hartcher K.M. and Jones B. 2017. The welfare of layer hens in cage and cage-free housing systems. Worlds. Poult. Sci. J. 73, 767–781.

Haslam S.M., Knowles T.G., Brown S.N., Wilkins L.J., Kestin S.C., Warriss P.D. and Nicol C.J. 2007. Factors affecting the prevalence of foot pad dermatitis, hock burn and breast burn in broiler chicken. Br. Poult. Sci. 48, 264–275.

Heck A., Onagbesan O., Tona K., Metayer S., Putterflam J., Jego Y., Trevidy J.J., Decuypere E., Williams J., Picard M. and Bruggeman V. 2004. Effects of ad libitum feeding on performance of different strains of broiler breeders. Br. Poult. Sci. 45, 695–703.

Heinrich A., Icken W., Thurner S., Wendl G., Bernhardt H. and Preisinger R. 2014. Nesting behaviour - a comparison of single nest boxes and family nests. Eur. Poult. Sci. 78.

Hermans P.G., Fradkin D., Muchnik I.B. and Morgan K.L. 2006. Prevalence of wet litter and the associated risk factors in broiler flocks in the United Kingdom. Vet. Rec. 158, 615–622.

Hester P.Y. 1994. The role of environment and management on leg abnormalities in meat-type fowl. Poult. Sci. 73, 904–915.

Hocking P.M., Maxwell M.H. and Mitchell M.A. 1993. Welfare assessment of broiler breeder and layer females subjected to food restriction and limited access to water during rearing. Br. Poult. Sci. 34, 443–458.

Holcman A., Malovrh Š. and Štuhec I. 2007. Choice of nest types by hens of three lines of broiler breeders. Br. Poult. Sci. 48, 284–290.

Hughes B.O. 1983. Headshaking stimuli in fowls: the effect of environmental. Appl. Anim. Ethol. 11, 45–53.

Hunniford M.E. and Widowski T.M. 2018. Curtained nests facilitate settled nesting behaviour of laying hens in furnished cages. Appl. Anim. Behav. Sci. 202, 39–45.

Jambui M., Honaker C.F. and Siegel P.B. 2017. Correlated responses to long-term divergent selection for 8-week body weight in female White Plymouth Rock chickens: sexual maturity. Poult. Sci. 96, 3844–3851.

Jones R.B. and Roper T.J. 1997. Olfaction in the domestic fowl: a critical review. Physiol. Behav. 62, 1009–1018.

de Jong I.C. and Guémené D. 2011. Major welfare issues in broiler breeders. Worlds. Poult. Sci. J. 67, 73–82.

de Jong I.C., van Harn J., Gunnink H., Hindle V.A. and Lourens A. 2012. Footpad dermatitis in Dutch broiler flocks: prevalence and factors of influence. Poult. Sci. 91, 1569–74.

de Jong I.C., van Harn J., Koene P., Ellen H., van Emous R.A., Rommers J.M. and van den Brand H. 2016. Risicobeoordeling waterverstrekking aan vleeskuikens en vleeskuikenouderdieren.

de Jong I.C., Lourens A., Gunnink H., Workel L. and van Emous R. 2011. Effect van bezettingsdichtheid op (de ontwikkeling van) het paargedrag en de technische resultaten bij vleeskuikenouderdieren.

Kang H.K., Park S.B., Kim S.H. and Kim C.H. 2016. Effects of stock density on the laying performance, blood parameter, corticosterone, litter quality, gas emission and bone mineral density of laying hens in floor pens. Poult. Sci. 95, 2764–2770.

Kaukonen E., Norring M. and Valros A. 2016. Effect of litter quality on foot pad dermatitis, hock burns and breast blisters in broiler breeders during the production period. Avian Pathol. 9457, 1–15.

Keeling L.J., Estevez I., Newberry R.C. and Correia M.G. 2003. Production-related traits of layers reared in different sized flocks: the concept of problematic intermediate group sizes. Poult. Sci. 82, 1393–1396.

Keer-Keer S., Hughes B.O., Hocking P.M. and Jones R.B. 1996. Behavioural comparison of layer and broiler fowl: measuring fear responses. Appl. Anim. Behav. Sci. 49, 321–333.

Keppler C. and Knierim U. 2017. MTool Managementtool © - Beurteilungskarten Legehennen. Retrieved on 7 May 2020, from *https://www.mud-tierschutz.de/mud-tierschutz/beratungsinitiativen/etablierung-eines-managementtools-bei-legehennen/mtool-fuer-jung-und-legehennen/*.

Kestin S.C., Su G. and Sørensen P. 1999. Different commercial broiler crosses have different susceptibilities to leg weakness. Poult. Sci. 78, 1085–1090.

Kirkden R.D. and Pajor E.A. 2006. Using preference, motivation and aversion tests to ask scientific questions about animals' feelings. Appl. Anim. Behav. Sci. 100, 29–47.

Kjaer J.B., Su G., Nielsen B.L. and Sørensen P. 2006. Foot pad dermatitis and hock burn in broiler chickens and degree of inheritance. Poult. Sci. 85, 1342–1348.

Kjaer J.B. and Vestergaard K.S. 1999. Development of feather pecking in relation to light intensity. Appl. Anim. Behav. Sci. 62, 243–254.

Kocaman B., Esenbuga N., Yildiz A., Laçin E. and Macit M. 2006. Effect of environmental conditions in poultry houses on the performance of laying hens. Int. J. Poult. Sci. 5, 26–30.

Koo T.K. and Li M.Y. 2016. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J. Chiropr. Med. 15, 155–163.

Kratzer D.D. and Craig J. V. 1980. Mating behavior of cockerels: effects of social status, group size and group density. Appl. Anim. Ethol. 6, 49–62.

Kruschwitz A., Zupan M., Buchwalder T. and Huber-Eicher B. 2008. Nest preference of laying hens (Gallus gallus domesticus) and their motivation to exert themselves to gain nest access. Appl. Anim. Behav. Sci. 112, 321–330.

Lay D.C., Fulton R.M., Hester P.Y., Karcher D.M., Kjaer J.B., Mench J.A., Mullens B.A., Newberry R.C., Nicol C.J., O'Sullivan N.P. and Porter R.E. 2011. Hen welfare in different housing systems. Poult. Sci. 90, 278–294.

Leeson S. and Summers J. 2000. Broiler breeder production. Nottingham University Press, Nottingham.

Leeson S. and Walsh T. 2010. Feathering in commercial poultry II. Factors influencing feather growth and feather loss. Worlds. Poult. Sci. J. 60, 52–63.

Lentfer T.L., Gebhardt-Henrich S.G., Fröhlich E.K.F. and von Borell E. 2011. Influence of nest site on the behaviour of laying hens. Appl. Anim. Behav. Sci. 135, 70–77.

Leone E.H. and Estévez I. 2008. Economic and welfare benefits of environmental enrichment for broiler breeders. Poult. Sci. 87, 14–21.

Lewis P.D., Danisman R. and Gous R.M. 2008. Illuminance, sexual maturation, and early egg production in female broiler breeders. Br. Poult. Sci. 49, 649–653.

Lewis P.D., Danisman R. and Gous R.M. 2010. Photoperiods for broiler breeder females during the laying period. Poult. Sci. 89, 108–14.

Lewis P.D. and Gous R.M. 2006. Constant and changing photoperiods in the laying period for broiler breeders allows normal or accelerated growth during the rearing period. Poult. Sci. 85, 321–325.

Lien R.J., Hess J.B., Conner D.E., Wood C.W. and Shelby R.A. 1998. Peanut hulls as a litter source for broiler breeder replacement pullets. Poult. Sci. 77, 41–46.

Lin S.J., Wideliz R.B., Yue Z., Li A., Wu X., Jiang T.X., Wu P. and Chuong C.M. 2013. Feather regeneration as a model for organogenesis. Dev. Growth Differ. 55, 139–148.

Lindholm C., Johansson A., Middelkoop A., Lees J.J., Yngwe N., Berndtson E., Cooper G. and Altimiras J. 2018. The quest for welfare-friendly feeding of broiler breeders: effects of daily vs. 5:2 feed restriction schedules. Poult. Sci. 97, 368–377.

van Loon M. 2020a. Met verlaagde beun minder kans op grondeieren. Pluimveeweb. Retrieved on 10 July 2020, from *https://www.pluimveeweb.nl/artikel/257169-met-verlaagde-beun-minder-kans-op-grondeieren/*.

van Loon M. 2020b. In toekomst mogelijk korting bij voetzoollaesies. Pluimveeweb. Retrieved on 21 October 2020, from *https://www.pluimveeweb.nl/artikel/365444-in-toekomst-mogelijk-korting-bij-voetzoollaesies/*.

Maclnnis D.J. and Jaworski B.J. 1989. Information processing from advertisements: toward an integrative framework. J. Mark. 53, 1–23.

Magdelaine P., Spiess M.P. and Valceschini E. 2008. Poultry meat consumption trends in Europe. Worlds. Poult. Sci. J. 64, 53–63.

Maguire R.O., Plumstead P.W. and Brake J. 2006. Impact of diet, moisture, location, and storage on soluble phosphorus in broiler breeder manure. J. Environ. Qual. 35, 858.

Martland M.F. 1985. Ulcerative dermatitis in broiler chickens: the effects of wet litter.

Avian Pathol. 14, 353–364.

Mason G.J. 1991. Stereotypies: a critical review. Anim. Behav. 41, 1015–1037.

McGary S., Estevez I. and Russek-Cohen E. 2003. Reproductive and aggressive behavior in male broiler breeders with varying fertility levels. Appl. Anim. Behav. Sci. 82, 29–44.

McGibbon W.H. 1976. Floor laying—a heritable and environmentally influenced trait of the domestic fowl. Poult. Sci. 55, 765–771.

Meijsser F.M. and Hughes B.O. 1989. Comparative analysis of pre-laying behaviour in battery cages and in three alternative systems. Br. Poult. Sci. 30, 747–760.

Mench J.A. 2002. Broiler breeders: feed restriction and welfare. Worlds. Poult. Sci. J. 58, 23–29.

Millman S.T., Duncan I.J. and Widowski T.M. 2000. Male broiler breeder fowl display high levels of aggression toward females. Poult. Sci. 79, 1233–1241.

Moyle J.R., Yoho D.E., Harper R.S. and Bramwell R.K. 2010. Mating behavior in commercial broiler breeders: female effects. J. Appl. Poult. Res. 19, 24–29.

National Chicken Council 2020. U.S. Broiler performance. Retrieved on 9 April 2020, from *http://www.nationalchickencouncil.org/about-the-industry/statistics/u-s-broiler-performance/*.

Newcombe M., Fitzsimmons R.C. and Decolongon J. 1991. Effect of floor type and energy source on growth and productivity of broiler breeders. Poult Sci 70, 2246–2252.

Nicol C.J., Gregory N.G., Knowles T.G., Parkman I.D. and Wilkins L.J. 1999. Differential effects of increased stocking density, mediated by increased flock size, on feather pecking and aggression in laying hens. Appl. Anim. Behav. Sci. 65, 137–152.

Ochs D., Wolf C.A., Widmar N.J. and Bir C. 2019. Is there a cage-free lunch in U.S. Egg production? Public views of laying-hen housing attributes. J. Agric. Resour. Econ. 44, 345–361.

OECD/FAO 2019. OECD-FAO Agricultural Outlook 2019-2028.

Onagbesan O.M., Vleugels B., Buys N., Bruggeman V., Safi M. and Decuypere E. 1999. Insulin-like growth factors in the regulation of avian ovarian functions. Domest. Anim. Endocrinol. 17, 299–313.

Osczevski R.J. 1995. The basis of wind chill. Arctic 48, 372–382.

Perry G.C., Charles D.R., Day P.J., Hartland J.R. and Spencer P.G. 1971. Egg-laying behaviour in a broiler parent flock. Worlds. Poult. Sci. J. 27, 162.

Pitsillides A.A., Rawlinson S.C.F., Mosley J.R. and Lanyon L.E. 1999. Bone's early responses to mechanical loading differ in distinct genetic strains of chick: selection for enhanced growth reduces skeletal adaptability. J. Bone Miner. Res. 14, 980–987.

Pizzari T. and Birkhead T.R. 2001. For whom does the hen cackle? The function of postoviposition cackling. Anim. Behav. 61, 601–607.

Prescott N.B. and Wathes C.M. 1999. Spectral sensitivity of the domestic fowl (Gallus g. domesticus). Br. Poult. Sci. 40, 332–339.

Puterflam J., Merlet F., Faure J.M., Hocking P.M. and Picard M. 2006. Effects of genotype and feed restriction on the time-budgets of broiler breeders at different ages. Appl. Anim. Behav. Sci. 98, 100–113.

Rath N.C., Huff G.R., Huff W.E. and Balog J.M. 2000. Factors regulating bone maturity and strength in poultry. Poult. Sci. 79, 1024–1032.

Reddy P.R.K. and Siegel P.B. 1977. Chromosomal abnormalities in chickens selected for high and low body weight. J. Hered. 68, 253–256.

Rendell L., Fogarty L., Hoppitt W.J.E., Morgan T.J.H., Webster M.M. and Laland K.N. 2011. Cognitive culture: Theoretical and empirical insights into social learning strategies. Trends Cogn. Sci. 15, 68–76.

Renema R.A. and Robinson F.E. 2005. Defining normal: comparison of feed restriction and full feeding of female broiler breeders. Worlds. Poult. Sci. J. 60, 508–522.

Renema R.A., Robinson F.E., Beliveau R.M., Davis H.C. and Lindquist E.A. 2007. Relationships of body weight, feathering, and footpad condition with reproductive and carcass morphology of end-of-season commercial broiler breeder hens. J. Appl. Poult. Res. 16, 27–38.

Riber A.B. 2010. Development with age of nest box use and gregarious nesting in laying hens. Appl. Anim. Behav. Sci. 123, 24–31.

Riber A.B. 2012. Gregarious nesting - an anti-predator response in laying hens. Appl. Anim. Behav. Sci. 138, 70–78.

Riber A.B. 2017. Alternatives to mutilation of the outermost joint of the backward-facing toe in broiler breeder males.

Riber A.B., de Jong I.C., van de Weerd H.A. and Steenfeldt S. 2017. Environmental enrichment for broiler breeders: an undeveloped field. Front. Vet. Sci. 4.

Riber A.B. and Nielsen B.L. 2013. Changes in position and quality of preferred nest box: effects on nest box use by laying hens. Appl. Anim. Behav. Sci. 148, 185–191.

Rietveld-Piepers B., Blokhuis H.J. and Wiepkema P.R. 1985. Egg-laying behaviour and nest-site selection of domestic hens kept in small floor-pens. Appl. Anim. Behav. Sci. 14, 75–88.

Rijkdienst voor Ondernemend Nederland 2014. Voetzoollaesies bij vleeskuikens. Retrieved on 21 October 2020, from *https://www.rvo.nl/onderwerpen/agrarisch-ondernemen/dieren-houden/dierenwelzijn/regels-voor-huisvesting-en-verzorging/vleeskuikens/aanvullende-eisen-categorie-2-en-3*.

Ringgenberg N., Fröhlich E.K.F., Harlander-Matauschek A., Toscano M.J., Würbel H. and Roth B.A. 2015a. Nest choice in laying hens: effects of nest partitions and social status. Appl. Anim. Behav. Sci. 169, 43–50.

Ringgenberg N., Fröhlich E.K.F., Harlander-Matauschek A., Toscano M.J., Würbel H. and

Roth B.A. 2015b. Effects of variation in nest curtain design on pre-laying behaviour of domestic hens. Appl. Anim. Behav. Sci. 170, 34–43.

Ringgenberg N., Fröhlich E.K.F., Harlander-Matauschek A., Würbel H. and Roth B.A. 2014. Does nest size matter to laying hens? Appl. Anim. Behav. Sci. 155, 66–73.

Robinson F.E., Wilson J.L., Yu M.W., Fasenko G.M. and Hardin R.T. 1993. The relationship between body weight and reproductive efficiency in meat-type chickens. Poult. Sci. 72, 912–922.

Romanov M.N., Talbot R.T., Wilson P.W. and Sharp P.J. 2002. Genetic control of incubation behavior in the domestic hen. Poult. Sci. 81, 928–931.

Sander J.E., Wilson J.L., Cheng I.H. and Gibbs P.S. 2003. Influence of slat material on hatching egg sanitation and slat disinfection. J. Appl. Poult. Res. 12, 74–80.

Sanotra G.S.C., Berg D. and Lund J. 2003. A comparison between leg problems in Danish and Swedish broiler production. Anim. Welf. 12, 677–683.

Savory C., Maros K. and Rutter S. 1993. Assessment of hunger in growing broiler breeders in relation to a commercial restricted feeding programme. Anim. Welf. 2, 131–152.

Schakel L. 2015. The chamber of secrets: nest design of individual trap nest boxes in a group housing system of laying hens. Wageningen University.

Shepherd E.M. and Fairchild B.D. 2010. Footpad dermatitis in poultry. Poult. Sci. 89, 2043–2051.

Sheppard K.C. and Duncan I.J.H. 2011. Feeding motivation on the incidence of floor eggs and extraneously calcified eggs laid by broiler breeder hens. Br. Poult. Sci. 52, 20–29.

Sherlock L., Demmers T.G.M., Goodship A.E., McCarthy I.D. and Wathes C.M. 2010. The relationship between physical activity and leg health in the broiler chicken. Br. Poult. Sci. 51, 22–30.

Shi H., Li B., Tong Q. and Zheng W. 2019. Effects of different claw-shortening devices on claw condition, fear, stress, and feather coverage of layer breeders. Poult. Sci. 98, 3103–3113.

Singh R., Cheng K.M. and Silversides F.G. 2009. Production performance and egg quality of four strains of laying hens kept in conventional cages and floor pens. Poult. Sci. 88, 256–264.

Stämpfli K., Buchwalder T., Fröhlich E.K.F. and Roth B.A. 2012. Influence of front curtain design on nest choice by laying hens. Br. Poult. Sci. 53, 553–560.

Stämpfli K., Roth B.A., Buchwalder T. and Fröhlich E.K.F. 2011. Influence of nest-floor slope on the nest choice of laying hens. Appl. Anim. Behav. Sci. 135, 286–292.

Struelens E., Van Nuffel A., Tuyttens F.A.M., Audoorn L., Vranken E., Zoons J., Berckmans D., Ödberg F., Van Dongen S. and Sonck B. 2008. Influence of nest seclusion and nesting material on pre-laying behaviour of laying hens. Appl. Anim. Behav. Sci. 112, 106–119.

Tahamtani F.M., Hinrichsen L.K. and Riber A.B. 2018. Laying hens performing gregarious nesting show less pacing behaviour during the pre-laying period. Appl. Anim. Behav. Sci. 202, 46–52.

Tahamtani F.M. and Riber A.B. 2020. The effect of qualitative feed restriction in broiler breeder pullets on fear and motivation to explore. Appl. Anim. Behav. Sci. 228, 105009.

Tallentire C.W., Leinonen I. and Kyriazakis I. 2016. Breeding for efficiency in the broiler chicken: A review. Agron. Sustain. Dev. 36.

Väisänen J. and Jensen P. 2003. Social versus exploration and foraging motivation in young red junglefowl (Gallus gallus) and White Leghorn layers. Appl. Anim. Behav. Sci. 84, 139–158.

Vivaldi G., Silversides F.G., Boily R., Villeneuve P. and Joncas R. 1996. Effects of chopped sinusoidal voltages on the behavior and performance of laying hens. Can. Agric. Eng. 38, 99–105.

Waddington D., Hocking P.M., Walker M.A. and Gilbert A.B. 1989. Control of the development of the ovarian follicular hierarchy in broiler breeder pullets by food restriction during rearing. Br. Poult. Sci. 30, 161–173.

Wageningen UR Livestock Research 2020. KWIN 2020-2021.

Walzem R.L. and Chen S. ei 2014. Obesity-induced dysfunctions in female reproduction: lessons from birds and mammals. Adv. Nutr. 5, 199–206.

Weaver W.D. and Meijerhof R. 1991. The effect of different levels of relative humidity and air movement on litter conditions, ammonia levels, growth, and carcass quality for broiler chickens. Poult. Sci. 70, 746–755.

Welfare Quality® 2009. Welfare Quality® assessment protocol for poultry (broilers, laying hens). Welfare Quality® Consortium, Lelystad, the Netherlands.

Whyte R.T. 1993. Aerial pollutants and the health of poultry farmers. Worlds. Poult. Sci. J. 49, 139–156.

Wilkins L.J., McKinstry J.L., Avery N.C., Knowles T.G., Brown S.N., Tarlton J. and Nicol C.J. 2011. Influence of housing system and design on bone strength and keel bone fractures in laying hens. Vet. Rec. 169, 414.

Williams B.A. 1992. Inverse relations between preference and contrast. J. Exp. Anal. Behav. 58, 303–312.

Wilson W. 1964. Photocontrol of oviposition in gallinaceous birds. Ann. N. Y. Acad. Sci. 117, 194–202.

Wolanski N.J., Renema R.A., Robinson F.E. and Wilson J.L. 2004. End-of-season carcass and reproductive traits in original and replacement male broiler breeders. J. Appl. Poult. Res. 13, 451–460.

Worley J.W. and Wilson J.L. 2000. Effects of stray voltage on laying habits of broiler breeders. Am. Soc. Agric. Eng. 16, 723–729.

Xie Y.L., Pan Y.E., Chang C.J., Tang P.C., Huang Y.F., Walzem R.L. and Chen S.E. 2012.

Palmitic acid in chicken granulosa cell death-lipotoxic mechanisms mediate reproductive inefficacy of broiler breeder hens. Theriogenology 78, 1917–1928.

Yue S. and Duncan I.J.H. 2003. Frustrated nesting behaviour: relation to extra-cuticular shell calcium and bone strength in White Leghorn hens. Br. Poult. Sci. 44, 175–181.

Zimmerman P.H., Koene P. and Van Hooff J.A.R.A.M. 2000. Thwarting of behaviour in different contexts and the gakel-call in the laying hen. Appl. Anim. Behav. Sci. 69, 255–264.

Zuidhof M.J., Renema R.A. and Robinson F.E. 2007. Reproductive efficiency and metabolism of female broiler breeders as affected by genotype, feed allocation, and age at photostimulation. 3. Reproductive efficiency. Poult. Sci. 86, 2278–2286.

Zupan M., Kruschwitz A., Buchwalder T., Huber-Eicher B. and Ŝtuhec I. 2008. Comparison of the prelaying behavior of nest layers and litter layers. Poult. Sci. 87, 399–404.

Summary

Broiler breeders have a different genetic background from laying hens, since they have been selected for fast growth of their offspring. Selection for fast growth negatively affects the reproductive system, which is apparent from the later onset of egg production and a higher number of defective eggs in broiler breeders compared to laying hens. The behaviour of broiler breeders also seems affected by the genetic selection for fast growth, since they tend to lay more eggs outside the provided nests (so called floor eggs) compared to laying hens. Floor eggs are a sign of reduced welfare, as the broiler breeder hens are not willing or able to visit the nests whilst chickens are known to be highly motivated to lay their eggs in nests. Moreover, the collection of floor eggs is time consuming and floor eggs have a lower saleability and hatchability than clean nest eggs. Many factors have been suggested to be involved in floor laying behaviour, ranging from intrinsic individual characteristics to management factors and housing factors. This study aimed to expand and update our knowledge on the factors involved in nesting behaviour of broiler breeders. The experimental work was structured around the Motivation-Ability-Opportunity (MAO) model, which states that the motivation to perform a certain behaviour is modified by the ability and opportunity to perform it. The behaviour studied in this thesis is laying an egg in the nest.

Motivation

The motivation of a broiler breeder hen to lay an egg in the nest is likely dependent on nest design. In Chapter 2, we provided groups of broiler breeder hens with four different nest designs: a plastic control nest, a plastic partition nest with a low wall in the middle of the nest floor dividing the nest in two smaller areas, a plastic ventilator nest with a ventilator underneath the nest creating an air flow inside the nest and a wooden nest with wooden instead of plastic walls. The hens had a clear preference for the wooden nests with 69% of the eggs laid in these nests, followed by the control nest (15%), the partition nest (10%) and lastly the ventilator nest (5%). This preference was confirmed in another preference test with plastic and wooden nests described in Chapter 5, where 63% of all eggs were laid in the wooden nests. The behaviour in the wooden nest was more settled, as the hens spend more time sitting instead of walking and required fewer nest inspections and visits per egg. However, the strong preference for the wooden nest caused crowding, leading to a high

frequency of piling, pecking and head shaking. Since the hens continued to prefer the wooden nests, apparently they were willing to pay this price to lay an egg in the wooden nest. The reasons behind the preference for wooden nests remain unknown, although we did find differences in air temperature and electrostatic properties between the plastic and wooden nests. The low preference for the ventilator nest suggests that hens find draughts unpleasant for egg laying, emphasizing the importance of good climate control in poultry houses to avoid floor eggs.

After the preference for wooden nests was stable for six weeks, these nests were closed at 32 weeks of age and the hens were forced to choose a new nest to lay their eggs (Chapter 2). The order of preference of the other nest designs remained intact after closing the wooden nests, but the nests directly next to the wooden nests were more preferred than the nests further away. These results confirm that broiler breeders are conservative in their nesting location. We also analysed data from 102 commercial flocks in the Netherlands from the period of 2014-2019 and found a mild positive correlation between the mean floor egg percentage until 27 weeks of age and the mean floor egg percentage during the rest of the production period (Chapter 6). So if many hens lay floor eggs at an early age, this might persist for the entire production period.

Ability

If the hens are motivated to lay an egg in the nest, they must be physically able to reach the nest. Leg health problems, including foot pad dermatitis and lameness, or a high body weight might limit this ability. Foot pad dermatitis, i.e. an acute inflammation of the skin, is considered to be painful and can lead to lameness of chickens. As described in Chapter 3, foot pad dermatitis is a major issue for broiler breeders housed in commercial floor systems with a partially slatted area. The incidence of foot pad dermatitis ranged from 78-95% of the birds and 8-35% had severe foot pad dermatitis between the ages of 32-60 weeks. Poor litter quality was correlated to higher levels of foot pad dermatitis, so attention should be given to housing features (climate control, perches, slats) or management practices (water provision) that could help to maintain a good litter quality. Severe hock burns and gait deviations were observed much less, with maximum 0.5% and 2.7% of broiler breeder hens affected respectively.

The incidence and severity of foot pad dermatitis, hock burn or gait deviations did not affect the percentage of floor eggs, so it seems that these painful conditions are not limiting the ability of the hens to get to the nest or are overruled by the motivation to lay an egg in the nest. Nests are provided on raised slatted areas in most regions of the world, which requires the hens to jump up to the slats before being able to lay an egg in the nest. Although this was expected to be more difficult with a higher body weight, we found no correlation between floor egg percentage and body weight in Chapters 3 and 5. Furthermore, pens with nests on a raised slatted area had a lower percentage of floor eggs compared to pens with nests directly accessible from the litter (Chapter 5). On the basis of our results, it seems unlikely that floor laying behaviour is caused by leg health problems. While genetic lines differ in their body weight goals and maximum egg production, we found that even within line the negative correlation between body weight and egg production is present (Chapter 3). A higher body weight was correlated to a higher mortality rate, showcasing the health risks of a high body weight.

Opportunity

Since reduced leg health does not seem to limit ability of a broiler breeder hen to reach the nest, it was studied whether flock mates hamper the opportunity to enter the nest. Gregarious nesting, where a hen prefers a nest with other hens present over an empty nest, can lead to insufficient nest space. We found that broiler breeders of three genetic lines divided themselves equally over the nests, while two other lines laid 2-5 times more eggs in the nests at the end of the row compared to the middle nests (Chapter 4). A more uneven distribution over the nests was correlated to a higher percentage of floor eggs, so excessive gregarious nesting seems to contribute to floor laying. Distribution over the nests was more uneven at earlier ages, suggesting that inexperience might add to gregarious nesting. We also studied fear of humans, general fearfulness and tendency to cluster, but we did not find evidence for a correlation with the occurrence of gregarious nesting.

Broiler breeders hens are housed with 7-10% males to ensure good fertilisation of the eggs. These males are known to be aggressive in their mating behaviour, contributing to feather loss and wounding of hens. During our study, over 30% of females had wounds at a certain point during the production period and the majority of females had severe feather damage from 40 weeks of age onwards (Chapter 4). Mating mainly happens in the litter area and we found that an increased proportion of wounded hens was correlated to a lower use of the litter area, which suggests that wounded hens try to avoid males. An increased proportion of wounded hens was also correlated to fewer floor eggs, which could be indirectly caused by a lower use of the litter area since floor eggs are mostly laid in the litter area. Males spend less time on slatted areas, so the effects of having a slatted area in front of the nest on nesting behaviour were studied in Chapter 5. Pens with nests directly on the litter had more floor eggs than pens with nests with a raised slatted area in front of it, but due to technical difficulties it was not possible to see whether this was due to male interference near the nest. A larger litter area also leads to an increased frequency of general mating activity (i.e. copulations and unsuccessful attempts combined), but not of the number of copulations. We observed on average 40 copulations per day in pens with 33 hens, so the hens were likely inseminated daily. Chickens can lay fertilised eggs for at least 10 days after insemination, so perhaps the current advised proportion of males could be lowered without compromising egg fertilisation.

Conclusions & recommendations

Broiler breeders have a clear preference for wooden nests compared to plastic nests, although the exact causes behind this preference remain unknown and should be studied further. Moreover, hens choose their nest site location during the first weeks of egg production and are unlikely to alter this location. The prevention of floor eggs should thus get extra attention during the start of the egg production. Foot pad dermatitis was found to be a common problem in broiler breeders and more attention should be given to improve litter quality as a means to reduce the occurrence of this condition. However, no relationship was found between leg health and of floor egg percentage. Excessive gregarious nesting seems to be partially caused by the genetic background of broiler breeders and can contribute to floor laying behaviour. Mating activity is dependent on the size of the litter area and is more frequent than necessary for fertilisation of the eggs. The optimal proportion of males in a flock should be investigated, to reduce floor eggs as well as stress and damage of hens caused by mating behaviour without compromising fertilisation rates. This thesis only studied fast-growing breeders, but future research should evaluate the welfare benefits and possible risks of slow-growing broiler breeders. Lastly, more research should be conducted on commercial scaled flocks in collaboration with farmers to translate scientific knowledge into practical advice. To conclude, nest design influences the motivation of broiler breeders to lay an egg in the nest, but the opportunity to visit the nest might be constrained by excessive gregarious nesting and male behaviour. Leg health problems do not seem to limit the ability to reach the nest or are overruled by the high motivation to get to the nest.

Samenvatting

Vleeskuikenouderdieren hebben een andere genetische achtergrond dan leghennen, omdat ze zijn geselecteerd voor een snelle groei van hun nageslacht. Selectie voor snelle groei heeft negatieve gevolgen voor het voortplantingssysteem, wat te zien is aan een latere start van eiproductie en een groter aantal afwijkende eieren bij vleeskuikenouderdieren in vergelijking met leghennen. Het gedrag van vleeskuikenouderdieren lijkt ook beïnvloed te zijn door deze selectie voor snelle groei, omdat ze de neiging hebben om meer eieren buiten het nest (zogenaamde grondeieren) te leggen. Grondeieren wijzen op een verminderd welzijn, omdat vleeskuikenmoederdieren niet gemotiveerd of in staat zijn de nesten te bezoeken terwijl het bekend is dat kippen zeer gemotiveerd zijn om eieren in het nest te leggen. Daarnaast kost het verzamelen van grondeieren veel tijd, brengen grondeieren weinig geld op en is de uitkomst van deze eieren lager. Veel factoren zijn in verband gebracht met het veroorzaken van grondeieren, variërend van intrinsieke individuele eigenschappen tot management- en huisvestingsfactoren. Het doel van deze studie was het updaten en uitbreiden van onze kennis over de factoren die het nestgedrag van vleeskuikenouderdieren beïnvloeden. De experimenten waren gestructureerd aan de hand van het Motivation-Ability-Opportunity (MAO; motivatie-bekwaamheid-gelegenheid) model, dat beschrijft dat de motivatie om een gedrag uit te voeren onder invloed staat van de bekwaamheid en gelegenheid om het uit te voeren. Het gedrag dat in deze thesis is bestudeerd is het leggen van een ei in het nest.

Motivatie

De motivatie van een vleeskuikenmoederdier om een ei in het nest te leggen is waarschijnlijk afhankelijk van het nestontwerp. In Hoofdstuk 2 waren vier nestontwerpen aan groepen vleeskuikenouderdieren aangeboden: een plastic nest, een plastic nest met een lage scheidingswand in het midden van de nestmat die de nestmat in twee kleinere delen verdeelt, een plastic nest met een ventilator onder het nest dat een luchtstroming in het nest creëert en een houten nest met houten in plaats van plastic wanden. De hennen hadden een duidelijke voorkeur voor het houten nest waar 69% van de eieren gelegd werd, gevolgd door het controle nest (15%), het nest met de scheidingswand (10%) en als laatste het ventilator nest (5%). Deze voorkeur werd bevestigd in een andere voorkeurstest met plastic en houten nesten, waar 63% van alle eieren

in de houten nesten gelegd werden (Hoofdstuk 5). Het gedrag in de houten nesten was meer gesetteld, omdat de hennen meer tijd zittend dan lopend doorbrachten en minder nestinspecties en -bezoeken per ei nodig hadden. Echter zorgde de sterke voorkeur voor de houten nesten voor drukte, wat leidde tot meer stapelen, pikken en kopschudden. De voorkeur voor de houten nesten bleef constant met de tijd, dus blijkbaar waren de hennen bereid om deze prijs te betalen om een ei in het houten nest te leggen. Wat de voorkeur voor het houten nest veroorzaakt is onbekend, hoewel we verschillen vonden in de luchttemperatuur en de elektrostatische eigenschappen van de plastic en houten nesten. De ventilator nesten hadden de minste voorkeur, wat suggereert dat hennen de luchtstroom onaangenaam vinden tijdens het leggen van een ei en dit benadrukt het belang van goede klimaatcontrole in de pluimveestal om grondeieren te voorkomen.
Toen de voorkeur voor de houten nesten zes weken stabiel, zijn deze nesten afgesloten op 32 weken leeftijd en werden de hennen gedwongen om een nieuw nest te kiezen (Hoofdstuk 2). De volgorde van voorkeur voor de andere nestontwerpen bleef intact, maar de nesten die direct naast het houten nest hadden meer de voorkeur dan de nesten verder weg. Dit resultaat bevestigt dat vleeskuikenouderdieren conservatief zijn in hun nestlocatie. We hebben data van 102 commerciële koppels geanalyseerd die gedurende 2014-2019 in Nederland gehouden zijn. Hierbij vonden we een milde positieve correlatie tussen het percentage grondeieren tot 27 weken leeftijd en het percentage grondeieren vanaf 27 weken leeftijd tot het eind van de productieperiode (Hoofdstuk 6). Dus als veel hennen op vroege leeftijd grondeieren leggen, dan kan dit aanhouden gedurende de rest van de productieperiode.

Bekwaamheid
Als de hennen gemotiveerd zijn om een ei in het nest te leggen, dan moeten ze fysiek bekwaam zijn om het nest te bereiken. Pootproblemen, inclusief voetzoollaesies en kreupelheid, of een hoog lichaamsgewicht kan de bekwaamheid van de hennen verminderen. Voetzoollaesies, i.e. een acute huidontsteking, worden pijnlijk beschouwd en kunnen leiden tot kreupelheid bij kippen. Zoals beschreven in Hoofdstuk 3, zijn voetzoollaesies een groot probleem bij vleeskuikenouderdieren die in een commerciële grondhuisvesting met gedeeltelijke roostervloer worden gehouden. Op 32-60 weken leeftijd had 78-95% van de dieren voetzoollaesies, waarvan 8-35% ernstig. Slechte strooiselkwaliteit was gecorreleerd aan ernstigere laesies, dus aandacht is nodig voor manieren om een goede strooiselkwaliteit te behouden in de vorm

van huisvesting (klimaatcontrole, zitstokken of roosters) en management (watergift). Ernstige haklaesies of loopafwijkingen waren veel minder waargenomen met maximaal 0.5% en 2.7% van de dieren die respectievelijk waren aangedaan.

De incidentie en ernst van voetzool-, haklaesies en loopafwijkingen waren niet gecorreleerd aan het percentage grondeieren, dus deze pijnlijke aandoeningen lijken de hennen niet de belemmeren in hun gang naar het nest of de motivatie om naar het nest te gaan is overheersend. Nesten worden in de meeste delen van de wereld op verhoogde roosters geplaatst, waardoor de hennen moeten springen om de nesten te bereiken. Hoewel we verwachtten dat vermoeilijkt wordt door een hoog lichaamsgewicht, vonden we geen correlatie tussen het percentage grondeieren en lichaamsgewicht in Hoofdstukken 3 en 5. Daarnaast hadden hokken met nesten op een verhoogd rooster een lager percentage grondeieren dan hokken met nesten direct op het strooisel (Hoofdstuk 5). Op basis van onze resultaten is het onwaarschijnlijk dat grondeieren worden veroorzaakt door pootproblemen. Genetische lijnen verschillen in beoogd lichaamsgewicht en eiproductie, maar we vonden zelfs binnen de lijnen een negatieve correlatie tussen lichaamsgewicht en eiproductie (Hoofdstuk 3). Een hoger lichaamsgewicht was ook gecorreleerd aan een hogere uitval, wat de gezondheidsrisico's van een hoog lichaamsgewicht demonstreert.

Gelegenheid

Aangezien verminderde pootgezondheid de gang naar het nest niet lijkt te limiteren, is bestudeerd of groepsgenoten de gelegenheid belemmeren voor het moederdier om het nest te betreden. Gezamenlijk nesten, waarbij een hen een nest prefereert met andere hennen erin boven een leeg nest, kan leiden tot te weinig nestruimte. We observeerden dat drie genetische lijnen van vleeskuikenouderdieren zich gelijk verdelen over de beschikbare nesten, maar dat twee lijnen 2-5 keer meer eieren in de nesten op het eind van de rij legden ten opzichte van de middelste nesten (Hoofdstuk 4). Een ongelijkere nestverdeling was gecorreleerd aan een hoger percentage grondeieren, dus overdadig gezamenlijk nesten kan bijdragen aan grondeieren. De nestverdeling was slechter op jongere leeftijd, wat suggereert dat weinig ervaren de neiging tot gezamenlijk nesten versterkt. Angst voor mensen, algemene angst en neiging tot clusteren waren niet gecorreleerd aan de nestverdeling.

Vleeskuikenmoederdieren worden gehuisvest met 7-10% hanen om een goede bevruchting van de eieren te borgen. Het is bekend dat deze hanen agressief zijn in hun paargedrag, wat bijdraagt aan veerschade en verwondingen van hennen. In onze studie had meer dan 30% van de hennen verwondingen op een bepaald moment in de productieperiode en de meerderheid van de hennen had ernstige veerschade vanaf 40 weken leeftijd (Hoofdstuk 4). Paringen gebeuren voornamelijk in het strooisel en we vonden een correlatie tussen een lager strooiselgebruik en een hogere proportie verwonde hennen, wat suggereert dat verwonde hennen hanen proberen te mijden. Een hogere proportie verwonde hennen was ook gecorreleerd aan minder grondeieren, wat indirect veroorzaakt kan zijn door een lager strooiselgebruik waar grondeieren meestal gelegd worden. Hanen spenderen minder tijd op de roosters en daarom is het effect van roosters voor het nest op nestgedrag bestudeerd in Hoofdstuk 5. Hokken met nesten direct op het strooisel hadden meer grondeieren dan hokken met nesten met een verhoogd rooster ervoor, maar door technische problemen kon niet worden bepaald of dit kwam door verstoring door hanen. Een groter strooiselgebied leidde tot een grotere paaractiviteit (i.e. geslaagde en mislukte paringen), maar niet tot meer geslaagde paringen. We observeerden gemiddeld 40 geslaagde paringen per dag per hok met 33 hennen, dus de hennen werden waarschijnlijk dagelijks geïnsemineerd. Kippen kunnen bevruchte eieren leggen voor tenminste 10 dagen na inseminatie, dus wellicht is de geadviseerde proportie aan hanen hoger dan nodig voor optimale bevruchting van de eieren.

Conclusies & aanbevelingen

Vleeskuikenouderdieren hebben een sterke voorkeur voor houten boven plastic nesten, maar de exacte oorzaken achter deze voorkeur is nog onbekend en vergt verder onderzoek. Bovendien kiezen hennen hun nestlocatie gedurende de start van eiproductie, wat later niet makkelijk veranderd. Het voorkomen van grondeieren moet dus extra aandacht krijgen aan de start van de productieperiode. Voetzoollaesies zijn een veelvoorkomend probleem bij vleeskuikenouderdieren, waarbij meer aandacht gegeven moet worden aan het behouden van een goede strooiselkwaliteit om deze problemen te verminderen. Echter, pootproblemen waren niet gerelateerd aan het percentage grondeieren. Overdadig gezamenlijk nesten lijkt gedeeltelijk genetisch bepaald bij vleeskuikenouderdieren en kan bijdragen aan grondeieren. Paargedrag is afhankelijk van de strooiseloppervlakte en is frequenter dan noodzakelijk voor de bevruchting van eieren. De optimale proportie hanen moet bestudeerd worden om grondeieren, stress en verwondingen van hennen te verminderen met behoud van de bevruchting van eieren. In deze studie zijn alleen snelgroeiende vleeskuikenouderdieren bestudeerd, maar toekomstig onderzoek zou moeten evalueren wat de voor- en nadelen van traaggroeiende rassen zijn voor het welzijn. Tenslotte is meer onderzoek nodig op commerciële schaal in samenwerking met boeren om wetenschappelijke kennis te vertalen naar praktische adviezen. Afrondend kunnen we concluderen dat nestontwerp de motivatie van vleeskuikenouderdieren om een ei in het nest te leggen beïnvloedt, maar de gelegenheid om dit nest te bezoeken kan verminderd worden door overdadig gezamenlijk nesten en het gedrag van hanen. Pootproblemen lijken de gang naar het nest niet te verstoren of zijn overstemd door de hoge motivatie om het nest te bereiken.

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About the author

Anne van den Oever was born on the 8th of April in Veldhoven, the Netherlands, where she also spent her childhood. In 2007 she started her studies in Veterinary Science at the University of Antwerp in Belgium. After two years she decided to change to the bachelor study of Biology at Wageningen University, which suited her interests better. She continued at Wageningen University for her master studies in Biology and obtained her degree in Biology in 2014, specializing in Animal Adaptation and Behavioural Biology. For her thesis at the Behavioural Ecology Group, she studied how mate preference was related to maternal investment in terms of egg size, brooding behaviour and care for the offspring in Chinese quail. During her research internship she joined the Behavioural Science Unit of Taronga Zoo in Sydney, Australia. At this zoo, she observed the behaviour of red-tailed black cockatoos kept under two different housing and husbandry conditions. In 2016 she started working as a researcher for Vencomatic Group, where she began her PhD project in January 2017 in collaboration with the Adaptation Physiology Group of Wageningen University. After finishing her PhD, Anne will continue to work for Vencomatic Group.

Publications

Peer-reviewed scientific journals

van den Oever A.C.M., Rodenburg T.B., Bolhuis J.E., van de Ven L.J.F., Hasan M.K., van Aerle S.M.W. and Kemp B. 2019. Relative preference for wooden nests affects nesting behaviour of broiler breeders. Appl. Anim. Behav. Sci. 104883.

van den Oever A.C.M., Bolhuis J.E., van de Ven L.J.F., Kemp B. and Rodenburg T.B. 2020. High levels of contact dermatitis and decreased mobility in broiler breeders, but neither have a relationship with floor eggs. Poult. Sci. 99:7, p. 3355-3362.

van den Oever A.C.M., Kemp B., Rodenburg T.B., van de Ven L.J.F. and Bolhuis J.E. 2021. Gregarious Nesting in Relation to Floor Eggs in Broiler Breeders. Animal.

van den Oever A.C.M., Candelotto L., Kemp B., Bolhuis J.E., Graat E.A.M., van de Ven L.J.F., Guggisberg D. and Toscano M.J. 2021. Influence of a raised slatted area in front of the nest on leg health, mating behaviour and floor eggs in broiler breeders. Animal.

Conference proceedings and abstracts

van den Oever A.C.M., Rodenburg T.B., Bolhuis J.E., van de Ven L.J.F and Kemp B. 2017. Nesting behaviour of broiler breeders, Xth European Symposium on Poultry Welfare, Ploufragan, France. p. 23.

van den Oever A.C.M., Rodenburg T.B., Bolhuis J.E., van de Ven L.J.F and Kemp B. 2017. Nesting behaviour of broiler breeders, Proceedings of the 51st Congress of the International Society for Applied Ethology, Aarhus, Denmark. p. 110.

van den Oever A.C.M., Rodenburg T.B., Bolhuis J.E., van de Ven L.J.F and Kemp B. 2017. Nesting behaviour of broiler breeders, in: Proceedings of the 7th International Conference on the Assessment of Animal Welfare at Farm and Group Level, Ede, the Netherlands. p. 132.

van den Oever A.C.M., Rodenburg T.B., Bolhuis J.E., van de Ven L.J.F and Kemp B. 2018. Effects of different nest designs on nesting behaviour in broiler breeders, ASAB Winter Meeting, London, United Kingdom. p. 15.

van den Oever A.C.M., Rodenburg T.B., Bolhuis J.E., van de Ven L.J.F and Kemp B. 2019. Relative preference for wooden nests affects nesting behaviour in broiler breeders, Proceedings of the 53rd Congress of the International Society for Applied Ethology, Bergen, Norway. p. 310.

Education and training certificate

Approved by graduate school WIAS

The Basic Package	3 ECTS
Introduction Day	2017
Research Integrity & Ethics and Animal Science	2017
Essential Skills	2017

Disciplinary Competences	17 ECTS
Laboratory Animal Science (Utrecht, the Netherlands)	2014
Preparing own PhD research proposal	2017
Advanced Statistics: Design of Experiments	2017
Statistics for the Life Sciences	2018
Training School COST-Action Group House Net (Bratislava, Slovakia)	2018
The Fundamentals of Animal Emotion	2019
Extended training period abroad (Center for Proper Housing: Poultry and Rabbits (ZTHZ), Zollikofen, Switzerland)	2019

Professional Competences	5 ECTS
Information Literacy	2017
PhD Workshop Carousel	2017
Mobilising Your -Scientific- Network	2017
Stress Identification Management	2018
Scientific Artwork	2018
Supervising Thesis Students	2019
Scientific Writing	2019
The Final Touch: Writing the General Introduction and Discussion	2019

Teaching Competences	5 ECTS
MSc minor thesis - Stephanie van Aerle	2018
MSc minor thesis - Md. Kamrul Hasan	2018
BSc thesis - Anne de Bruijn	2019

Presentation Skills	4 ECTS
Poster - Xth European Symposium on Poultry Welfare, Ploufragan, France	2017
Poster - 51st Congress of the International Society of Applied Ethology (ISAE), Aarhus, Denmark	2017
Poster - 7th International Conference on the Assessment of Animal Welfare at Farm and Group Level (WAFL), Ede, the Netherlands	2017
Oral - The Association for the Study of Animal Behaviour (ASAB) Winter Meeting, London, United Kingdom	2018
Oral - WIAS Science Day, Wageningen, the Netherlands	2019
Oral - 53rd Congress of the International Society of Applied Ethology (ISAE), Bergen, Norway	2019

Education and Training Total	34 ECTS

One ECTS credit equals a study load of approximately 28 hours

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