LIFTING LAYING HEN WELFARE IN AVIARIES TO A HIGHER LEVEL

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‘Turn your face toward the sun and the shadows will fall behind you'

Maori saying

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LIST OF ABBREVATIONS

DW: Dekalb White

FPD: foot pad disorders

IB: ISA Brown

FP: feather pecking

ILVO: Institute for Agricultural and Fisheries Research

KBD: keel bone disorders

LSM: least squares mean

PABAK: prevalence adjusted bias adjusted kappa

SE: standard error

TKS: total keel score

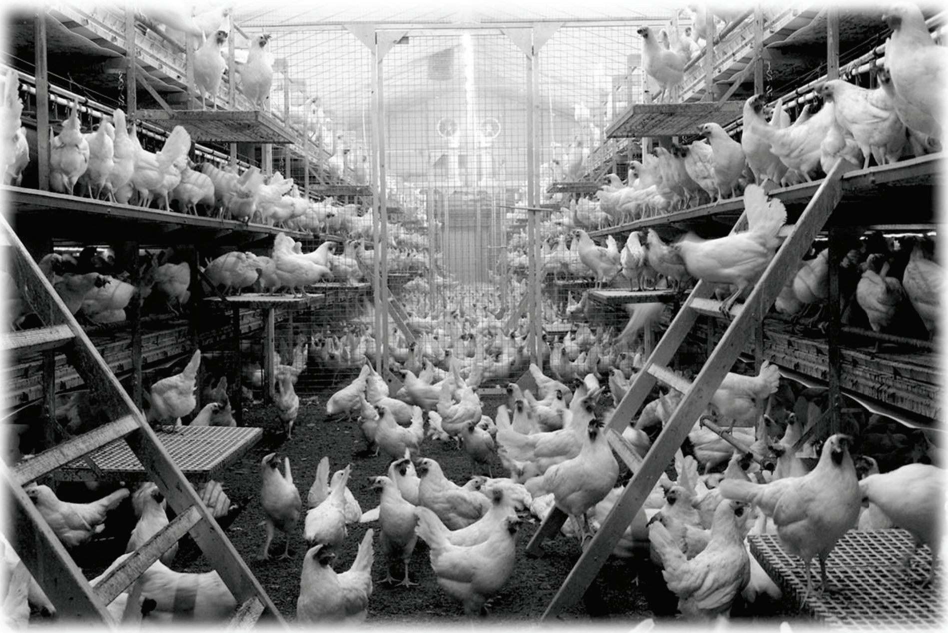
TPS: total plumage score

VP: vent pecking

WAIR: wing assisted incline running

CHAPTER 1

GENERAL INTRODUCTION



1.1 INTRODUCTION

Widespread public concern and debate about the welfare of laying hens (*Gallus gallus domesticus*) resulted in a ban on conventional cages in the European Union per January 1, 2012. This ban on conventional cages, often referred to as 'battery cages', is part of the 'European Council Directive 1999/74/EC Laying down minimum standards for the protection of laying hens'. Hence, since January 2012, all laying hens in flocks larger than 350 hens in the EU must be housed in either 'furnished or colony cages' or 'alternative systems' (Appleby, 2003; Council Directive 1999/74/EC, 1999; LayWel, 2006). However, furnished and colony cages are in the industry often referred to as an alternative to the conventional cage and thus could be considered in the category of alternative systems. In order to avoid confusion about the term alternative systems, the terms 'non-cage systems' and 'cage systems' will be used throughout this manuscript, unless explicitly mentioned as a particular cage type (furnished cages, colony cages or conventional cages).

Non-cage housing systems have the potential to offer a better laying hen welfare compared with cage systems, mainly because non-cage systems typically provide more space and opportunities to perform highly-motivated behaviours (Appleby and Hughes, 1991; Duncan, 2001; Freire et al., 2013; Rodenburg et al., 2012; Shimmura et al., 2010). In practice, however, serious concerns have been expressed concerning the welfare of hens in commercial non-cage systems due to reports on high flock mortality, feather pecking and cannibalism, and keel bone and foot pad disorders These welfare problems may originate from a discrepancy between hen and housing environment, resulting in inability to cope when not being able to perform highly-motived behaviours. A mismatch between the physiological abilities of the hen and the required abilities to move and perform in such housing systems may also lead to some of the above mentioned welfare problems (EFSA, 2005; FAWC, 2010; Fossum et al., 2009; Freire et al., 2003; Lay et al., 2011; Rodenburg et al., 2005, 2012; Sandilands et al.,2009; Sherwin et al., 2010; Shimmura et al., 2010; Tauson et al., 1999; Wang et al, 1998; Wilkins et al., 2011). These concerns especially apply to the modern aviary housing systems (Figure 1) as farms with aviary housing systems grow both in number and size as a direct result of the ban on conventional cages in the EU (Tuyttens et al., 2011). This increase will continue as several countries are phasing out furnished cages by implementing a ban on all cage systems for laying hens (e.g. The Netherlands, Germany), and aviaries are generally considered the best non-cage substitute for commercial large scale egg production (Rodenburg et al., 2005; Tuyttens et al., 2011; Van Horne and Achterbosch, 2008). The Belgian Federal Service of Public Health, Food Chain Safety and Environment funded the project 'LAYERHOUSE' (Contract RT11/12 Layerhouse) to investigate the suitability of aviary systems as a substitute for cage systems prior to considering a ban on all cage systems in Belgium.

Figure 1. Laying hens in a multi-tiered non-cage system, also called an aviary or aviary system. The hens can move freely from the littered floor to all the tiers and perform highly-motivated behaviours.

Animal welfare and economic concerns in aviary systems are also current in non-EU countries (e.g. Tasmania and New Zealand) that are in transition from conventional cages towards non-cage systems. Concerns about laying hen welfare will continue to increase given worldwide societal awareness. In the USA such pressure has led to an increasing number of states considering to enforce a ban on conventional cages. Moreover, in North-America several large retailers, egg-industries and companies like McDonalds and Starbucks are moving towards the use of cage-free eggs (McDonalds, 2015; Rembrandt Foods, 2015; Sonderlin, 2015). Ultimately, a tremendous number of farms, with in total hundreds of millions of laying hens, will undergo the transition from battery cages to aviaries between now and the year 2030. This thesis will therefore not only focus on demonstrating the current magnitude of certain welfare problems, but also contribute to the improvement of aviary systems by testing an innovative remedial measure that, ultimately, may lead to improved laying hen welfare in aviary housing systems.

In 2014, approximately 385 million laying hens were kept in the EU, of which 44% were housed in non-cage systems. In that same year, Belgium kept approximately 3.3 million laying hens in non-cage systems, which accounted for 39% of all registered hen places in Belgium (EC-CIRCABC, 2015). Although there were fewer non-cage hens compared to hens in furnished and colony cages, 60% of all registered Belgian holdings (registered farms) were henhouses with a non-cage system. In 2010, 67% of all Belgian hens (in 56% of all holdings) were still held in conventional cages, indicating that two-thirds of all Belgian henhouses underwent the transition from conventional cages to the new housing systems within two to four years. This demonstrates the magnitude of the transition in housing systems caused by the ban on conventional cages (EC-CIRCABC, 2015; Tuyttens et al., 2011). Laying hens housed in Belgium are held according to the minimum standards that are laid down in the European Directive 1999/74/EC, because Belgium has translated almost literally the Directives into national legislation (Koninklijk Besluit 2005-2772 tot vaststelling van de minimumnormen voor de bescherming van legkippen, 2005).

The main concerns against the barren conventional cage housing system were the small amount of available space (550 cm^2) and the lack of possibilities to perform highly-motivated behaviours, such as foraging, nesting, perching, dustbathing, running, and wing-flapping. The alterations to the housing systems for laying hens were based partially on the desire to fulfil the behavioural needs of the laying hen (Sandilands et al., 2009). Performing highly-motivated behaviours is considered as one of the five freedoms (1. from hunger and thirst; 2. from discomfort; 3. from pain, injury, and disease; 4. to express highly-motivated behaviour; and 5. from fear and distress) (Brambell, 1965). Limitations to any of these five freedoms may negatively affect the welfare of the animals. In non-cage systems the freedom to express most highly-motivated behaviours is largely fulfilled as the hens can freely move on the littered floor and the tier(s), perch and roost on the provided aerial perches, perform nest seeking, pre-laying and egg laying behaviours in the nest boxes, 'forage' for feed and water at feeders and drinker lines spread-out throughout the henhouse, and perform other highly-motivated behaviour such as ground pecking and scratching, dustbathing and comfort behaviour (e.g. wing flapping, preening, and stretching) (Lay et al., 2011). To guarantee space and opportunity to perform those behaviours, the EU Directive stipulates maximum stocking densities in non-cage systems, minimal perch and nest box space, and feeder and drinker space per bird. Even more foraging, exploring and other active behaviours can be performed if a non-compulsory covered veranda or free-range is provided (COUNCIL DIRECTIVE 1999/74/EC, 1999; LayWel, 2006).

Given that birds have more opportunity to perform highly-motivated behaviours in aviary systems, the aviary system has the potential to be more animal-friendly compared to cage systems. However, this potential may not be fully realised in practice as some characteristic aspects of the aviary system may impair the welfare of the laying hens. Major risks for the welfare of the hens include the development and consequences of feather pecking (FP), vent pecking (VP) and cannibalism, the occurrence of keel bone and foot pad disorders (KBD and FPD), increased mortality as well as poorer hygienic conditions and red mite infestations compared with cage systems (Blatchford et al., 2016; Freire et al., 2003; Heerkens et al., 2015a, 2016a; Petrik et al., 2015; Rodenburg et al., 2012; Tauson et al., 1999). Furthermore, several studies that compared different housing systems demonstrated that egg production performance (e.g. egg laying rate, egg shell quality) and proportion of dirty eggs in non-cage systems can be inferior to the egg performance in cage systems (e.g. Abrahamsson and Tauson, 1995; Fossum et al., 2009; Shimmura et al., 2010). However, results are inconsistent due to large variation within housing systems (De Reu et al., 2009). Some studies even found higher prevalences of dirty and cracked eggs in furnished cages compared to non-cage systems (Abrahamsson and Tauson, 1995; Mertens et al., 2006).

The introduction of this doctoral thesis will focus on the characteristics of aviary housing systems, how these aviary system characteristics interact with laying hen behaviour, after which the most important laying hen welfare aspects in aviaries will be explained further. This structure will emphasize the scope of this doctoral thesis concerning the relationship between aviary system characteristics and laying hen welfare.

1.2 THE AVIARY SYSTEM

Non-cage systems come in many different names as they include several types of housing systems, such as deep litter systems, floor housing systems, percheries and **aviaries**. Such housing systems can be placed in either fixed henhouses or mobile houses. What characterizes non-cage systems is that those systems are operated from the inside, meaning that the stockperson enters the living space where the hens are kept and is in direct contact with the hens, contrary to cage systems where there is a barrier between the hens and the stockperson (LayWel, 2006). Non-cage systems can roughly be divided in two types; (i) single-tier systems often referred to as floor housing; and (ii) multi-tier systems often referred to as aviary housing or aviaries (Figure 1). In both non-cage types the laying hens are generally kept in large flocks (ranging from 350 hens to more than 70,000 hens per henhouse). The larger henhouses are divided in compartments housing groups of 3,000 to 6,000 hens per compartment. The EU Directive 1999/74/EC states that hens in non-cages systems should have access to a littered area (at least 250 cm^2 per hen and the litter must occupy at least one-third of the floor surface), feeder and drinker lines, aerial perches and nest boxes. In floor housing, a littered area and a single elevated tier, under which a manure pit or belt is placed, is provided to the laying hens. This single elevated tier is normally equipped with the feeder and drinker lines, aerial perches and nest boxes. Aviaries increase the total floor surface in the henhouse with the use of tiers (a maximum of four tiers according to EU Directive 1999/74/EC), enabling higher stocking densities per m^2 ground floor surface, as the hens can disperse across several levels. The first aviaries were developed in the UK in the late 1970's for economic reasons, using the volume of the henhouse more costs-efficiently compared to the single-tier systems. The higher stocking density per m^2 ground floor surface made aviaries more profitable and suitable for keeping large flocks for commercial purposes. Aviaries were developed further in the 1980's in the UK and The Netherlands, and soon afterwards also Germany and Sweden developed several types of aviaries in the early 1990's (Elson 2004). Already in 1992 the Swiss Government prohibited the use of conventional cages in Switzerland (Fröhlich and Oester, 2001). Therefore, also the Swiss developed commercial aviaries in that same decade. The four decades of developments in aviaries resulted in aviaries with very different sizes, designs, features, configurations, materials, perch types, feeder and drinker types and so on.

Besides the economic benefits of the higher stocking density in aviaries, the aviary system may also improve the welfare of the laying hens by stimulating more highly-motivated behaviours (e.g. perching, wing-flapping) compared to cages systems. There are a few typical features of aviary and floor systems that show more resemblance with the ancestral natural habitat of the laying hen, compared to cage systems. The tiers and perches sort of mimic the natural habitat of bushes, shrubs and trees in which the wild fowl lives. Nest boxes facilitate a more natural-like and closed off area for the laying hens to lay their eggs. Furthermore, laying hens are highly motivated to perch and this behaviour originates from the ancestral red jungle fowl (*Gallus gallus*). The provision of perches mimics the presence of branches to allow the performance of day-time perching and night-time roosting (Newberry, 2001; Weeks and Nicol, 2006; Wood-Gush et al., 1978). Foraging behaviours, such as ground pecking and scratching, are of great importance to laying hens as these behaviours comprise approximately 40 to 60% of the time-budget of active behaviour (Dawkins, 1989; Klein et al., 2000). Non-cage systems offer plenty of space and opportunities to perform these foraging behaviours.

1.2.1. TIERS

Tiers are the most typical feature of the spacious and complex character of aviary housing and provide living space at several heights for the laying hens. Each tier may be equipped with feeder and drinker lines, aerial perches and (integrated) nest boxes. The tier is a perforated floor that is placed into metal frames or stacks. Most tier floors are constructed of either rectangular welded wire mesh or plastic slats, because those materials are more durable and easier to clean between production cycles compared to wooden slats. The wire mesh is galvanised or treated otherwise for a smooth finish to prevent sharp edges that can damage the feet or misplaced eggs. The maximum number of tiers allowed by the EU Directive is four, with a maximum stocking density of 18 hens/m^2 useable floor space (accumulated space of ground floor and tiers) or 9 hens/m^2 ground floor space and the minimum distance between two tiers is at least 45 cm. The tiers may be interconnected with each other or with the floor by ladders or ramps to facilitate a continuous pathway for the hens to walk up and down to reach the littered floor or tiers (Figure 2).

Figure 2. Aviary system with ramps between tiers.

Under these perforated floors a manure belt is placed to prevent that manure that is trampled through from dropping on the hens below and to maintain a better environment and lower ammonia levels by regular manure removal compared with systems with no manure belts. In row-type aviaries the stockperson can walk in the aisle between the stacks for collection of floor eggs, removal of dead hens and inspection and maintenance of the housing system. Further, although less convenient, also the top tier is accessible for inspections. The width of the aisle is determined by the distance between two stacks. The portal-type aviary provides an extra level on top of the two tiers between two stacks, allowing the stockperson to walk on top of the highest tier, on the tiers on the side, as well as under the additional top tier. Figure 3 depicts the two types of aviaries.

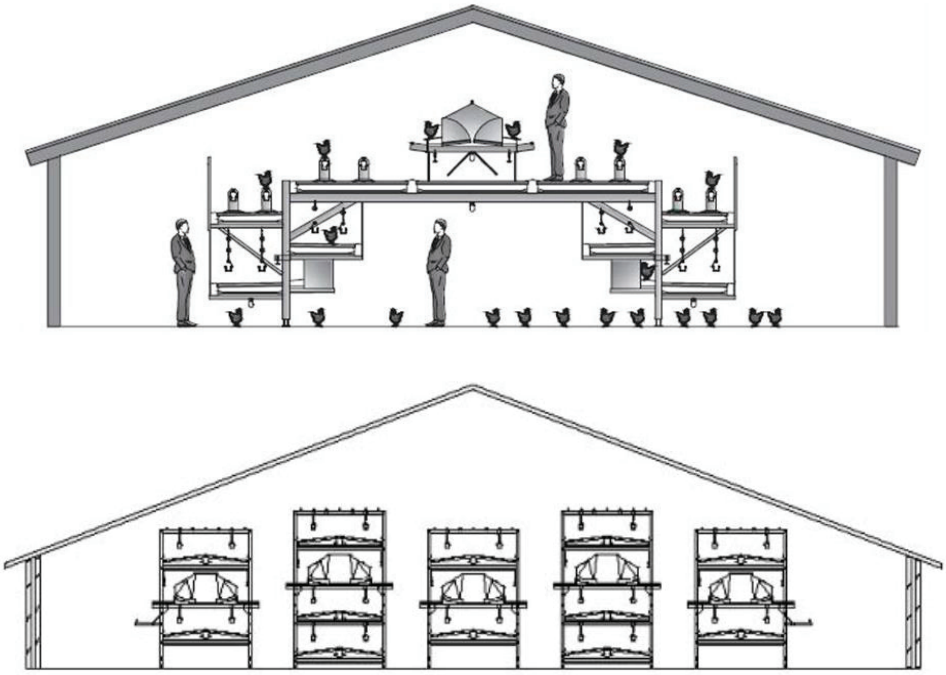


Figure 3. Cross sections of two different types of aviary systems. Above: Portal-type system (Red-L®, Vencomatic BV, Eersel, The Netherlands). Under: Row-type system with fives stacks and alternating three and four tiers (from LayWel 2006). Both aviary types provide (integrated) group nest boxes, feeder and drinker lines at several tiers, aerial perches, manure belts, wire mesh or plastic slatted flooring of the tiers and a litter area.

The lowest 'tier' in the henhouse is the ground floor providing a litter area that typically is covered with dried friable manure and litter substrates in which the hens can scratch, forage and dustbathe. Wet litter or large manure plaques may cause high ammonia levels and inferior hygiene, which can be detrimental for both human and animal health reasons (David et al., 2015). Therefore, wet litter and manure plaques should be prevented. At the start of a new production cycle, often a limited amount of litter substrate is provided on the ground floor, such as sand, wood shavings or saw dust. Also roughage can be offered on the floor or in baskets (e.g. bales of hay or alfalfa), as well as pecking blocks (e.g. aerated concrete, limestone) for the hens to peck at and to blunt the sharp tip of the beak. These substrates, roughage and pecking blocks can also be provided throughout the production cycle to ensure good dry and friable litter and to offer occupational material to the hens. Good litter quality and occupational material stimulate highly-motivated behaviours, such as scratching and foraging behaviours, and reduces damaging behaviours (e.g. feather pecking), and may prevent foot pad problems (Lambton et al., 2010; Nicol et al., 2001; Van Krimpen et al., 2005; Wang et al., 1998). The hens use the litter floor throughout the day, with peak occupancy during late morning and early afternoon when the hens dustbathe in large groups (Campbell et al., 2016a).

The tiers create more freedom of movement and increase bird activity in aviary systems and this results in stronger leg, wing and keel bones (Rodenburg et al., 2008; Scholz et al., 2008b). However, this increased activity and ability to jump or fly longer distances also pose a threat for another aspect of the welfare of laying hens, namely the increased risk of bone fractures due to collisions and failed landings (Gregory et al., 1990; Wilkins et al., 2011). Investigation of aviary configurations in a field study and under controlled experimental conditions (e.g. strategic placement of platforms, ramps or perches) (Figure 3) may accomplish more successful landings, fewer collisions or avoid unnecessary long aerial descents and ultimately reduce bone fractures (EFSA, 2005; Sandilands, 2009; Stratmann et al., 2015a).

1.2.2 PERCHES

Elevated perches allow hens to perch during daytime, for monitoring the environment or seeking refuge from other hens, and to roost during night-time. Hens are highly motivated to perform this perching and roosting behaviour, and during night-time more than 90% of the hens may use the perches for roosting (Abrahamsson and Tauson, 1995; Olsson and Keeling, 2000, 2002). According to EU Council Directive 1999/74/EC, each hen in a non-cage system ought to be provided with at least 15 cm perch per hen to facilitate space for the perching and roosting behaviours. The perches should not have sharp edges, cannot be installed above the litter and the horizontal distance between perches must be at least 30 cm and the horizontal distance between the perch and the wall must be at least 20 cm. The EU Directive does not require the perches to be of specific size, shape, diameter or material. Generally, aviaries are equipped with round steel pipes and the majority of perches is provided on the highest tier for roosting during the night (EFSA, 2015). Other types of perches with different shapes, diameters and materials are used as well, such as wooden rectangular, circular, and plastic mushroom-shaped perches. Although round metal perches are less preferred by the hens and may hamper highly-motivated perching behaviour and impair keel bone and foot pad health (Appleby et al., 1992; Pickel et al., 2010, 2011; Struelens et al., 2009), they are currently the most commonly used type of perch in commercial aviaries. Round steel perches have the advantage to be cheap and easily installed in new systems, they are durable, and are easy to clean and disinfect between production rounds and offer less attractive hiding places for red mites.

Besides the welfare benefits of being able to display perching and roosting behaviour, perches also have positive effects on the physiology of the hens by increasing leg bone strength (Barnett et al., 1997; Hester et al., 2013). However, perches also pose a threat to the welfare of laying hens by increasing the risk of keel bone disorders, such as keel bone fractures and keel bone deviations (Freire et al., 2003; Sandilands et al., 2009; Wilkins et al., 2004). According to the EU Directive (1999/74/EC), the horizontal distance between perches must measure at least 30 cm and there must be at least 20 cm horizontal distance between the perch and the wall. The risk of fractures increases if the distance between perches is more than 80 cm either horizontally, vertically or diagonally or if the angle the hens have to jump between perches is between 45 and 90º (Scott et al., 1997). Commercial aviary configuration often allows hens to jump or fly very short distances and steep angles (e.g. jumping downwards from perch to tier), but also the opportunity to fly several meters over long diagonal distances (e.g. from highest perch to litter floor) is not uncommon. Perch size, shape, material and hygiene have also been associated with foot pad disorders, such as foot pad hyperkeratosis, foot pad dermatitis and bumble foot (Oester, 1994; Rönchen et al., 2008; Tauson and Abrahamsson, 1994, 1996; Wang et al., 1998,). More recent research demonstrated that round steel perches covered with polyurethane material could reduce keel bone disorders and footing stability during perching (Pickel et al., 2010; Stratmann et al., 2015b). Investigating perch material and configuration may thus contribute to improved laying hen welfare.

1.2.3 NEST BOXES

Nest boxes are provided for hens to lay their eggs in and to improve their welfare by facilitating the opportunity to perform highly motivated egg laying behaviours, such as nest seeking, nest building, and pre-laying behaviours (Keeling, 2004). The floor of the nest boxes is sloped so that eggs will roll onto a collection belt to allow efficient egg collection. To prevent overcrowding or too much competition for a nest site, at least one nest box should be provided for every seven hens, or a minimum of 1 m^2 of nest space for a maximum of 120 hens as regulated by the EU Directive 1999/74/EC. The nest box floors are mainly lined with either artificial turf mats, rubber mats or plastic mesh (Laywel, 2006). Seclusion of the nest boxes with a plastic curtain is beneficial for the welfare of the laying hens, because these curtains stimulate pre-nesting and nest-building behaviours. The curtains appear to have no effect on the proportion of eggs laid in the nest boxes (Struelens et al. 2005; 2008). The nest boxes may be closed during certain periods of the day and during the night via an expulsion system to prevent hens soiling the nest lining, because soiled nest box lining leads to more dirty eggs that cannot be sold for a premium price. Appropriate nesting materials and configuration is believed to benefit laying hen welfare by preventing disturbed nesting and frustration behaviour (Hunniford et al., 2014; Struelens et al., 2008).

Optimal use of the nest boxes is crucial to the farmer and effort should be taken to prevent eggs being laid outside of the nest boxes. Besides the strategic placing of lights and the light regime in the housing system, floor eggs can be prevented by improving the attractiveness of the nest box. In non-cage systems, eggs that are not laid in the nest boxes, but on the litter or in the system (on the tiers) are so-called floor eggs. Collection of floor eggs increases labour, because those eggs have to be collected manually. These eggs are also likely to be downgraded eggs due to dirt and cracks (EFSA, 2005). In non-cage systems reported percentages of floor eggs vary from 0.6% (Abrahamsson and Tauson, 1995), 1.2% (Emous and Fiks-van Niekerk, 2003), 4.6% (Van Horne, 1996) to approximately 10% (Nicol et al., 2006). In practice many efforts have been taken to reduce the number of floor eggs, as it is too time consuming and almost unfeasible to collect more than 2% floor eggs per day in flocks of 100,000 hens or more. Identifying factors that influence floor egg laying may prevent floor egg laying even further.

The aviary system with the above-mentioned stacks with tiers, perches, nest boxes and other structural elements provides many hiding places for the poultry red mite *(Dermanyssus gallinae*). During day-time, the mites hide in cracks and crevices of the housing systems and during the night the mites feed on the hens' blood (Mul et al., 2009). The feeding leads to blood loss and can result in anaemia (to varying degrees). This ectoparasite poses a serious threat in laying hen husbandry, both economically as well as for the health and welfare of the hens. The red mites cause irritation and restlessness during the night. The restlessness and stress caused by the red mites can lead to feather pecking and cannibalistic behaviours, increased feed and water intake, reduced egg quality and increased mortality. Red mites can also be vectors for various diseases (Chirico et al., 2003; Kilpinen et al., 2005; Sparagano et al. 2009, 2014). Therefore, thorough cleaning and disinfection of the entire housing system between production cycles is a necessity. Red mite infestations are often a persistent problem and have become endemic in some countries (Sparagano et al, 2014). Belgium was not included in the review by Sparagano et al. (2014), but the red mite problem is most likely endemic to Belgium as well.

1.2.4 VERANDA AND FREE-RANGE

Optionally, the farmer can choose to provide a covered veranda or outdoor access on a free-range (at least 4 m^2/hen according to the EU-Directive 1999/74/EC) for the laying hens. Free range access is obligatory for free-range and organic farms. The veranda and free range provide hens with even more opportunities to perform behaviours such as wing flapping, running, dustbathing and foraging. Popholes in the wall of the henhouse or veranda allow the hens access to these areas and sufficient popholes with a sufficient width should be provided to prevent blocking the passage for other hens (Elson, 2004; Laywel, 2006). According to the EU Directive, popholes should at least be 35 cm high and 40 cm wide, spread out along the entire length of the henhouse, and add up to 2 m total width of popholes per 1,000 hens. A covered veranda is accessible during the daylight hours and the temperature is similar to the outside temperature as the outer wall is most often made of ventilation curtain. Materials that allow the rainwater to drain, such as wood chips or pebbles are usually placed near the henhouse to maintain good hygiene as they prevent the hens from carrying too much dirt and mud into the henhouse that could negatively affect foot health (Wang et al., 1998). The free range should mainly be covered with grass, but to encourage hens to range further the free range should also contain other vegetation (trees, hedges, etc.) or artificial elements (sewage pipes, shelters and other structural elements) (Willet et al., *in press*; Zeltner and Hirt, 2003). Stimulating the hens to range is underlined by the results of Bestman and Wagenaar (2014), who found that flocks that used the free range more had a better plumage condition. Disadvantages of providing a free range are the risk of avian influenza, bacterial and parasitic diseases and predation leading to increased mortality and production losses (Fossum et al., 2009; Tauson, 2005a).

To conclude on the elements of aviary housing, it is clear that most structural elements in aviary housing offer more opportunities for highly-motivated behaviours compared to cages systems, but due to the complex environment, the materials used and the large group sizes those elements also pose threats to the welfare of laying hens. Investigation of the appropriate materials and configurations could contribute to improved laying hen welfare in aviary systems.

1.3 LAYING HEN WELFARE

There are various ways to approach or define animal welfare, such as: i) the normal functioning of the animals' biological systems and basic health, ii) the subjective feelings of the animal (how the animal experiences life) and its affective states (e.g. pain, distress and pleasure), or iii) the ability or freedom to perform its full behavioural repertoire and having natural elements in their environment (Appleby and Hughes, 1997; Fraser, 2008). All approaches are not mutually exclusive and overlap substantially as they share several indicators, such as health, physiology, behaviour and zootechnical performance (often referred to as production). Behaviour and health as indicators for welfare can be assessed as i) detecting signs or behaviours of poor coping or clinical disorders in experimental situations or in the environment under investigation, ii) investigating the needs and priorities of the animal, and iii) comparison of behaviours performed in natural environment and the behaviour in the environment under investigation (EFSA, 2005). In this thesis the welfare of laying hens is not approached through one specific of the above mentioned criteria, but is more generally approached by using a variety of the non-mutually exclusive indicators (Mendl, 2001).

The major benefits for laying hen welfare in non-cage systems are the freedom of movement and the opportunities to perform highly-motivated behaviours. When kept in a restricted environment under high stocking densities, domestic chickens have difficulties to perform all highly-motivated behaviours, leading to welfare problems. Breeding and domestication of chickens has resulted in some behavioural differences between domestic chickens and the wild-type fowl, but these differences are mainly attributed to changed behavioural thresholds, whereas nearly all behavioural traits and activities have been preserved (Duncan et al., 1978). The major welfare issues for laying hens in non-cage systems are mortality, damaging pecking behaviours (feather pecking, vent pecking, cannibalism) and keel bone disorders (EFSA, 2005; FAWC, 2010, 2013; Harlander-Matauschek et al., 2015; Rodenburg et al., 2013). Freedom of pain and discomfort is one of the five freedoms. Like mammals, laying hens possess nociceptors, supporting the evidence that hens are capable of perceiving pain (Gentle and Wilson, 2004). Laying hen welfare is impaired by feather pecking (FP), vent pecking (VP) and cannibalism, as these behavioural problems inflict damage to the plumage and skin of the victims, which ultimately can lead to the death of the victim, and these behaviours indicate inability of the pecker to cope with its environment. Keel bone disorders (KBD) are physiological consequences mainly caused by the ability to freely move around and perform natural locomotory behaviour, although the causes of KBD need further investigation, as this problem also occurs in systems with less freedom of space (e.g. in furnished cages). To a lesser extent also foot pad disorders are a pressing issue in laying hen welfare, of which the prevalence may be underestimated (EFSA 2005; Lay et al., 2011). All the above mentioned welfare issues are known to have multi-factorial underlying causes, such as housing characteristics, management procedures, group size, hygiene, rearing and genetic predisposition (Lay et al., 2011; Rodenburg et al. 2005). Assessing welfare problems and identifying underlying causes allows to adapt housing designs which may lead to large improvements in laying hen welfare (Fraser, 2008; Tauson, 1998). Investigating the housing system that is considered to provide improved laying hen welfare compared to cage systems, and which is considered as the best non-cage alternative to cage systems for large scale egg production, may provide further evidence that transition from cages to aviaries may answer the consumer demand for better laying hen welfare. Identifying beneficial and/or harmful aviary housing characteristics may result in recommendations or further improvements to this housing system. **A multifactorial approach by investigating a broad spectrum specifically aimed at aviary housing systems and management characteristics at farm level may confirm known risk factors and reveal new factors that affect laying hen welfare in aviary systems. Examining specific aviary housing conditions under controlled experimental settings allows demonstration of beneficial effects of innovative adaptations to the aviary housing systems that contribute to better laying hen welfare.**

Morbidity and mortality are the opposite of good health. Hens tend to suffer to varying degrees during the period of morbidity preceding death, and mortality and morbidity are considered as very important indicators of poor welfare (EFSA, 2005, Nicol et al., 2006). Hens in non-cages systems can be at a higher risk for increased mortality due to diseases and infections, smothering, feather and vent pecking, cannibalism, or heat stress compared with hens in cage systems (Fossum et al., 2009; Matthews and Sumner, 2015; Rodenburg et al., 2008; Van Emous and Fiks-van Niekerk, 2003), although other studies found no differences or even lower mortality in non-cage systems (Aerni et al., Singh et al., 2009; Stadig et al., 2016). Besides the welfare concerns related to mortality, increased loss of animals obviously negatively affects the farm economics too. **Identifying risk factors that affect mortality in aviary systems and modifying the system and management accordingly may improve laying hen welfare and farm economics.**

Zootechnical performance is inevitably most important for the farm profitability. The zootechnical performance, including egg laying rate, number of floor eggs, egg weight, egg shell characteristics, feed intake and feed conversion, differs between hybrids and between housing systems (Abrahamsson and Tauson, 1998; Mignon-Grasteau et al., 2015; Singh et al., 2009; Tumová et al., 2011). Alterations in production parameters can also be indicators for the welfare of the laying hen as stress and impaired welfare can have negative effects on egg production (Barnett et al., 1992; Shini et al., 2009). The underlying causes for deviating production parameters can be very diverse, such as overcrowding in the nest boxes, unbalanced feed composition, poor plumage condition, bone fractures, draught, too few drinkers and feeders or heat stress (EFSA, 2005). Also FP, red mite infestations, restlessness and poor foot pad health can affect the zootechnical performance of laying hens. Hence, the zootechnical performance can be used as an indication of possible welfare problems, nevertheless is not reliable as a sole indicator for laying hen welfare. **Investigating risk factors that affect zootechnical performance in aviary systems may offer a scope to improve both laying hen welfare as well as the farm economics.**

1.3.1 FEATHER PECKING, VENT PECKING AND CANNIBALISM

Laying hens have to perform well in the aviaries' complex environment and under several management procedures. The aviary characteristics and management procedures may conflict with the ability of the hen to cope with its environment. Discrepancy of highly-motivated behaviours that cannot be performed or fulfilled to satisfactory, such as foraging, may result in coping problems that manifest as redirected or maladaptive behaviours, such as feather pecking (FP), vent pecking (VP) and cannibalism. Feather pecking, vent pecking and cannibalism affect the integument of the laying hen and are associated with stress, pain, fear, and mortality. FP is defined as the pecking at feathers of conspecifics and ranges from gentle FP to severe FP. Gentle FP consists of pecks that damage the feathers to a lesser extent without removing the feathers, and is most often ignored by the recipient. Although the damage caused by gentle FP is not so harmful, stereotyped gentle FP can be an indication of a welfare problem of the pecker. Severe FP involves a vigorous head movement of the pecker and often removal of the feather and a painful response of the victim. Severe FP is mostly directed at the back, rump, vent and tail, ultimately leading to denuded areas (Bilčík and Keeling, 2009; Pötzsch et al. 2009; Rodenburg et al., 2013; Savory, 1995). VP is pecking directed at the vent or cloaca and often occurs during the onset of egg laying when young hens have difficulty finding the nest and cloacal prolapse exposes the caudal end of the oviduct. Conspecifics are attracted to this prolapse and may start to peck at it as well as at the surrounding tissue (Kjaer and Hocking, 2004). Under natural conditions, a hen lays her egg in bushes and shrubs, without any other hens nearby that would be attracted to peck at the prolapsing oviduct. Cannibalism is when hens peck at bleeding feather follicles or damaged skin and is often preceded by severe FP. VP and cannibalism damage the skin and underlying tissues and organs and the damage may vary from small superficial wounds to big gaping wounds, ultimately leading to death of the victim (Pötzsch et al., 2001; Savory, 1995). Pecking wounds can also be sources of infection, leading to increased mortality. Severe FP, VP and cannibalism are often the main causes of mortality in non-cage systems (Fossum et al., 2009; Nicol et al., 2013; Rodenburg et al., 2008). The victims of severe FP, cannibalism or VP respond to the pecks by either confronting the pecker or by moving away and avoiding the pecker. In some cases victims seem to surrender to the peckers and remain still while being pecked. Avoiding being pecked is more difficult in cage systems compared to in non-cage systems due to the limited amount of space (Rodenburg et al., 2013). However, FP, VP and cannibalism are of greater concern in non-cage systems compared to cage systems, in particular when compared to conventional cages (Fossum et al., 2009; Lay et al., 2011; Sherwin et al., 2010; Tauson, 2005). In conventional cages it is easy to identify the pecker which then can be removed from the cage to prevent further damage and at most three victims can be targeted. In non-cage systems the hens are kept in large groups of several thousands of hens per section. In such large groups a pecker is more difficult to identify and can also cause more damage. Furthermore, the maladaptive behaviours may spread through the flock via social transmission (Newberry et al., 2004; Rodenburg et al., 2013; Zeltner et al., 2000).

Recording damage to different parts of the body can explain the cause of the plumage damage (Bilčík and Keeling, 1999), because the plumage not only deteriorates due to feather pecking, but also abrasion and wear against the housing equipment damages the plumage. Furthermore, the natural process of moulting can affect the plumage condition during the production cycle. The tail, rump, belly, and back are known target areas for feather pecking, whereas damage of the wings may be attributed to abrasion. Damage to the lower neck is often caused by abrasion during feeding, whereas damage to the upper neck may be attributed to damaging pecking behaviours (Bilčík and Keeling, 1999; Nicol et al., 1999; McAdie and Keeling, 2000). Damaging behaviours can be assessed by scoring the occurrence of the behaviour itself (e.g. pecking bouts directed at the feathers of conspecifics) or by the scoring its consequences (e.g. plumage condition and pecking wounds). The integument in turn can be assessed using methods in which the hen is caught and handled or the plumage can be assessed from a distance. Both integument scoring methods show strong correlation and are both correlated to the incidence of the damaging pecking behaviours. A disadvantage of recording plumage damage is that the consequences of FP are scored on the victims and the perpetrator that inflicts the damaging pecking remains unidentified (Bestman and Wagenaar, 2003; Bilčík and Keeling, 1999; Bright et al., 2006; Lambton et al., 2010; 2013; Tauson, 2005).

FP has been reported to occur in the majority of flocks in non-cage systems, e.g. 57% of the investigated non-cage flocks by Green et al. (2000), 71% of investigated organic flocks (Bestman et al., 2009), up to 86% of free-range flocks in Lambton et al. (2010).

Several studies demonstrated a high prevalence of hens with damaged plumage in flocks housed in non-cage systems, ranging from an average 58% of hens/flock with damaged plumage (Ekstrand et al., 1997), to 62% (Gunnarsson et al., 1999), and 83% (Algers et al., 1995). Bestman and Wagenaar (2003) reported that 52% of the investigated Dutch organic flocks showed severe plumage damage due to FP. VP has been found to vary from 6 to 42% hens/flock being affected in non-cage systems (EFSA, 2005). Pötzsch et al. (2001) reported that 37% of the investigated flocks showed VP behaviour and/or VP damage and also reported a high and positive correlation between the presence of VP and flock mortality.

Removal of feathers is painful, but also leads to difficulties to maintain body temperature, leading to thermal discomfort. Not only the pecked hen has an impaired welfare, but the maladaptive behaviour also indicates that the 'pecker' is unable to cope with its environment (as reviewed by Rodenburg et al., 2013). Furthermore, FP can be an economic problem due to increased mortality, increased feed uptake to compensate for increased body heat loss, less efficient conversion of energy from feed into egg mass, lower egg weight and reduced egg production (EFSA, 2005; El-Lethey et al., 2000; Nicol et al., 2013; Rodenburg et al., 2013). FP is believed to be redirected foraging behaviour, mainly ground pecking (Blokhuis, 1986; Huber-Eicher and Wechsler, 1997) and may also be related to other behavioural characteristics, such as fearfulness, sociality, coping ability and reaction to stress (Rodenburg and Koene, 2004a). Gentle FP may already occur during rearing, whereas severe FP generally develops later in the production cycle and increases with age during the production cycle (De Haas et al., 2014b; Lambton et al., 2010).

Beak trimming is currently the most common practice to control FP, VP and cannibalism in most countries worldwide. This measure does not really prevent the behaviour from occurring or developing. Instead, it reduces the damage as the sharp tip of the beak is removed. Beak trimmed flocks can still develop high levels of FP, VP and cannibalism, so the effectiveness of this method is definitely not absolute (Hartcher et al., 2015; Nicol et al., 2013). Beak trimming is considered to be a painful procedure, and the formation of neuromas may lead to chronic pain. Other consequences of beak trimming are sensory loss in the tip of the beak, and reduced ability to manipulate objects, as reviewed by Janczak and Riber (2015). Beak trimming is considered a mutilation as it affects the integrity of the animal and is therefore not allowed in organic laying flocks, and several member states of the EU have banned this procedure (Sweden, Norway, Finland) also in conventional egg production or are phasing out beak trimming (The Netherlands and some states in Germany) for all laying hens (Van Horne and Achterbosch, 2008; Van Krimpen et al., 2005). Therefore, alternative measures should be developed to prevent high flock mortalities due to FP, VP and cannibalism. Commercial hybrids can be very different in the display of damaging behaviours, and inconsistent results between brown and white hybrids have been found (De Haas et al., 2014a,b; Odén et al., 2002; Mahboub et al., 2004). Promising selection techniques have been developed that already have been proven to decrease mortality with 10% and to make hens less fearful or sensitive to stress (Bolhuis et al., 2009; Ellen et al., 2007, Rodenburg et al., 2009). Another example of a measure to control FP, VP and cannibalism is reducing the light intensity and/or using light with specific wavelengths (e.g. red, blue or ultraviolet) in the henhouse (Nicol et al., 2013). These light measures may diminish the colours and details of their flock mates' plumage, thereby reducing the detection of blood and bold patches, consequently making it less attractive to peck at (Mohammed et al., 2010). However, keeping the hens in the dark cannot be considered as an animal friendly solution. Other measures that have proven to reduce FP, VP and cannibalism and that can be applied easily in non-cage systems are providing roughage (e.g. silage or alfalfa) and aerated pecking blocks, providing music in the house to reduce fear or a free range with sufficient canopy cover and shelter to stimulate foraging (Bestman and Wagenaar, 2003; De Haas et al., 2014b; Lambton 2013).

FP, VP and cannibalistic behaviour in laying hens are considered to be multifactorial problems originating from risk factor interactions between the laying hens, the environment and management procedures (Drake et al., 2010; Nicol et al., 2013, Rodenburg et al., 2013). Several risk factors that have been identified are the rearing phase, beak treatment, individual characteristics by genetic predisposition (e.g. hybrid, parent flock), environmental conditions (e.g. housing system, group size, litter substrate, light regime), management (e.g. 'standard' or 'modified in order to reduce the risk for feather pecking'), and feed (e.g. feed composition, feed form, diet changes) (Ambrosen and Petersen, 1997; Bilčík and Keeling, 1999; De Haas et al., 2013, 2014a, 2014b; Lambton et., 2010, 2013; Nicol et al., 1999; Odén et al., 2002; Rodenburg et al., 2013; Zimmerman et al., 2006). Identification of these diverse risk factors allows us to deal with the multifactorial problem via a multifactorial approach (e.g. via rearing conditions, hybrid selection, housing conditions, feed). An approach proven to effectively reduce these damaging pecking behaviours is applying bespoke management procedures and selection of appropriate housing environments based on identified risk factors (De Haas et al., 2014b; Lambton et al., 2013).

To improve laying hen welfare in aviary systems, the identification of typically aviary system related risk factors for FP, VP and cannibalism may contribute to select the most appropriate aviary design and to formulate bespoke management procedures. As any damage to the body may be considered a welfare issue, also the identification of risk factors for plumage damage due to abrasion offer opportunities to improve laying hen welfare in aviaries.

1.3.2 KEEL BONE DISORDERS

The keel bone, a structural bone, is a very pronounced ventrally directed structure of the sternum that extends axially from the midline of the sternum and anchors the muscles that are needed to flap the wings, the *pectoralis* major and *pectoralis* minor (Figure 4). Due to its thin and flattened structure and prominent position, the keel bone is susceptible to injuries of the keel bone, as well are its surrounding soft tissues (Casey-Trott et al., 2015; Heerkens et al., 2016a).

Figure 4. The keel bone, indicated by arrow, is situated ventral to the heart and is the largest bone in a laying hen. (Source: Prof. T. Widowski. University of Guelph)

The different disorders of the keel bone are generally divided into two different types: fractures and deviations (Figure 5c and 5d). These two disorders differ in appearance and likely originate from different causes. Most studies have not discriminated between these two disorders, used different definitions (fractures, breaks, curvature, deformities) and were recorded with various methods, making comparison between studies difficult (Harlander-Matauschek et al., 2015). Discrimination between the two disorders allows more precise investigation of the causal factors. Casey-Trott et al. (2015) offered definitions and terminology for the two keel bone disorders, as well as recording and training methods for KBD assessment to improve accuracy and allowing better comparison across (future) studies. Also hematomas and wounds (or scabs) are often found on the soft tissue and skin surrounding the keel bone (Figure 5a and 5b). These disorders likely originate from collisions.

Figure 5. Keel bone disorders on live hens. a. hematoma, b. wound (scab), c. fracture (severe), d. deviation (severe)

To emphasize the welfare concern associated with KBD, Nasr et al. (2012a,b) demonstrated that laying hens with KBD experience pain. KBD has been acknowledged only recently as one of the greatest welfare problems that the laying hen industry faces (FAWC, 2010; 2013; EFSA, 2015). KBD most likely received little attention from laying hen farmers with non-cage systems as it has not directly been associated with increased mortality or decreased egg production. Although not assessed in commercial systems, Nasr et al. (2012b, 2013) have reported that hens with keel bone fractures showed reduced egg quality and increased feed intake, thereby making the issue of KBD also of economic interest.

Keel bone disorders in white and brown Leghorns have already been described in the 1930's (Carstens et al., 1936; Warren, 1937). In the 1990's KBD attracted increased attention during which mainly the differences in prevalences of KBD between housing systems and hybrids were described, as well as the effects of perch presence (Gregory and Wilkins, 1996; Gunnarsson et al., 1995; Tauson and Abrahamsson, 1994, 1996). It was only more recently that studies have put more focus on actually resolving this painful issue by gaining knowledge on the causes of KBD as well as by investigating measures and solutions that could reduce the prevalence and severity of KBD in laying hens (Pickel et al., 2011; Richards et al., 2012; Stratmann et al. 2015a, 2016; Scholz et al., 2014; Tarlton et al., 2013; Toscano et al., 2012). It is assumed that collisions are the main cause of keel bone fractures in laying hens housed in non-cage systems and that keel bone deviations result from a prolonged pressure load on the keel during perching. However, there is still a lack of evidence to support these assumptions, as well as a lack of knowledge on other causes of KBD that could also explain the high prevalence of keel bone fractures and deviations in cage systems (Harlander-Matauschek et al., 2015). No other studies have considered scoring wounds and hematomas of the soft tissues surrounding the keel bone, even though these injuries likely are inflicted by collisions or prolonged pressure. **This thesis, therefore, aimed to identify factors of aviary housing that affect keel bone disorders in laying hens housed in aviary systems.**

1.3.2.1 KEEL BONE FRACTURES

The majority of laying hens housed in non-cage systems sustain one or more keel bone fractures during the production cycle, with reported prevalence ranging from 48% up to 97% of the hens in a flock towards the end of the production (Freire et al., 2003; Heerkens et al., 2015a; Käpelli et al., 2009; Petrik et al., 2015 Rodenburg et al., 2008; Sherwin et al., 2010; Wilkins et al., 2004). Also, in organic production systems, keel bone fractures are highly prevalent (Bestman and Wagenaar, 2014). Consequently, the keel bone is one of the bones most affected by fractures (Gregory et al., 1990; Wilkins et al., 2004). Laying hens in cage systems may sustain keel bone fractures as well, with prevalences reported of 25% (Petrik et al., 2015), 30% (Sandilands et al., 2007) and 42% (Sherwin et al, 2010) in conventional cages, and 23% (Habig and Distl, 2013), 35% (Sherwin et al., 2010) 36% (Wilkins et al., 2011), 62% (Rodenburg et al., 2008), and 92% (Hester et al. 2013) in furnished cages. These high prevalences of keel bone fractures in both non-cage and cage systems demonstrate the magnitude of this welfare issue.

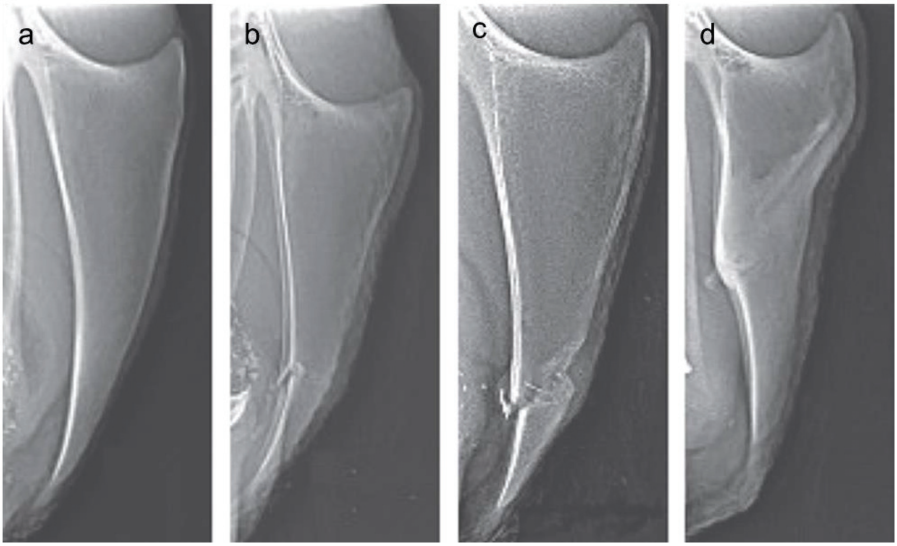


Figure 6. Radiographic images of various keel bone fractures and deviation. a. intact keel without fracture; b. fresh mild fracture; c. fresh severe fracture; d. deviation (adapted from Richards et al., 2011).

Keel bone fractures are (old) breaks manifested as callus formation on the ventral surface of the keel, or as sharp bends, shearing and/or fragmentation of the bone (Figure 5c, 6b, 6c). Keel bone fractures on live birds are best detected via the well described and validated method of palpation and is generally scored either binomial (absent or present) or on a severity scale (Figure 7) (Casey-Trott et al., 2015; Petrik et al., 2013; Scholz et al., 2008a; Wilkins et al., 2004, 2011). Palpation of the keel bone is performed by running down two or three fingers along the protruding keel to detect callus deposition or deformations.

The callus has formed as a result of the healing process following the fracture. The healing process consists of several phases (Einhorn, 2005), starting with an inflammatory response that results in the formation of a hematoma and granulation tissue. Also, the rupture of blood vessels during the event of fracturing causes a hematoma. The presence of this hematoma can be recorded by visual inspection (Figure 5a). On the fracture site, soft callus formation will occur, after which this soft callus will ossificate to form a bony callus. Eventually, this bony callus is replaced by lamellar bone tissue during the remodelling phase (Chanoit et al., 1999; Einhorn, 2005). A fresh (mild) fracture is more difficult to detect by palpation as the distinct callus may not have formed yet. Therefore fractures identified by palpation are often referred to as ‘old breaks’ or old fractures (Wilkins et al., 2004).

In non-cage systems keel bone fractures are most likely caused by dynamic events (e.g. flying, jumping) that result in collisions with structural elements of the housing system (e.g. perches, feeding troughs, platforms, supporting beams, tiers or floors) or collisions with other hens (Campbell et a., 2016b; Gregory and Wilkins, 1996; Moinard et al., 2004a; Scott et al., 1997). Collisions may occur following failed landings and misjudgements when flying or jumping up- or downwards from and to perches, tiers or the floor (Campbell et al., 2016b,c; Sandilands et al., 2009). These collisions result in high kinetic energy impacts on the keel bone. Reduction of the collision energy may result in a decreased risk of fractures and/or fractures of less severity (Toscano et al., 2013). The use of softer and/or more compressible materials, such as polyurethane coated perches are likely to better absorb the kinetic energy upon collision impact, thereby reducing the incidence keel bone fractures and keel bone severity (Pickel et al., 2011; Stratmann et al., 2016).

Figure 7. Fracture of the keel bone with increasing severity of fracture from left to right, as found in adult laying hens (adapted from Wilkins et al., 2011).

More high-impact collisions may occur in aviaries compared to other housing systems, because hens find it more difficult to negotiate movements at steeper angles (more than 45^{o}) and wider distances (more than 100 cm), especially with downward movements (as reviewed by Sandilands et al., 2009). Such movements are not possible in some floor systems and not in any cage system. In aviaries, however, it is common that hens have the opportunity to travel for both short and long distances in either flat or very steep angles. Galliforms have been found to prefer wing-assisted incline running (WAIR) over flight to reach elevated levels when moving through a complex natural environment. During WAIR the bird uses flapping forelimbs (wings) to aid the running hind limbs to climb a slope (Dial, 2003; Dial and Jackson, 2011; Tobalske and Dial, 2007). Laying hens most likely also prefer WAIR and walking movements over flight to reach the different levels in aviary systems. A beneficial trait of the WAIR and walking movements over flight is that the whole-body kinetic energy is less during those movements. Training of WAIR early in life could also help to strengthen the leg muscles. Stronger leg muscles may result in better take-off and landing abilities, thereby reducing accidental high impacts with the structural elements of the housing or with other hens, consequently reducing the risk of keel bone fractures (Harlander-Matauschek et al. 2015; Kozak et al., 2015; LeBlanc et al., 2015; Stratmann et al., 2015a; Tobalske and Dial, 2007).

In contrast to hens in non-cage systems, hens in cage systems have limited space to move. Several studies demonstrated reduced bone strength in cage systems compared to non-cage systems, which may be attributed to the lack of exercise and movements in cage systems (Leyendecker et al., 2005; Regmi et al., 2016; Rodenburg et al., 2008; Scholz et al., 2008b; Silversides et al., 2012; Wilkins et al., 2011). In conventional cages, the lack of exercise may result in ‘cage layer fatigue’ or osteoporosis (Whitehead and Fleming, 2000). As the lack of bone mass is an underlying cause of keel bone fractures (Fleming et al., 2004), it is more likely that keel bone fractures in cage systems occur due to more static events (Harlander-Matauschek, et al., 2015), and especially in conventional cages where even no perches, feed troughs or other structural elements are present to collide with.

Non-cage systems, and in particular aviary systems, come in many different designs equipped with a variety of structural elements and different materials. So far, mainly the effects of perch design, perch material, perch configuration and ramps on keel bone fractures have been investigated (as reviewed by Harlander-Matauschek et al., 2015). Hence, there is a lack of knowledge on other potential housing system related risk factors for keel bone fractures in aviary systems. Disorders of the soft tissue surrounding the keel bone (hematomas and wounds) have received little to no attention. Hens are capable of feeling pain, leading to the general thought that soft tissue disorders are most likely associated with pain and discomfort.

Identification of risk factors for keel bone fractures (and associated soft tissue damage) in aviary systems may contribute to improving laying hen welfare in aviary systems through system and management improvements. Aviary system risk factors may be extrapolated to floor housing as there are similarities between those types of non-cage systems and possibly even cage systems due to some similarities, thereby potentially revealing causes of keel bone fractures other than high impact collisions.

1.3.2.2 KEEL BONE DEVIATIONS

Keel bone deviation is defined as an abnormally shaped keel bone (Figure 5d, 6d) that deviates from a 2-dimensional straight plane in either the sagittal or transverse planes (Figure 8) (Casey-Trott et al., 2015). There seems to be a lack of knowledge on how (severely) these keel bone deviations affect laying hen welfare. However, as keel bone deviations may already occur at the onset of lay and last throughout the whole production cycle, this can be considered a major welfare problem as it may inflict chronic discomfort and reduced mobility (Fleming et al., 2004; Stratmann et al., 2015a, 2016).

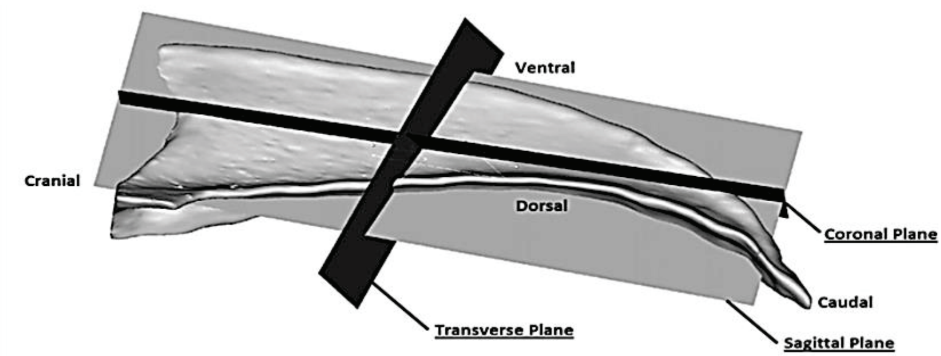


Figure 8. Anatomical planes of the keel bone (adapted from Casey-Trott et al., 2015)

This form of KBD is assumed to originate from prolonged pressure load on the keel bone during perching and roosting (EFSA, 2005; Moe et al., 2004; Pickel et al., 2011). During seated perching the majority of the bodyweight rests on the keel bone (Pickel et al., 2011) and perching is performed for prolonged periods on a daily basis. This may lead to remodelling of the keel bone, ultimately resulting in deviation of the keel bone in either the sagittal or transversal plane (Figure 8), or in both directions (Casey-

Trott et al., 2015; Harlander-Matauschek et al., 2015). Newly developed soft perches increase the contact area between the keel bone and the perch, thereby reducing the pressure load. This reduction of pressure load resulted in reduced deviations (Stratmann et al. 2016). It has been suggested that vigorous wing-flapping may play a role in keel bone deviations as well (Harlander-Matauschek, 2015). Only perch design and material have been demonstrated to affect keel bone deviation. The many different aviary types and designs are equipped with various materials with various shapes. **Therefore, identification of housing system characteristics that affect keel bone deviation may contribute to improving laying hen welfare in aviary systems.**

Throughout the laying cycle, structural bone loss occurs progressively and results in weakening of the skeleton and the resorption of calcium also makes the keel bone weaker and more susceptible to fractures, remodelling, deformation or deviation (Casey-Trott et al., 2015; Whitehead, 2004). Bone material in general is a dynamic tissue that is subjected to age, physiological, nutritional, and physical factors (Fleming et al., 2015; Rath et al., 2000; Whitehead, 2004). Egg and egg shell formation is one of those factors. During the process of egg and egg shell formation there is an increased demand for calcium (Kim et al., 2012). This demand peaks at night when the egg shell is formed, whereas circulating calcium supplied from the digestive system is low at night. A high proportion of the calcium is resorbed from medullary bone. However, resorption can also occur from exposed structural bone surfaces, such as from the keel bone. As high egg production may lead to weakened keel bones, making it more prone to fractures, the concerns of KBD may increase as hens are bred to produce even more eggs and for a longer period of time, demanding more from the bone structures of the laying hen (Bain et al., 2015; Whitehead and Fleming, 2000). Associations between egg production and KBD have only been demonstrated in experimental studies (Nasr et al., 2012a, 2013), but there seems to be a lack of information obtained from commercial flocks in aviary systems. **Therefore investigating KBD prevalence and egg production at farm level may contribute to the understanding of the relationship between bone quality and egg production and the possible effects of KBD on farm economics.**

1.3.3 FOOT PAD DISORDERS

The most common foot pad disorders (FPD) in laying hens are hyperkeratosis, dermatitis of the foot pads and bumble foot (Figure 9). These FPD affect laying hen welfare as they are associated with infections, impaired mobility and cause of pain and discomfort (Lay et al., 2011). FPD can be recorded on live birds by macroscopically examining the foot pads or more histo-pathologically via skin biopsy (Rönchen et al., 2008; Weitzenbürger et al., 2006). Hyperkeratosis has the smallest impact on hen welfare, whereas severe foot pad dermatitis and bumble foot are considered to reduce hen welfare substantially (EFSA, 2005; Tauson 2002; Wang et al., 1998).

Proliferative hyperkeratosis (Figure 9a) in laying hens is the result of hypertrophy of the corneus layer of the foot pad skin and is caused by increased compression load of the toe- and foot pad (Weitzenbürger et al., 2006). The compression load occurs to some extent during perching (Pickel et al., 2011), but hyperkeratosis is mainly associated with the permanent compression load when housed on wire flooring as occurs in cage systems (Abrahamsson and Tauson, 1995; Abrahamsson et al., 1996; Tauson and Abrahamsson, 1994, 1996; Tauson et al., 1999).

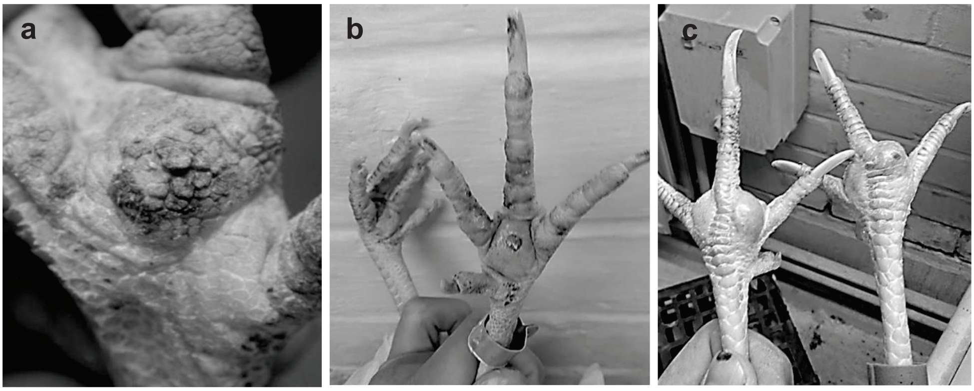


Figure 9. a. hyperkeratosis, b. foot pad dermatitis and c. bumble foot

Direct contact with litter and faeces in combination with pressure load may lead to infiltration of bacteria into the epithelium. This infiltration can result in foot pad dermatitis (Figure 9b), which is an inflammation or infected lesioning of the epithelium accompanied with mild to severe swelling of the foot pad (Wang et al., 1998). When the bulbous swelling of the foot pad is visible from the dorsal side it is called bumble foot (Figure 9c). Poor litter quality (wet litter), improper design of perches, inferior hygienic conditions, type of flooring, and genetic predisposition have all been associated with foot pad dermatitis and bumble foot (Abrahamsson and Tauson, 1995; Pickel et al. 2011; Tauson, 2002; Wang et al., 1998; Weitzenbürger et al., 2006). Several studies demonstrated that bumble foot is more prevalent in white hybrids compared to brown hybrids, whereas hyperkeratosis is more prevalent in brown hybrids (Abrahamsson and Tauson, 1995; Abrahamsson et al., 1996; Mahboub et al., 2004, Tauson and Abrahamsson, 1994,1996).

Identification of risk factors for foot pad disorders that are specifically related to aviary housing systems may contribute to improving laying hen welfare in aviary systems and has the potential of being extrapolated to floor housing and cage systems.

1.4 COMMERCIAL HYBRIDS

Breeding companies have been selecting laying hens for many decades, mainly with the aim to improve egg laying performance. Nowadays, three large breeding companies provide the vast majority of all laying hens worldwide. Each breeding company has specific hybrids that can be divided in three main groups: 1. white hybrids, 2. brown hybrids, and 3. silver hybrids. The white hybrids are white feathered hens, lay eggs with white egg shells, and have a lower bodyweight, generally lay slightly more eggs and consume less food compared to brown hybrids. Brown hybrids have brown feathers and lay brown eggs. Silver layers are a cross between white cockerels and brown hens and have white feathers, but lay brown eggs (Leenstra et al., 2012).

Genetic selection of laying hens resulted in hens that show early sexual maturation, no broodiness, and that start to produce a new egg immediately after the previous one (Appleby et al., 2004; Keeling, 2002). The wild ancestor, the red jungle fowl, produces approximately 6-30 eggs per breeding season, whereas the modern hybrid is capable of producing over 320 eggs per laying cycle (up to approximately 72 weeks of age) and breeding companies are currently still breeding for more eggs per hen in a prolonged laying cycle (500 eggs/100weeks/hen) while maintaining good egg shell quality, health and welfare (Bain et al., 2015).

Selection has also changed the physiology and behaviour of the laying hen. Compared to its ancestor, the laying hen, and especially the brown hybrids, has become larger and heavier (Figure 10). However, the wing size has not grown proportionally, resulting in a less optimal bodyweight-ratio with more wing loading (i.e., more weight per wing area), decreasing the flight capabilities of the modern laying hen (Moinard et al., 2004a). Brown and silver hybrids generally are more docile, less fearful and less flighty than white hybrids (De Haas et al., 2013, 2014a; Uitdehaag et al., 2009). Especially white hybrids are known for hysteric withdrawal, panic and violent escape reactions when startled, which can cause stress, pain, injuries and increased mortality (Jones, 1993; Odén et al., 2002).

Figure 10. From left to right: red jungle fowl, commercial white hybrid laying hen, commercial brown hybrid laying hen.

Consumers associate egg shell colour to the way of keeping, nutritious value or taste (Johnston et al., 2011). These egg shell attributes vary demographically between countries or even between regions. This consumer attitude towards egg shell colour, often directed by retail companies and commercial brands, thereby determine which hybrids are kept in certain housing systems in the respective region or country. In Sweden, white eggs are associated with non-cage, free-range and organic systems, therefore predominantly white hens are kept in those systems. In contrast, in other countries, such as the Belgium, US, Canada, and France, brown eggs are generally associated with free-range systems, subsequently generally brown hens are kept in those systems (Bejaei et al., 2011; Johnston et al., 2011; Leenstra et al., 2012).

Hybrids may show differences in susceptibility to physiological disorders, such as KBD (e.g. Fleming et al., 2004; Kapelli et al., 2011a; Kjaer et al., 2000; Vits et al., 2005) and

FPD (e.g. Mahboub et al 2004, Abrahamsson and Tauson, 1994,1995,1996), as well as behavioural differences in FP, VP, and cannibalism (Kjaer and Hocking, 2004). Susceptibility to certain disorders, however, are not consistent between the different studies. This inconsistency also accounts for the comparison of white and brown hybrids in the present study. Furthermore, mortality and feather cover can vary considerably between hybrids within housing systems and underlines the importance of the match between the hybrid and the housing system (EFSA, 2005; Leenstra et al., 2012). This indicates that genetic predisposition can make hybrids more suitable for certain housing conditions and therefore selection of the most appropriate hybrid for housing in aviary systems can contribute to improved laying hen welfare.

Identification of hybrid susceptibility for the welfare problems investigated may contribute to selection of the most suited laying hen hybrid for aviary systems.

1.5 CONCLUSION

Aviary systems are equipped with resources to stimulate highly-motivated behaviours in the laying hen. However, the intensive housing environment may affect laying hen welfare in various ways, such as the development of damaging pecking behaviours, high prevalences of keel bone and foot pad disorders, and high mortality.

The different housing equipment companies produce a variety of aviaries with all sorts of configurations and materials. Alongside differences in management procedures, specific aviary housing characteristics may affect laying hen welfare. Identification of risk factors related to housing and management will inform about possible improvements of aviary systems and management procedures. Innovative adaptations of the aviary system as remedial measure may ultimately lead to improved laying hen welfare

Breeding companies offer a variety of laying hen hybrids. Although the different hybrids originate from only few grandparent lines, hybrid selection resulted in genetic predisposition for higher susceptibility concerning the development of damaging pecking behaviours, keel bone and foot pad disorders, and mortality. Investigating which hybrids are most susceptible for which risk factors could lead to selection of the most suitable hybrid characteristics for aviary housing systems.

The expected worldwide increase in the number of laying hens housed in aviaries emphasizes the need to improve the welfare of laying hens kept in such a housing system.

CHAPTER 2

RESEARCH OBJECTIVES

Worldwide, many commercial laying hen farms underwent, or foresee to undergo, a transition from conventional cages to aviary systems. Challenges in housing laying hens in aviary systems include welfare issues concerning damaging pecking behaviours, mortality levels, keel bone disorders and foot pad health. An interplay of aviary housing characteristics, management, as well as genetic predisposition, may affect aspects of laying hen welfare. Identification of appropriate housing system characteristics, management and hybrid opens opportunities to improve laying hen welfare and profitability in aviary systems.

The objectives of this thesis were to identify aviary housing system characteristics, management procedures and hybrid-related risk factors that affect specific aspects of laying hen welfare. Furthermore, we aimed to test an innovative remedial measure to reduce keel bone and foot pad disorders in two commercial hybrids.

Firstly, during a field study on Belgian farms with aviary housing systems, the housing characteristics, management procedures, hybrid, egg production and mortality were obtained from an extensive questionnaire. Furthermore, flock prevalence of plumage condition, wounds, keel bone and foot pad disorders was obtained from assessing a sample of individual hens (chapter 3 and 4).

Secondly, an experimental study was conducted to test the effects of ramps between perches and nest boxes on keel bone and foot pad disorders. To demonstrate possible genetic predisposition effects, we used two commercial hybrids, namely ISA Brown and Dekalb White hens (chapter 5).

The main objectives of this thesis were to:

1. Assess plumage condition and wounds as indicators of damaging pecking behaviours (chapter 3)
2. Determine the prevalence of keel bone and foot pad disorders (chapter 4)
3. Identify aviary system characteristics and management procedures that affect aspects of laying hen welfare and mortality (chapter 3 and 4)
4. Test the influence of welfare issues and risk factors on egg production parameters (chapter 3 and 4)
5. Assess the effect of ramps on keel bone and foot pad disorders (chapter 5)
6. Assess the effect of hybrid on keel bone and foot pad disorders (chapter 5)

CHAPTER 3

SPECIFIC CHARACTERISTICS OF THE AVIARY HOUSING SYSTEM AFFECT PLUMAGE CONDITION, MORTALITY AND PRODUCTION IN LAYING HENS

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ABSTRACT

Feather pecking and high mortality levels are significant welfare problems in non-cage housing systems for laying hens. The aim of this study was to identify husbandry-related risk factors for feather damage, mortality and egg laying performance in laying hens housed in multi-tier non-cage housing, so called aviaries. Factors tested included type of system flooring, degree of red mite infestation and access to free-range areas. Information on housing characteristics, management and performance in Belgian aviaries (N = 47 flocks) were obtained from a questionnaire, farm records and farm visits. Plumage condition and pecking wounds were scored in 50 randomly selected 60-week-old hens per flock. Associations between plumage condition, wounds, performance, mortality, and possible risk factors were investigated using a linear model with a stepwise model selection procedure. In general, many flocks had poor plumage condition and a high prevalence of wounds, with considerable variation between flocks. Better plumage condition was found in wire mesh aviaries ($P < 0.001$), in aviaries with no red mite infestation ($P = 0.004$) and in free-range systems ($P = 0.011$) compared to plastic slatted aviaries, in houses with red mite infestations and those without free-range area. Furthermore, hens in aviaries with wire mesh flooring had fewer wounds on the back ($P = 0.006$) and vent ($P = 0.009$), reduced mortality ($P = 0.003$), and a better laying performance ($P = 0.013$) as compared to hens in aviaries with plastic slatted flooring. Flocks with better feather cover had lower levels of mortality ($P < 0.001$). Red mite infestations were more common in plastic slatted aviaries ($P = 0.043$). Other risk factors associated with plumage condition were genotype, number of diet changes and presence of nest perches. Wire mesh flooring in particular seems to have several health, welfare and performance benefits in comparison to plastic slats, possibly related to decreased feather pecking, better hygiene and fewer red mite infestations. This suggests that adjustments to the aviary housing design may further improve laying hen welfare and performance.

Key words: feather pecking, mortality, housing system, aviary, red mite

3.1 INTRODUCTION

The European Union Council Directive 1999/74/EC stipulates that from 2012 onwards laying hens in the EU can only be housed in either furnished cages or non-cage systems (EU, 1999). Over 160 million laying hens in the EU are currently kept in non-cage systems for commercial egg production (EuroStat, 2014). Non-cage housing systems have the potential to be more animal-friendly, mainly because they offer the hens more space and opportunities to perform highly-motivated behaviours than cage systems (Appleby and Hughes, 1991; Duncan, 2001; Freire et al., 2013; Rodenburg et al., 2012; Shimmura et al., 2010). In practice, however, there are still reasons for serious concern about the welfare of hens in commercial non-cage systems. Several recent studies demonstrated that hens housed in those non-cage systems have a higher risk for increased mortality and feather pecking compared with cage systems (Fossum et al., 2009; Freire et al., 2003; Lay et al., 2011; Rodenburg et al., 2005, 2012; Sherwin et al., 2010; Shimmura et al., 2010). Because non-cage systems are growing both in number and size, the related concern for layers' welfare is also growing (Tuyttens et al., 2011).

Non-cage systems can be divided roughly into the traditional single-tier floor housing systems and the more recently developed aviary systems. Aviaries are multi-tier systems that consist of a littered ground floor and a metal structure with up to four tiers (row-type aviaries). A portal-type aviary provides a single level on top of two stacks (Tauson, 2005a). Aviaries vary in design, but all systems typically have feeders, drinkers and perches located on one or more tiers. Earlier aviary types have separate units for nest boxes, whereas, in the more recent types, the nest boxes are integrated into the tiers. The tiers' flooring is either constructed of wire mesh or plastic slats (LayWel, 2006). Access to the ground floor and multiple levels increases the surface accessible to the hens and allows them to perform more highly-motivated behaviours such as running, wing flapping, flying, nesting, perching and dustbathing as compared with cage environments (Leyendecker et al., 2005). According to EU Directive 1999/74/EC, up to 18 hens/m^2 house floor area can be kept, with a stocking density of 9 hens/m^2 on the useable area (including the tiers). Additionally, a covered veranda or a free-range area accessible through popholes may be provided. Free-range surface requirements are typically 4 m^2/hen. Increased feather pecking and cannibalism as well as poorer hygienic conditions and red mite infestations have been suggested as cause for variability in egg production and mortality in aviary systems; these measures are more unpredictable and disadvantageous compared to cage systems (Aerni et al., 2005; Lay et al., 2011; Sparagano et al., 2014; Tauson et al., 1999; Tauson, 2002). Furthermore, eggs laid outside the nest, so-called floor and system eggs, can be problematic in non-cage systems because of related increases in labour requirements and reductions in egg quality (Appleby, 1984).

Maladaptive pecking behaviours form serious animal welfare and economic problems in poultry production. Feather pecking is defined as pecking at and pulling out the feathers of conspecifics, whereas both cannibalistic and vent pecking damage the skin and underlying tissue of the conspecific. These behaviours may be associated with stress, pain and fear, as well as increased mortality, and increased feed consumption due to heat loss (Bilčík and Keeling, 1999; Gunnarsson et al., 1999; Lambton et al., 2013). These undesirable behaviours are considered to be multifactorial and unpredictable problems that are present in all housing systems (Gunnarsson et al., 1999; Lambton et al., 2010; Nicol et al., 2013; Rodenburg et al., 2013; Pötzsch et al., 2001). Feather pecking is believed to be redirected ground pecking in relation to foraging behaviour (Blokhuis, 1986; Huber-Eicher & Wechsler, 1997). Although aviary systems provide more opportunities to perform highly-motivated behaviours compared to cage systems, feather pecking remains a serious welfare issue (Blokhuis et al., 2007; Huber-Eicher & Wechsler, 1997; Rodenburg et al., 2013). When hens are kept in large groups, as in the aviary systems, feather pecking is more difficult to control, as a feather-pecker can cause more damage and is more difficult to identify in a larger group versus a smaller one (McAdie and Keeling, 2000; Rodenburg et al., 2005).

Feather pecking, variable production results and relatively high mortality are still problems associated with laying hens housed in aviary housing systems. The aviary system has surpassed traditional floor housing as the most common non-cage housing system in Belgium and many other countries. The aims of this study were therefore (1) to quantify feather damage and pecking wounds, mortality and egg production rate in laying hens housed in a cross-sectional sample of commercial aviary systems in Belgium, and (2) to identify risk factors within aviary systems for these outcome variables.

3.2 MATERIALS AND METHODS

3.2.1 FLOCK RECRUITMENT AND FARM VISITS

Main stakeholders and representatives from the Belgian egg industry were informed about the project aims and their public support of the project was requested. Egg producers were informed by presenting the project at agricultural fairs and by an announcement in the main Belgian poultry stakeholders' magazine. Egg producers were then approached by telephone and asked for their participation in the study. Participation was encouraged by offering a financial compensation for their time and effort. A total of 47 henhouses with aviary housing on 33 commercial farms were included in this study. Farm visits were conducted between August 2012 and December 2013 by two observers from the Institute for Agricultural and Fisheries Research (ILVO), who jointly received extensive training in integument scoring prior to the start of the farm visits. Both observers of the present study received the training as part of the CORE organic II project HealthyHens in which both observers of the present study were involved (www.coreorganic2.org). The laying hens were approximately 60 weeks of age at the time of the farm visit (ranging from 58 to 64 weeks of age).

3.2.2 MANAGEMENT, PRODUCTION RESULTS AND FLOCK MORTALITY

A questionnaire was sent to the farmer two weeks before the farm visit. It contained approximately 110 questions on management (e.g. light- and feeding regime, farm hygiene, manure disposal), aviary system characteristics (e.g. age, manufacturer, perch orientation, feeder- and drinker types), housing enrichments, and laying hen information (e.g. hybrid, breeder, rearing, flock size) (see Appendix I). During each farm visit this questionnaire was checked to complete any missing answers. Egg production rate (expressed as average number of eggs laid per day per hen present), percentage of downgraded eggs, percentage of floor and system eggs, and percentage of cumulative mortality of the flock (percentage of dead hens since arrival on production farm) were obtained from farm records. If the farm visit was conducted before or after the flock was 60 weeks of age, the production results and mortality at 60 weeks of age were used for the statistical analyses by retrieving this information from the farm records provided or by contacting the farmer afterwards.

3.2.3 AVIARY SYSTEM CHARACTERISTICS

For each aviary system details on manufacturer and specific aviary type were recorded during the interview. A distinction was made between row- and portal-type aviary systems, and whether or not the hens had access to a covered veranda or free-range area. Henhouses with both a free-range area and a covered veranda were considered as free-range henhouses only. Further details of the aviaries (Table 1), such as flooring material of the aviary tiers (wire mesh vs. plastic slats), age of the barn and the aviary system and presence or absence of a perch or platform in front of the nest were derived from the questionnaire and interview, or were recorded during the visit to the henhouse. Infestation of red mites was scored on an ordinal scale as either 'absent', 'mild infestation' (some red mites visible, but not in large quantities), or 'severe infestation' (clearly visible clumps or large quantities of red mites in cracks, crevices and structural elements) (adapted from Welfare Quality® protocol; Welfare Quality®, 2009).

3.2.4 LAYING HEN CHARACTERISTICS AND MEASUREMENTS

Information on hen age, hybrid and rearing conditions were obtained from the questionnaire and interview. For animal-based parameters (plumage condition and wounds), 50 randomly selected laying hens per aviary henhouse were caught from all tiers of the aviary system, scored and then immediately released. Each observer scored approximately half of the hens per farm visit. If a covered veranda or a free-range area was present, an approximate representative number within the sample of 50 birds was scored in those areas (according to an estimation of the proportion of hens present in the covered veranda and/or free-range compared to the flock size). Plumage condition of five body parts (neck, back, wings, tail, vent) was scored on an ordinal 4-point scale, adapted from the LayWel project (Blokhuis et al., 2007, Tauson et al., 2005b). For the wings, the wing with the worst plumage condition was scored. A score ranging from 1 to 4 was given to each body part, in which the highest score indicated the best plumage condition. For a total plumage score (TPS) the scores of the five individual body parts were summed to form a non-equidistant score. Thus the lowest TPS score was 5, representing an extremely poor plumage condition, and the highest TPS score was 20, which represents a more or less perfect plumage condition.

Wounds on the back and the vent area were scored per body part on a 4-point scale. A score 4 was given when no wounds or ≤ 2 small wounds were present, score 3 for

> 2 wounds with diameter < 0.5 cm, score 2 for wound(s) present with a 0.5 to 2.2 cm diameter, and score 1 for presence of larger wound(s) > 2.2 cm. Cloacal discharge was scored on a binary scale (1 = present, 2 = absent).These scores were based on protocols used within the HealthyHens project and the LayWel project (Brenninkmeyer et al., *under review*, Tauson et al., 2005b).

3.2.5 DATA ANALYSES

Descriptive statistics for means, variation and range were calculated for plumage condition, wounds, production and mortality. Associations between animal-based measures (plumage condition and wounds), production (production rate, downgraded eggs, floor and system eggs), mortality, and possible risk factors were investigated using linear models. For each dependent variable a separate model was built using a forward stepwise model selection procedure. The models selected from the following factors as independent variables: all characteristics in Table 1 and times fed per day, nest entrance (perch vs. platform), daily time spent in henhouse by stockperson (minutes), daily time spent in henhouse by stockperson per 1,000 hens (minutes), red mite infestation (absent, mild infestation, severe infestation). The flock was the experimental unit for production results and mortality scores. The average of individual hen scores per flock was the experimental unit for plumage and wound scores. For hybrid as risk factor, only the two predominant hybrids (respectively Lohmann Brown Classic and ISA Brown) were included. All other hybrids were pooled together to form a diverse group consisting of five hybrids (respectively Lohmann Brown Lite, Hy-line Brown, Bovans Brown, Novogen Brown and Dekalb White) and included both white and brown hybrids. This group was included as a separate hybrid in the risk factor analysis, but due to the diversity of hybrids within this group, it was excluded from any further interpretation of results. Only significant risk factors were included in the final model per outcome variable (significance level of 5%). The means provided for the scores in the text are the least squares means (± SE). The analysed data were considered sufficiently normally distributed, based on the graphical evaluation (histogram and QQ-plot) of the residuals. In case of post-hoc pairwise testing, p-values were corrected with the Tukey-Kramer adjustment for multiple comparisons. Analyses were performed using R 3.0.1 for Windows and StatSoft. Inc. (2012) STATISTICA, version 11.

3.3 RESULTS

3.3.1 FLOCK, FARM, AVIARY AND MANAGAMENT INFORMATION

All hen places in this study sum up to 1,479,036 commercially held laying hens in Belgian aviaries, which corresponds with 44.4% of all registered non-cage laying hens in Belgium in December 2013 (EuroStat, 2014). Because floor systems are also registered as non-cage systems, the actual percentage of all laying hens kept in Belgian aviaries that were reached in this study is much higher than 44.4%.

General flock and farm information is summarized in Table 1. The average flock age during the visit was 60.7 ± 0.3 weeks. At 60 weeks of age the average cumulative mortality was 4.1% (range 0.9 to 12.8%) and average laying rate was 87.6% (range 72.0 to 94.1%), with 2.2% downgraded quality eggs, and 1.0% floor and system eggs (range 0.9 to 12.8%). The main causes of mortality, as reported by the farmers, were feather pecking and cannibalism, salpingitis, *Escherichia coli* infections and smothering. The highest mortality (12.8%) found in the present study was due to an outbreak of infectious laryncho tracheitis at 32 weeks of age. The examined laying hen hybrids were Lohmann Brown Classic (N = 21 flocks) and ISA Brown (N = 13), plus five other hybrids. Beak trimming is commonly applied in Belgian laying hen husbandry. Of the 47 flocks in the study, 46 had trimmed beaks, while one organic flock had intact beaks.

Table 1. Summary information on nominal, binomial and ordinal variables included in the aviary system field-study (N = 47 houses)

Factor	Feature	Frequency
Aviary type	Row	37 / 47 (79%)
	Portal	10 / 47 (21%)
Covered veranda	Yes	22 / 47 (28%)
	No	25 / 47 (53%)
Free-range area[1,2]	Yes	9 / 47 (19%)
	No	38 / 47 (81%)
Aviary system flooring	Wire mesh	31 / 47 (66%)
	Plastic slats	16 / 47 (34%)
Age of barn[3]	0 to 4 years	27 / 46 (59%)
	5 to10 years	5 / 46 (11%)
	≥ 11 years	14 / 46 (30%)
Age of aviary[3]	0 to 4 years	39 / 46 (85%)
	5 to10 years	7 / 46 (15%)
	≥ 11 years	0 / 46 (0%)
Nest entrance	Perch	25 / 47 (53%)
	Platform	22 / 47 (47%)
Hens at start	0 to 20,000	11 / 47 (23%)
	20,001 to 40,000	24 / 47 (51%)
	40,001 to 60,000	10 / 47 (21%)
	≥ 60,001	2 / 47 (4%)
Hybrid	Lohmann Brown Classic	21 / 47 (45%)
	ISA Brown	13 / 47 (28%)
	Other	13 / 47 (28%)
Phases of feed	1	10 / 46 (22%)
	2	13 / 46 (28%)
	3	22 / 46 (48%)
	≥ 4	1 / 46 (2%)
Red mite infestation[3]	Absent	16 / 43 (37%)
	Mild infestation	20 / 43 (47%)
	Severe infestation	7 / 43 (16%)

[1] All henhouses with a free-range area also provided a covered veranda
[2] Two free-range farms were organic laying hen farms
[3] Missing data on some farms

The farmers were on average 42.1 ± 1.0 years of age (range 24 to 54 years) with 18.7 ± 1.3 years of experience in laying hen farming. The average corridor width between rows (portal-type aviaries were excluded from this measurement) was 163 ± 10 cm. On average hens were fed 5.4 ± 0.1 times per day and had been fed 2.3 ± 0.1 different diets since arrival on the production farm. Red mites were found in 63% of the henhouses and were found in varying quantities on supporting beams, in nest boxes or near perches. Red mites were rarely detected on the birds. The self-reported average time spent daily in the henhouse amongst the hens (for e.g. collecting floor eggs and carcasses, system maintenance, etc.) by the farmer or stockperson was on average 108 ± 9 minutes per flock per day. Weighted for flock size, the time spent for every 1,000 hens placed was on average 3.9 ± 0.3 minutes per day.

3.3.2 PLUMAGE CONDITION AND WOUNDS

The strength of agreement for the inter-observer reliability between the two observers for scoring plumage condition was considered moderate with a score of 0.55 measured by prevalence adjusted bias adjusted kappa (PABAK). The strength of agreement for wounds was considered substantial with a PABAK of 0.71. According to Fleiss et al. (2003), a PABAK of ≥ 0.4 is considered acceptable.

The plumage condition of a total of 2150 hens from only 43 flocks was scored (Table 2). Four flocks were not scored for plumage condition due to induced moulting before or during the farm visit. Signs of feather pecking and/or feather damage were present on all observed farms. The maximum TPS of 20 points (no damage) was observed in 22 (1.0%) of the 2150 observed hens and the minimum TPS of 5 (severe damage) was observed in 11 hens (0.5%). The average TPS at flock level was 13.4 ± 0.4 with the worst flock having a TPS of 9.1 ± 0.4; the best flock had a TPS of 19.3 ± 0.1 (Figure 1). Table 2 displays the average prevalence of plumage damage and wounds to the different body parts as well as variation within and between flocks of the different scores for feathers and wounds. The mean flock prevalence range was considerable: some flocks had hardly any feather damage or wounds, whereas in other flocks (almost) all birds had feather damage or wounds. Within-flock variation was almost equal between the feather scores for the different body parts and also between the wound scores. Between-flock variation was the largest for feather scores on the back and the vent. Cloacal discharge was found in only 1.0% of all observed hens.

Table 2. Mean flock level prevalence of feather damage and wounds, average score for plumage and wounds (1 = worst score, 4 = best score), and variation within- and between-flocks.

Feathers	N flocks[1]	Mean flock prevalance[2] (%)	Range (%)	Mean flock score[3]	Within-flock variation[4]	Between-flock variation[5]
Neck	43	80.0 ± 2.8	14 to 100	2.77 ± 0.02	0.73	0.47
Back	43	70.1 ± 4.5	2 to 100	2.62 ± 0.02	0.78	0.83
Wings	43	66.8 ± 3.0	2 to 92	3.05 ± 0.02	0.75	0.37
Tail	43	91.6 ± 1.8	38 to100	2.06 ± 0.02	0.80	0.50
Vent	43	56.4 ± 4.7	2 to 100	2.83 ± 0.03	0.81	0.93
TPS[6]				13.4 ± 0.4	2.50	2.34
Wounds						
Back	47	29.9 ± 3.5	0 to 80	3.61 ± 0.01	0.58	0.35
Vent	47	26.5 ± 3.5	0 to 94	3.61 ± 0.02	0.62	0.42

[1]Only 43 from 47 flocks could be scored for plumage because 4 flocks underwent induced moulting
[2]Percentage of hens per flock with affected plumage or wounds (hens scored with either a score 1, 2 or 3)
[3]Mean score (± SE) on ordinal 4-point scale (1 to 4) at flock level
[4]Within-flock variation calculated by random effects model
[5]Between-flock variation calculated by random effects model
[6]TPS: Total Plumage Score, the scores of the five individual body parts were summed to form a non-equidistant score

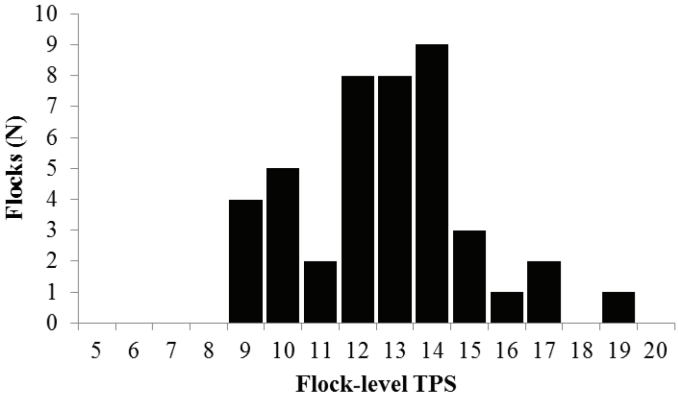

Figure 1. Distribution of total plumage score (TPS) at flock level as scored on 50 hens per flock. TPS is the cumulative score from five body parts which are given a score between 1 (heavily deteriorated plumage condition) and 4 (no feather damage).

3.3.3 RISK FACTORS

System flooring material was identified as risk factor for plumage condition, wound severity, laying rate and mortality (Table 3). Hens housed in systems with wire mesh flooring instead of plastic slatted flooring showed better feather scores on the back (P = 0.006), the wings (P = 0.002) and the vent (P = 0.010). Consequently, the TPS was also better in wire system flooring compared to plastic system flooring (P < 0.001). Hens in systems with wire mesh flooring had less severe wounds on the back (P = 0.01) and the vent (P = 0.009). In systems with wire mesh flooring, egg laying rate was higher (P = 0.013) and mortality lower (P = 0.003).

Red mite infestation was identified as risk factor for plumage condition, cloacal discharge and mortality (Table 4). Flocks in henhouses with no red mite infestation had a better plumage condition, as indicated by a higher TPS, than flocks with a mild or severe red mite infestation. Cloacal discharge score and mortality were worse in flocks with mild red mite infestation compared to flocks with no or severe red mite infestation. A red mite infestation was more frequently found in systems with plastic slatted flooring than in systems with wire mesh flooring (85.7% vs. 51.7%, P = 0.043).

Table 3. Effect of wire mesh vs. plastic slats flooring on plumage score, wound score, egg production rate and mortality at 60 weeks of age

Feather score	Wire mesh LSM ± se	Plastic slats LSM ± se	*P*-value
Neck			NS
Back	2.91 ± 0.18	2.52 ± 0.21	0.006
Wing	3.20 ± 0.06	2.88 ± 0.07	0.002
Tail			NS
Vent	2.92 ± 0.17	2.52 ± 0.24	0.010
TPS	13.8 ± 0.4	12.4 ± 0.6	<0.001
Wounds score			
Back	3.78 ± 0.07	3.49 ± 0.09	0.010
Vent	3.72 ± 0.07	3.39 ± 0.10	0.009
Egg production rate (%)	88.3 ± 0.7	85.3 ± 0.9	0.009
Mortality (%)	3.3 ± 0.4	5.4 ± 0.5	0.003

LSM = Least Squares Means; NS = Not significant; TPS = Total Plumage Score

Hens with access to a free-range area had better feather scores on the back (2.86 ± 0.25 vs. 2.57 ± 0.15, P = 0.029) and better TPS scores (14.7 ± 0.7 vs. 12.6 ± 0.4, P = 0.011) compared to hens without access to a free-range area. Better plumage scores for the tail were found with a platform in front of the nest rather than only a perch in front of the nest (2.32 ± 0.10 vs. 2.02 ± 0.10, P = 0.016). Hens housed in row-type aviaries compared to hens in portal-type aviaries showed better neck plumage scores (2.93 ± 0.07 vs. 2.26 ± 0.13, P < 0.001). The score for vent plumage improved with increasing time per day spent in the henhouse by a stockperson ($F_{4,34}$ = 4.858, P = 0.043). Mortality decreased with a better TPS ($F_{1,40}$ = 20.05, P < 0.001) and egg production rate increased with better neck plumage scores ($F_{1,41}$ = 10.61, P = 0.002).

Table 4. Effect of red mite infestation on plumage score, cloacal discharge and mortality at 60 weeks of age

	No infestation	Mild infestation	Severe infestation	
	LSM ± se	LSM ± se	LSM ± se	*P*-value
Back	3.14 ± 0.20^a	2.32 ± 0.20^b	2.32 ± 0.30^a	0.020
Vent	3.25 ± 0.23^a	2.49 ± 0.19^b	2.43 ± 0.33^a	0.045
TPS	14.7 ± 0.5^a	12.4 ± 0.4^b	12.2 ± 0.8^b	0.004
Cloaca discharge	1.998 ± 0.005^a	1.982 ± 0.004^b	1.999 ± 0.007^a	0.032
Mortality (%)	3.1 ± 0.4^a	4.6 ± 0.4^b	2.8 ± 0.7^a	0.015

LSM = Least Squares Means; TPS = Total Plumage Score
Shared superscripts indicate no significant difference (*P* > 0.05)

Furthermore, the percentage of downgraded eggs increased with increasing age of the barn ($F_{1,29}$ = 11.97, P = 0.002). Increasing numbers of feed changes during the first 42 weeks of the flocks' production period on the laying farm (e.g. transition feed, pre-start feed, peak-of-lay feed) resulted in a better plumage score in the neck ($F_{1,39}$ = 7.93, P = 0.008). Lohmann Brown Classic hens showed a higher production rate than ISA Brown hens (88.5 ± 0.9 % vs. 84.0 ± 1.0 %, P = 0.005). ISA Brown showed a better plumage score on the wings than Lohmann Brown Classic (3.08 ± 0.08 vs. 2.76 ± 0.07, P = 0.013).

We found no significant effects or interactions of the outcome variables with number of feedings per day, years of farming experience, presence of a covered veranda, age of the aviary system or flock size on any of the indicators measured.

3.4 DISCUSSION

This study shows that some health and welfare aspects for laying hens in aviaries are often not optimal and that a large variation in plumage condition, wounds, mortality and production exists between farms. The variation ranging from hardly any affected hens on a single farm up to all hens affected on another particular farm implies that there is room for improving several welfare aspects on commercial aviary farms. Previous studies on non-cage systems had also reported large variation ranging from very low to extremely high prevalence of the measured health and welfare problems (Bestman and Wagenaar, 2003; Bilčík and Keeling, 1999; Gunnarsson et al., 1999; Lambton et al., 2010; Nicol et al., 2013). We were able to confirm some previously described risk factors, but we have also identified new risk factors for plumage condition, egg production and mortality linked to housing system and management.

To demonstrate the prevalence and variation of the investigated variables between farms, this study examined a representative cross-sectional sample population of Belgian henhouses with aviaries. In our study, the aviary systems were installed on average less than 3 years before the start of this study, indicating that this study mainly represents the newer henhouses found in Belgium.

The average mortality of 4.1% at 60 weeks of age found in the present study is almost half of the 8.1% mortality from a Belgian field study conducted in 2005 and 2006 (Rodenburg et al., 2008), although the study of Rodenburg et al. studied a much smaller sample population. However, the mortality rate found in the present study is also lower than the mortality rate (%/hen housed/age) found in the systematic review by Aerni et al. (2005). This may indicate that mean mortality in aviaries has now declined to a level comparable to cage systems (Tactacan et al., 2009; Weitzenbürger et al., 2005). The comparatively low mean cumulative mortality found in the present study most likely has multi-factorial causes, such as better disease control, adjusted rearing conditions, reduced feather pecking and cannibalism, improved feeds and improved management by farmers due to increasing experience with the system.

The present study confirmed that feather pecking is still a very common problem with large variation between different henhouses, similar to many other studies as reviewed by Nicol et al. (2013). The farmers in our study reported that feather pecking and cannibalism were the main causes of hen mortality, which corresponds with the association we found, i.e. that better plumage condition was associated with decreased mortality. This finding is in accordance with other studies (Green et al., 2000; Whay et al., 2007). Although plumage condition is strongly correlated with feather pecking behaviour (Bilčík and Keeling, 1999), missing or damaged feathers are not necessarily solely the result of feather pecking. Abrasion against different parts of the environment may also lead to wear, feather damage, feather loss and increased feather pecking (Guinebretière et al., 2013; McAdie and Keeling, 2000; Tactacan et al., 2009; Tauson, 1998). The feather damage we found on the neck, back, tail and vent, though, can more confidently be attributed to pecking (Bilčík and Keeling, 1999; Uitdehaag et al., 2008). The high between-flock variation for feather scores on the back and the vent (Table 2) indicates that those body parts are possibly most affected by different risk factors encountered as compared to lower between-flock variation for the other scored body parts that apparently had more similar scores among all flocks. Wounds on the vent and the surrounding skin are typically caused by injurious vent pecking, a behaviour closely related to feather pecking that is associated with stress (Gunnarsson et al., 1999; Pötzsch et al., 2001). Even though beak trimming is the main method for reducing feather damage caused by feather pecking (Nicol et al., 2013), our results prove that this method is far from sufficient to prevent severe injury.

The present study demonstrated that wire mesh as flooring material in the aviary was associated with better plumage scores, fewer wounds, higher production rates and lower mortality compared to plastic slats as flooring material. Whay et al. (2007) reported increased feather loss in single-tier floor housing systems with plastic slats compared to wire and wooden slatted floors. However, that study pooled data from wooden and wire floors, as there was no suggestion of any difference between these two. Pötzsch et al. (2001) found that feeders located on wire mesh and drinkers located on plastic slats were risk factors for increased feather pecking and vent pecking in non-cage systems. However, the comparison between wire mesh and plastic slats alone, without feeder or drinker location, was not analysed. Differences found in plumage score between different housing systems as found in previous studies (Freire and Cowling, 2013; Lay et al., 2011; Sherwin et al., 2010; Shimmura et al., 2010) may not be solely due to being housed in either a non-cage system or a cage system, but might be caused by being housed on different flooring materials. In the quantitative comparison review by Freire and Cowling (2013), they state that hens in furnished cages are predominantly held on wire mesh floors, whereas plastic slatted flooring in cages is rare. In contrast to their findings, our study of Belgian aviary systems revealed that plastic slatted flooring in aviaries was fairly common, with one-third of all studied aviary systems having plastic slatted flooring. Previous studies might have unknowingly underestimated the effect of flooring type in the housing systems that were compared. However, the findings of Nicol et al. (2003), albeit on single-tier floor housing, were not in agreement with the present study as they demonstrated a tendency for less feather pecking in systems with plastic flooring compared to systems with wire flooring.

The effect of flooring material in the present study may have multiple explanations. Possible explanations are a difference in red mite infestation, dustbathing behaviour, in bird and system hygiene, or a combination of these factors. Deep-litter systems such as the aviary system have a high risk for infestation with the red mite ectoparasite, *Dermanyssus gallinae*; in many countries this pest has become endemic (Sparagano et al., 2009, 2014). The presence of red mites is associated with feather pecking, increased mortality due to cannibalistic behaviours, anemia, and the mites can also be vectors for several poultry diseases (Chauve, 1998; Sparagano et al., 2014; Wall and Tauson, 2013). The complex environment of an aviary is more difficult to disinfect between rounds, also providing more refuge places for the red mites as compared to cage systems (Chauve, 1998; Höglund et al., 1995; Lay et al., 2011). We found that 63% of all henhouses had a red mite infestation. Our results confirm the association between feather pecking, disease and mortality with red mite infestation, as hens in housing with red mite infestations had poorer plumage condition, increased cloacal discharge and increased mortality. Red mites can more easily find refuge under plastic slats than under wire mesh, thus rendering control more difficult (Zoons, 2015, *personal communication*). Our results show that red mite infestations were more present in aviaries with plastic slatted flooring compared to wire mesh flooring.

Although the motivation for laying hens to dustbathe is more satisfied in aviary systems than in cage systems, sham-dustbathing in the presence of litter still occurs (Colson et al., 2007; Lindberg and Nicol, 1997; Olsson et al., 2002). Sham-dustbathing on structural components of the housing system instead of in loose litter may causes abrasion of the feathers. Plastic slats are perceived to be more comfortable and give more support (Tauson, 1998), therefore sham-dustbathing may have occurred more often in those systems compared to wire mesh flooring systems, resulting in a poorer plumage condition of mainly the wings and the ventral part of the body. McAdie and Keeling (2000) demonstrated that conspecifics are more likely to peck at damaged feathers than intact feathers. Hence, increased feather damage in plastic slatted systems, caused by abrasion, may lead to increased feather pecking and thereby a worse plumage condition compared to the plumage condition of hens in wire mesh systems.

The hygiene in wire mesh flooring systems is better, because manure is more effectively trampled through the wire mesh onto the manure belt, whereas in more solid flooring types (wood, plastic) manure sticks more to the flooring surface (Akpobome and Fanguy,1992; Hughes and Black, 1974). Hens kept on plastic slats might therefore have dirtier plumage. This would be in line with Simpson and Nakaue (1987), who showed that broilers kept on wire showed less feather soiling than broilers kept on plastic-coated expanded metal. Further research is needed to demonstrate if dirty plumage indeed leads to increased dustbathing, excessive preening behaviour or makes feathers more attractive to be pecked at by conspecifics. Our study could not demonstrate this as neither dustbathing and feather pecking behaviour nor plumage cleanliness were measured during the farm visits. Poorer hygiene may also contribute to the higher mortality found in systems with plastic slatted flooring, as these systems are more difficult to clean and disinfect between successive production rounds (Shields and Greger, 2013). Comparing dustbathing and preening behaviour in systems with different flooring types and linking it with plumage cleanliness and plumage condition could elucidate the hygiene and the previously mentioned (sham) dustbathing hypotheses.

Another aspect we investigated was the presence of a free-range area. A free-range area reduces stocking density in the henhouse and also provides more opportunities to perform highly-motivated behaviours, such as exploratory behaviours and foraging, thereby reducing the motivation to feather peck (Bestman and Wagenaar, 2003; Lambton et al., 2010; Sherwin et al., 2010). Our study confirms that providing access to a free-range area had a positive effect on plumage condition. Contrary to Sherwin et al. (2010), we found no indications of a negative effect of a free-range area on the prevalence of vent pecking. In fact, we found wounds on the back to be less frequent in free-range systems. Our study did not show increased mortality due to the presence of a free-range area as reported in the review of Tauson (2005a).

Another novel housing effect we identified is the presence of a perch versus a platform in front of the nests. Hens in systems with a platform in front of the nests had better tail feather scores, suggesting that hens peck less at the tail when kept in those systems. Hens are highly motivated to explore the nest site prior to egg-laying. Agonistic and frustration behaviours occur during the nest exploration and pre-laying behaviour (Freire et al., 1996; Hunniford et al., 2014; Ringgenberg et al., 2014; Struelens et al., 2008; Wood-Gush and Murphy, 1969). A platform generally provides more space for hens to walk in front of the nest compared to a perch, and therefore creates less disturbance or competition in front of the nest when the hens need to choose a nest for egg laying.

Several dietary factors are known to influence feather pecking behaviour, such as energy levels, minerals, crude protein and fibre levels (Rodenburg et al., 2013). Switching to a new diet can change food palatability, resulting in increased or decreased food intake and perhaps in increased food competition. We found that more frequent dietary changes during the laying period were associated with decreased feather damage of the neck but made no difference to other body areas. These results are not in line with previous studies that showed that three or more changes of diet during the laying period increase the risk of feather pecking (Green et al., 2000; Pötzsch et al., 2001).

We cannot explain the difference in neck plumage score between row-type and portal-type aviaries. A possible confounding factor for the better neck feather score in row-type aviaries was system flooring, as no portal-type aviaries with plastic-slatted flooring system were included in this study. However, system flooring was not associated directly with neck feather score.

The higher laying rate found in the Lohmann Brown Classic hybrid compared to ISA Brown hens is in accordance with the production sheets provided by the respective breeders. This study found a better wing plumage score for the ISA Brown hens compared to the Lohmann Brown hens, whereas Nicol et al. (2003) found that flocks with ISA Browns were more likely to show feather pecking. Our study did not have adequate power to investigate differences between white or brown hybrids, because only three flocks had white hybrids (all Dekalb White).

3.5 CONCLUSIONS

Feather pecking and flock mortality remain common problems in aviary systems. Although the relationship between laying hen welfare, performance, and mortality and its environment is very complex and multifactorial (Appleby and Hughes, 1991), we identified several aviary housing characteristics as risk factors for feather pecking and feather damage, variability in production rate and mortality. Especially wire mesh flooring should be the preferred aviary flooring material for aviary systems as it can have both animal welfare and economic advantages as compared to plastic flooring. Investigation of the underlying background of this flooring effect and the possible effect of hygiene, disinfection and red mite control of the aviary system could further explain its effect on plumage condition, production and mortality. Providing a free-range area, adding platforms in front of the laying nests and selection of hen hybrid can also be effective in reducing certain welfare problems of laying hens.

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CHAPTER 4

RISK FACTORS ASSOCIATED WITH KEEL BONE AND FOOT PAD DISORDERS IN LAYING HENS HOUSED IN AVIARY SYSTEMS

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ABSTRACT

Aviary systems for laying hens offer space and opportunities to perform highly-motivated behaviours. However, hen welfare can be impaired due to increased risk for keel bone and foot pad disorders in those systems. This cross-sectional study (N = 47 flocks) aimed to assess prevalences of these disorders in laying hens housed in aviaries in Belgium to identify risk factors for these disorders and their relation to egg production. Information on housing characteristics and egg production were obtained through questionnaire-based interviews, farm records and measurements in the henhouse. Keel bone (wounds, hematomas, fractures, deviations) and foot pad disorders (dermatitis, hyperkeratosis) were assessed in 50 randomly selected 60-week-old laying hens per flock. A linear model with stepwise selection procedure was used to investigate associations between risk factors, production parameters and the keel bone and foot pad disorders. We found high prevalences for keel bone and foot pad disorders with considerable variation between flocks. Portal-type aviaries were associated with more keel wounds (P < 0.001), but fewer keel bone fractures (P = 0.005) and lower foot pad dermatitis severity (P = 0.004), compared with row-type aviaries. Wire mesh tiers were associated with fewer keel bone fractures (P = 0.003), compared with plastic slatted tiers. Lohmann Brown Classic hens had less foot pad hyperkeratosis (P < 0.001) and fewer caudal tip fractures (P = 0.027) compared with ISA Brown hens. Increasing corridor width was associated with fewer breast skin hematomas (P < 0.001). Presence of a nest box perch was associated with fewer keel wounds (P < 0.001) compared with a platform at the nest box entrance. Hyperkeratosis was less prevalent in free-range systems than in systems without outdoor access (P = 0.007). Percentage of downgraded eggs was negatively associated with keel bone deviations (P = 0.029) at the flock level. Keel bone and foot pad disorders are alarmingly high in aviary housing. The identification of various risk factors suggests improvements to aviary systems may lead to better welfare of laying hens.

Key words: keel bone, housing system, aviary, foot health

4.1 INTRODUCTION

Approximately 160 million laying hens were housed in non-cage systems in the EU in 2014 (EC-CIRCABC, 2014). Non-cage systems typically offer hens more space and opportunities to perform highly-motivated behaviours than cage systems (Freire et al., 2013; Lay et al., 2011; Rodenburg et al., 2005). However, due to the increased freedom of movement and access to litter, laying hens housed in non-cage systems are at a higher risk for some health problems such as keel bone and foot pad disorders (Rodenburg et al., 2008; Sherwin et al., 2010; Tauson 2005; Wang et al., 1998; Wilkins et al., 2011).

The commercial non-cage systems with the largest flocks are generally the multi-tiered aviary systems. Aviaries have a littered ground floor, on top of which a vertical metal structure with up to four tiers is erected. Aviaries typically provide resources and structural elements, e.g. feeders, drinkers and nest boxes and aerial perches, on one or more tiers (LayWel, 2006), thereby encouraging the hens to navigate through the aviary system both horizontally and vertically (Fröhlich et al., 2012). Access to the littered ground floor and multiple levels increases the total accessible surface and increases locomotory activities such as running and wing flapping. It also allows hens to perform a diverse range of highly-motivated behaviours, such as foraging, dustbathing and perching. Allowing hens the opportunity to perform those behaviours contributes to their welfare (Lister and van Nijhuis, 2012).

Keel bone injuries represent one of the most important and widespread welfare issues in commercial laying hens (Sandilands et al., 2011), as several studies have demonstrated alarmingly high prevalences of keel bone fractures in various non-cage systems (Freire et al., 2003; Käpelli et al., 2011; Rodenburg et al. 2008; Wilkins et al., 2004, 2011). Keel bone fractures are caused by high impact collisions with the housing structures, whereas deviations of the keel bone result from a prolonged pressure load on the keel during perching (Pickel et al., 2011; Sandilands et al., 2009; Scholz et al., 2008; Stratmann et al., 2015a; Toscano et al., 2013; Wilkins et al., 2011). Both high- and low-impact collisions with the system as well as prolonged pressure load can also cause hematomas and wounds of the surrounding tissue of the keel bone (Casey-Trott et al., 2015). Keel bone fractures are considered painful as well as linked to negative performance traits such as increased feed conversion, reduced egg quality, and shorter longevity (Nasr et al., 2012a, 2012b, 2013).

Hyperkeratosis, foot pad dermatitis and bumble foot are the most common foot health problems in laying hens housed in non-cage systems. Foot pad dermatitis is an inflammation of the subcutaneous tissue of the foot pad, leading to necrosis and ulcerations. If severe, this may ultimately may lead to a bulbous lesion and swelling called ‘bumble foot’ (Röngen et al., 2008; Wang et al., 1998; Weitzenbürger et al., 2006). Bumble foot is considered to be painful and critically impairs the hen’s welfare (Lay et al., 2011). In general, housing system (e.g. cage vs. non-cage), perching behaviour, wet litter, scratching, perch and flooring material and poor foot hygiene have been identified as causes of these foot health problems. Nevertheless, specific information on risk factors within aviary systems for the foot problems remains scarce (Blokhuis et al., 2007; Lay et al., 2011; Rönchen et al., 2008; Shimmura et al., 2010; Tauson and Abrahamsson, 1994, 1996; Wang et al., 1998, Weitzenbürger et al., 2006).

Farms with non-cage systems are growing in both number and size (Tuyttens et al., 2011), therefore risk factors must be identified that elicit keel bone and foot health problems and these issues should be resolved for this specific housing system. The primary aim of this study was first to perform a cross-sectional exploration of the prevalence of keel bone and foot disorders in laying hens housed in the different commercial aviary systems in Belgium. Second, we wanted to identify specific aviary system characteristics, husbandry procedures and hybrids as possible risk factors for these disorders. Finally, we wanted to investigate whether egg production is linked with these disorders.

4.2 MATERIAL AND METHODS

The 47 henhouses with aviary housing included in this study were part of a larger study on health and welfare problems of laying hens in aviaries (Heerkens et al., 2015a). Farms were visited when the laying hens were approximately 60 weeks of age (range 58 to 64 weeks of age). All visits took place between August 2012 and December 2013 and were carried out by the same two observers. During a questionnaire-based interview with the farmer, information on farm management (e.g. feeding regime, light-dark cycle, daily time spent in henhouse), henhouse (e.g. presence of covered veranda or free range, age barn), aviary system characteristics (e.g. type, manufacturer, age system, flooring type, perch orientation) and flock (e.g. hybrid, age, rearing, flock mortality, flock size) was collected (see Appendix I). Width of the corridor between two stacks was measured in henhouses with row-systems (N = 37). Portal-systems have no corridors between stacks, hence corridor width could not be measured as risk factor in portal-type aviaries. Egg production results (egg production rate, percentage downgraded eggs, percentage floor and system eggs) and cumulative flock mortality (percentage of dead hens since arrival on production farm) at 60 weeks of age were obtained from farm records.

Fifty randomly selected laying hens per henhouse were examined for keel bone and foot pad disorders. Birds were caught individually from various locations in the henhouse and from all tiers of the aviary system and were then released immediately after scoring. If a covered veranda or free-range area was available, a representative number of birds within the sample of 50 was scored. Each observer scored approximately half of the hens per farm to minimize observer bias.
Keel bones and the surrounding tissue were scored for four injuries. The skin surrounding the keel bone may show an inflammatory response (Marsell and Einhorn, 2011), which is visually scored by scoring hematomas (absent/present) and keel wounds (absent/present) on a dichotomous scale. One flock was assessed in the dark to prevent a panic reaction; this flock could therefore not be scored for hematomas due to the low light conditions. Both the middle section of the keel bone and the caudal keel tip (last 1 cm) were scored via palpation for fractures (absent/present). The palpation method has been described by Wilkins et al. (2004) and was validated for accuracy and repeatability by Petrik et al. (2013). Palpation of the keel bone reveals calcium deposits or other malformations indicative of previous fractures. The combination of both fracture scores is referred to below as ‘total fracture’. The keel bone was also examined, by both palpation and visual assessment, for deviations. Deviation from the normal shape occurs when the keel deviates from a theoretically perfect 2-dimensional straight plane in either the sagittal or transverse planes (Casey-Trott et al., 2015). Deviation was scored using a 3-point scale. A score 3 was assigned to a straight keel bone or with only a slight deviation (< 0.5 cm), a score 2 for moderate deviation of > 0.5 cm and < 1 cm, and a score 1 for severe deviation (> 1 cm). For a total keel score (TKS) the scores of hematoma, keel wounds, middle keel fracture, caudal fracture and

deviation were summed to form a non-equidistant score. For TKS the minimum score of 5 represents a wound, hematoma, middle fracture, caudal fracture and severe deviation being present, whereas the maximum TKS was 11, indicating the absence of any keel disorder.

Following inspection of the keel bone, both foot pads were inspected for foot pad dermatitis and hyperkeratosis. Foot pad dermatitis was scored on an ordinal scale (1 to 4); score 4 for no lesions on the foot pads, score 3 for a small lesion of the foot pad epithelium (< 0.2 cm), score 2 for a larger lesion (> 0.2 cm) and score 1 for swelling of the foot pad visible from dorsal view ('bumble foot'). Hyperkeratosis is proliferation of the corneus layer of toe- and metatarsal pad skin. Prevalence of hyperkeratosis was scored on a dichotomous scale: proliferation of the foot pad epithelium was either present or absent. For both the scoring of foot pad dermatitis and hyperkeratosis, the foot with the worst scores was used for further analyses. Both feet were also scored on a dichotomous scale for missing toes or wounds on toes.

Statistical analyses were performed using R 3.0.1 for Windows and StatSoft. Inc. (2012) STATISTICA, version 11. The data were considered sufficiently normally distributed, based on the graphical evaluation (histogram and QQ-plot) of the residuals. In case of post-hoc pairwise testing, *P*-values were corrected with the Tukey-Kramer adjustment for multiple comparisons. The average of 50 individual hen scores per flock for keel bone and foot pad disorder was used for statistical analyses. The presented means for the scores are the least squares means (± S.E.). Linear models were used to investigate associations between animal based scores at flock level (keel and foot pad disorders), egg production, mortality, and the possible risk factors (i.e. independent variable). A stepwise model selection procedure was used with a separate model for each dependent variable. Only significant risk factors were included in the final model (significance level of 5%). The following factors were selected as independent variables for the models: aviary type (row vs. portal), free range availability (yes or no), flooring material of the aviary tiers (wire mesh vs. plastic slats), age of the barn, age of aviary system, nest box entrance configuration (perch vs. platform), times fed per day, daily time spent in henhouse by stockperson, time spent in henhouse by stockperson per 1,000 hens present, presence of red mites ('absent'; 'mild infestation: some red mites visible, but not in large quantities'; or 'severe infestation: large clumps or quantities of red mites clearly visible') (Heerkens et al. 2015a). The two predominant hybrids, Lohmann Brown Classic (45%) and ISA Brown

(28%), were included for the risk factor analyses and interpretation of results. All other hybrids were pooled together in one group consisting of five hybrids, including four brown hybrids and one white hybrid (respectively Lohmann Brown Lite, Hy-line Brown, Bovans Brown, Novogen Brown and Dekalb White). Initially, this latter group was included in the risk factor analysis. However, due to its diversity it was excluded from any further interpretation of results. Only Lohmann Brown Classic and ISA Brown were used for further comparisons between hybrids.

4.3 RESULTS

The 47 henhouses in this study embody the majority of all aviary henhouses present in Belgium at the time this study was conducted (Heerkens et al. 2015a). The average flock age during the visit was 60.7 ± 0.3 weeks and cumulative flock mortality at 60 weeks was 4.1% (range 0.9 to 12.8%). Flock size ranged from 6,000 to 70,532 hens per henhouse (average of 31,469 hens). The mean egg production rate at 60 weeks was 87.6 ± 0.7%, with 2.2% downgraded eggs, and 1.0% “floor” or “system” eggs. All flocks except for one organic flock had been beak-trimmed and were reared in various commercial rearing housing systems that prepare pullets specifically for aviary housing during the production cycle. Nine henhouses provided access to a free-range area (19% of the flocks).

The inter-observer reliability for the palpation method between the two observers, calculated according to the PABAK values, was substantial with a score 0.85 for keel bone deviation, 0.89 for middle fracture and 0.79 for caudal fracture. Keel bone injuries were observed very frequently, with considerable variation between flocks (Table 1). Keel wounds had the lowest prevalence (17.6%), whereas overall keel bone fractures (defined as either a middle or caudal fracture or both) had the highest prevalence (82.5%). The mean TKS was 8.40 ± 0.03 with a within-flock variation of 1.26 and between-flock variation of 0.37 (calculated by random effects model). Only 4.3% of all hens had no keel injuries (TKS = 11), while 1.6% of all hens scored positive for all keel injuries (TKS = 5). Deviations of the keel bone (either mild or severe) were observed in 59.8% of all scored hens. There was also considerable variation between flocks for the prevalence of the different foot disorders (Table 1). Foot pad hyperkeratosis prevalence was the highest with 42.0%, foot pad dermatitis prevalence was 27.6%, whereas bumble foot prevalence was only 1.2%. Hens with missing toes or toe wounds were very rarely seen (both < 0.1%).

Table 1. Mean prevalence (± S.E.) at flock level for keel bone injuries and foot health as measured in 50 randomly selected hens at 60 weeks of age in 47 flocks housed in Belgian commercial aviary systems

Injuries	Prevalence (%)	Range
Keel		
Hematomas	41.2 ± 2.3	6 tot 74
Wounds	17.6 ± 2.0	0 to 62
Middle fracture	65.6 ± 1.9	36 to 92
Caudal fracture	44.3 ± 2.4	6 to 78
Overall fracture	82.5 ± 1.6	60 to 100
Mild deviation	31.8 ± 1.4	12 to 52
Severe deviation	28.0 ± 1.5	10 to 62
Foot pads		
Hyperkeratosis	42.0 ± 2.2	14 to 70
Dermatitis	27.6 ± 2.3	0 to 70
Bumble foot	1.2 ± 0.4	0 to 16

Aviary housing and husbandry characteristics that could be identified as risk factor for some of the keel and foot disorders were aviary type, system flooring material, hybrid, corridor width, nest box entrance configuration, free-range availability, and time of stockperson in henhouse. In portal-type aviaries, keel wounds were more prevalent (37.5% vs. 12.6%, P < 0.001), middle keel fractures were less prevalent (55.6% vs. 68.3%, P = 0.005), and foot pad dermatitis scores were better (3.78 ± 0.08 vs. 3.52 ± 0.04, P = 0.004) compared to row-type aviaries. In aviaries with wire mesh flooring, caudal tip fractures were less prevalent (36.5% vs. 50.0%, P = 0.019) and total fracture prevalence was also lower (76.1% vs. 85.5%, P = 0.003) compared to plastic slatted flooring. In Lohmann Brown Classic hens, fewer caudal tip fractures (36.4% vs. 50.6%, P = 0.027) and less foot pad hyperkeratosis (27.3% vs. 51.6%, P < 0.001) were found compared to ISA Brown hens. The corridor width was negatively associated with prevalence of hematomas on the keel bone skin ($F_{1,34}$ = 16,68, P <0.001). Keel wounds were less prevalent with a perch instead of a platform in front of the nest box (20.4%

vs. 29.7%, *P* = 0.006) and foot pad hyperkeratosis was less prevalent when hens had access to a free-range (33.5% vs. 46.7%, *P* = 0.007). A high TKS, representing fewer keel disorders, showed a positive association with the time the stockperson spent in the henhouse per day ($F_{3,42}$ = 5.291, *P* = 0.035). This appeared to suggest that the more time the stockperson spent in the henhouse, the fewer keel bone disorders would occur. However, this association disappeared when time was corrected for every 1,000 hens housed.

At flock level, the percentage of downgraded eggs was negatively associated with percentage of hens with a keel bone deviation ($F_{2,29}$ = 5.28, *P* = 0.029). A trend to a negative association between egg production rate and keel wounds (P = 0.059) was observed. We found no other significant associations between keel or foot disorders with egg production or mortality.

4.4 DISCUSSION

This field study demonstrated that mean flock level prevalences for several keel and foot disorders show large variation between different aviary henhouses in Belgium. This study also identified several commercial aviary system characteristics, management and hybrids as risk factors for some of these disorders in laying hens. The alarmingly high prevalence of keel bone fractures is similar to those found in previous studies in non-cage systems (Freire et al., 2003; Käpelli et al., 2011; Petrik et al., 2015; Rodenburg et al., 2008; Sherwin et al., 2010; Tarlton et al., 2013; Wilkins et al., 2004). Previous studies that assessed foot pad problems in non-cage systems report similar prevalences to those found in our field study (Simonsen et al., 1980; Wang et al., 1998; Weitzenbürger et al., 2006).

To our knowledge, this study is the first to report the type of aviary, (row vs. portal-type aviaries) as risk factor for keel and foot disorders. A much higher prevalence of keel wounds was found in portal-type aviaries, whereas middle keel fractures were less prevalent in those aviary types. These differences may relate to differences in bird movements between the aviary types. Laying hens originate from terrestrial birds that prefer ground-based movements, i.e. walking, running and jumping (Dial and Jackson, 2011). Hens also prefer wing-assisted incline running (WAIR) rather than flying to reach an elevated area. Moreover, flight is used almost exclusively for escape behaviour (Harlander-Matauschek et al., 2015). Compared to their ancestors, modern hybrids also have poorer flight control due to the higher wing loading and heavier body weight (Moinard et al., 2004). Due to the step-wise design of portal-systems, movements in portal-type aviaries seem to comprise shorter distances and allow more walking, jumping and WAIR-like movements, compared to movements in row-type aviaries, where hens make more long descents from the tiers to the ground floor. The type of movements within the portal-type aviary may lead to more low-impact collisions with the system, resulting in more keel wounds due the prominent forward positioning of the keel bone. The lower prevalence of middle keel fractures in portal-type aviaries is probably due to higher levels of WAIR and short-distance movements, leading to fewer high-impact collisions. Fewer flying behaviours and more WAIR between tiers may also explain the lower prevalence of keel bone fractures (Stratmann et al., 2015a). In that study, aviaries were equipped with ramps between tiers to allow more WAIR between tiers. Provision of those ramps resulted in 45% fewer falls, 59% fewer collisions and consequently 23% less keel bone fractures, although the prevalence of keel bone fractures remained high.

Hens in row-type aviaries appear to spend more time on the ground floor (*personal observations*, not measured). This could explain the higher prevalence for foot pad dermatitis we found in row-type aviaries, as contact with litter leads to poorer foot pad hygiene and has been reported to increase foot pad dermatitis and bumble foot (Tauson and Abrahamsson, 1994; Blokhuis et al., 2007).

Another characteristic that can differ between aviary systems is the flooring material of the tiers in the aviary stacks that are constructed of either metal wire or plastic slats. Effects of this flooring material on keel and foot disorders were also found. Hens probably can more effectively grasp the narrow wire mesh with their claws in comparison to the generally wider plastic slats. Grasping the wire mesh may improve control over the landing, leading to fewer accidents and collisions and consequently resulting in fewer caudal tip fractures and a lower total fracture prevalence. Further research is needed to investigate this hypothesis.

When possible, hens will directly jump to a designated area (e.g. a perch, tier or other stack); when the jumps comprise shorter distances, the chance of a correct landing increases. Failures in landings inevitably leads to collisions with the system (Scott and Parker, 1994; Scott et al., 1997; Stratmann et al., 2015a). The shorter the distance, the more hens may directly jump from stack to stack, thereby increasing the risk of a keel bone disorder. In the present study, we could only confirm this hypothesis by demonstrating the negative association between hematomas of the keel bone skin and corridor width between stacks. No other keel bone disorders were associated with the corridor width. Further studies of birds' movement patterns within and between the tiers and stacks in aviaries could possibly demonstrate the effect of long-, and short-distance jumps and flights on the prevalence of keel bone disorders.

Hens in systems with a perch in front of the nest had fewer keel wounds. Pre-nesting behaviours, such as nest-site inspection, are highly-motivated behaviours (Freire et al., 1996; Ringgenberg et al., 2014; Struelens et al., 2008) that involve repeatedly entering and exiting the nest boxes. Providing a perch rather than a platform in front of the nest box apparently causes fewer collisions or scraping of the keel skin, resulting in fewer wounds sustained when housed in those systems.

The hybrid effect on fractures of the caudal tip of the keel bone and on footpad hyperkeratosis might be due to genetic predisposition, differences in bone strength or differences in perching behaviour. However, further research is still needed to more specifically identify the behavioural, physiological and genetic traits and differences between those traits (Abrahamsson and Tauson, 1995, 1996; Käpelli et al. 2011; Kjaer, 2000; Schrader and Müller, 2009; Wilkins et al., 2011).

Flocks with access to a free-range area had a lower prevalence of foot pad hyperkeratosis, but there was no difference in foot pad dermatitis or bumble foot prevalence compared to hens without access to a free-range area. Hence we could not confirm the findings of Shimmura et al. (2010) who found higher foot pad dermatitis in free-range systems. This is probably also closely related with free-range quality (grass cover, moisture, cleanliness) and usage.

The negative association between keel bone deviations and downgraded eggs indicates that flocks with a higher percentage of straight keel bones produce fewer downgraded eggs. Nasr et al. (2012a) found that hens with reduced keel bone strength had reduced egg quality. Keel bone deviations were not measured in their study, but the reduced keel bone strength may have caused more keel bone deviation in combination with a prolonged pressure load during perching (Pickel et al., 2011). In turn, stronger keel bones could have been associated with fewer deviations and increased egg quality, similar to our findings.

4.5 CONCLUSIONS

Keel bone injuries can reach alarmingly high levels in flocks of laying hens housed in commercial aviary systems. Foot pad problems are also highly prevalent in those housing systems. Although the relations between these welfare problems and the hens' environment are very complex and multifactorial, several risk factors for keel bone and foot pad disorders have been identified. We could not demonstrate that the impaired welfare due to keel bone and foot pad disorders also resulted in noticeable effects on egg production. Based on our findings we conclude that keel disorders may be reduced by selecting an aviary design that best matches hens' preferred manner of moving. Foot disorders may be reduced by selecting the most appropriate aviary construction materials (e.g. perches, aviary flooring) and maintaining dry litter. Genetic selection of laying hen hybrids may also further reduce the genetic predisposition for sustaining keel and foot disorders.

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CHAPTER 5

RAMPS AND HYBRID EFFECTS ON KEEL BONE AND FOOT PAD DISORDERS IN MODIFIED AVIARIES FOR LAYING HENS

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ABSTRACT

Non-cage systems provide laying hens with considerable space allowance, perches and access to litter, thereby offering opportunities for highly-motivated behaviours. Conversely, these typical characteristics of non-cage systems also increase the risk of keel bone and foot pad disorders. The aim of this study was twofold: 1) to investigate if providing ramps between perches (factor 'Housing') reduces keel bone and foot pad disorders and 2) to test for genetic predisposition by comparing two different layer hybrids (factor 'Hybrid'). In a 2x2 design, 16 pens were equipped either with or without ramps between perches and nest boxes (8 pens/treatment), and housed with either 25 ISA Brown or Dekalb White birds per pen (in total 200 birds/hybrid). Keel bone injuries and foot health were repeatedly measured via palpation and visual assessment between 17 and 52 weeks of age (factor 'Age') and daily egg production was recorded. The relationships between the dependent response variables (keel bone and footpad disorders, egg production) and independent factors (age, ramps, hybrid) were analysed using generalized linear mixed models and corrected for repeated measures. Ramps reduced keel bone fractures ($F_{1,950}$=45.80, P < 0.001), foot pad hyperkeratosis ($F_{1,889}$=10.40, P = 0.001), foot pad dermatitis ($F_{1,792}$=20.48, P< 0.001) and bumble foot ($F_{1,395}$=8.52, P< 0.001) compared to pens without ramps. ISA Brown birds sustained more keel bone fractures ($F_{1,950}$=33.26, P < 0.001), had more foot pad hyperkeratosis ($F_{1,889}$=44.69, P < 0.001) and laid more floor eggs ($F_{1,1883}$= 438.80, P <0.001), but had fewer keel bone deviations ($F_{1,1473}$=6.73, P < 0.001), fewer cases of foot pad dermatitis ($F_{1,792}$= 19.84, P <0.001) and no bumble foot as compared to Dekalb White birds. Age, housing and hybrid showed several interaction effects. Providing ramps proved to be very effective in both reducing keel bone and foot pad problems in non-cage systems. Keel bone and foot pad disorders are related to genetic predisposition. These results indicate that adaptation of the housing systems and hybrid selection may be effective measures in improving laying hen welfare.

Key words: keel bone disorders, foot pad disorders, aviary housing, ramps, hybrids

5.1 INTRODUCTION

Laying hens in non-cage housing systems are offered more space and opportunities to perform highly-motivated behaviours in comparison to cage systems. At the same time, however, certain specific non-cage housing characteristics may also impair laying hen welfare by increasing the risk for keel bone and foot pad disorders (EFSA, 2005; Heerkens et al., 2016a; Rodenburg et al., 2008; Sandilands et al., 2009).

Keel bone disorders are a serious concern for the welfare of laying hens, particularly when housed in non-cage systems (FAWC, 2010; Harlander-Matauschek et al., 2015; Sandilands et al., 2009). This concern is based on field studies in non-cage systems reporting that keel bone disorders can reach extremely high prevalences, affecting from 56% up to 97% of a flock near the end of a production cycle (Heerkens et al., 2016a; Petrik et al., 2015; Rodenburg et al., 2008; Wilkins et al., 2011). A distinction can generally be made between keel bone fractures and keel bone deviations. After the healing process fractures typically manifest themselves as callus formations at the fracture sites and may involve sharp, unnatural shearing or folding and/or fragmentation of the bone. Keel bone deviations are abnormalities in the shape of the keel bone, manifested as the bone's deviation from a theoretically flat, 2-dimensional straight plane in either the transverse or sagittal plane (Casey-Trott et al., 2015). Other, more superficial injuries of the tissue surrounding the keel bone are skin wounds and hematomas (Heerkens et al., 2016a). Fractures and deviations are believed to have different causes. Due to the anatomical prominent position of the keel bone it is usually the first point of when the bird collides with an object or surface (due to a fall or bad landing). Consequently, fractures are likely the result of (repetitive) collisions with structural elements of the housing systems. In contrast, deviations seem to originate mainly from prolonged pressure-load during perching (Pickel et al., 2011; Sandilands et al. 2009; Wilkins et al. 2011). Bone strength is influenced by genetics, environment and physical exercise (Fleming et al., 2007; Leyendecker et al., 2005) and affects susceptibility to keel bone disorders.

The major foot pad disorders in laying hens are hyperkeratosis, dermatitis and bumble foot. Hyperkeratosis is proliferation of the corneus layer of the foot pad skin and is caused by prolonged pressure load on foot pads during standing, grabbing and perching on a wire floor (Rönchen et al., 2008; Weitzenbürger et al., 2005). Foot pad dermatitis is a term for infected lesioning of the epithelium of the metatarsal and toe pads, and is often accompanied with inflammation (Wang et al., 1998). Bumble foot manifests as severe swelling and inflammation of the foot pad. This ailment is perceived to be particularly painful (Tauson and Abrahamsson, 1994). The prevalences of these foot pad disorders vary in non-cage systems and are associated with perch design, (wet) litter and hybrid (Pickel et al. 2011; Abrahamsson and Tauson, 1995; Wang et al., 1998; Weitzenbürger et al., 2006).

Perch arrangements and ramps between perches affect the rate of successful landings and are associated with keel bone disorder prevalence (Scott et al., 1997; Stratmann et al., 2015a). Hens are not accomplished fliers, preferring wing-assisted-incline-running (WAIR) and walking to flying when attempting to reach higher or lower areas in their environment (Dial, 2003; Dial and Jackson, 2011; Sandilands et al., 2009; Stratmann et al. 2015a). Ramps facilitate walking and WAIR to all areas, eliminating the necessity to fly or jump and therefore reducing the number of falls and collisions (Kozak et al., 2015; Stratmann et al., 2015a).

The aim of our study was to investigate in more detail which particular keel bone disorders are affected by providing ramps in experimental aviary systems and how ramps may affect foot pad disorders. Genetic predisposition may affect the susceptibility for sustaining keel bone and foot pad disorders (Abrahamsson and Tauson, 1995; Vits et al., 2005). Therefore, a second aim was to assess how various keel bone and footpad disorders in a brown (ISA Brown, IB) and white (Dekalb White, DW) commercial hybrid are affected by the presence of ramps. We hypothesized that laying hens housed in pens equipped with ramps would sustain fewer keel bone fractures and deviations. We expected ramps to increase foot pad hyperkeratosis due to mechanical compression on load on the foot pads (Rönchen et al., 2008; Tauson and Abrahamsson, 1994; Weitzenbürger et al., 2006) and decrease the prevalence of dermatitis due to improved foot pad hygiene (Tauson and Abrahamsson, 1994, 1996). The direction of the genetic effect was harder to predict. White hybrids are considered to have better flight and 3D-movement skills and have a lower bodyweight than brown hybrids, and thus would possibly encounter fewer collisions and collisions with a lower impact (Scholz et al., 2014; Toscano et al., 2013). The white hybrids also have weaker bones and thus are at increased risk for fractures and deviations (Habig and Distl, 2013).

5.2 MATERIALS AND METHODS

5.2.1 BIRDS AND HOUSING

All animal procedures were approved by the Animal Ethics Committee of the Institute for Agricultural and Fisheries Research (ILVO) (EC2014/223). Dekalb White (DW) and ISA Brown (IB) chicks were reared on a commercial rearing farm in a commercial NivoVaria® system (Jansen Poultry Equipment, Barneveld, the Netherlands) from day 1 until 17 weeks of age and pullets had access to wood shavings on the litter floor. This rearing system provides feeders and drinker lines at higher levels which the pullets can reach by jumping or by using diagonally placed platforms. The strategical placement of feeders and drinker lines encourages and trains pullets to seek for system utilities at higher levels, thereby preparing them for aviary housing systems during the production phase. All hens were beak trimmed at the hatchery. At the age of 17 weeks, 200 hens per hybrid were transported from the rearing farm to ILVO's poultry experimental facility (Melle, Belgium) and distributed randomly across 16 pens containing wood shavings. The experimental facility was equipped with dynamic ventilation with lateral air inlets at both sides. The ventilation rate could vary from 0 m^3/hour to the maximum ventilation rate of 25,000 m^3/hour, depending on the temperature. The temperature was recorded by means of a min/max thermometer. The indoor temperature was kept as close as possible to approximately 20°C. The hens were kept under conventional conditions for lighting (6h dark, 18h light). There was some variation in temperature and light level within the house, but this was equally distributed across treatments. Each pen measured 220 (L) x 350 (W) x 220 (H) cm, and housed 25 hens (3080 cm^2/hen usable space). Each pen housed either DW or IB hens; all hens were banded with colored and numbered plastic leg rings. Each pens was equipped with three wooden perches placed stepwise at respectively 60, then 120 and at highest 180 cm above the littered floor (Figure 1). Two wooden perches were placed to enable stepwise access to the nest box at 50 and 100 cm above the wooden slatted tier. The slatted tier measured 220 x 100 x 45 cm. All perches were rectangular (104 x 5 x 3 cm) with rounded edges. Three nest boxes (35 x 35 x 30 cm) lined with AstroTurf® (www.astroturfpoultrypads.com/) were installed 110 cm above the slatted tier. Half of the pens were fitted with ramps that connected the floor, tier and perch with the adjacent perch, creating a continuous pathway that enabled hens to reach the littered floor, tier, perches or nest box without having to jump or fly (Figure 1).

Specifically, one ramp connected the litter floor with the lowest perch, one ramp connected the lowest and the middle perch, one ramp connected the middle and the highest perch, one ramp connected the slatted tier with the first perch leading toward the nest box and one ramp connected that perch with the perch in front of the nest box. The ramps were 20 cm wide and constructed of galvanized metal wire. They were placed at a 45^{o} angle between levels. Furthermore, pens were equipped with a feeding trough (125 cm) and a bell drinker (120 cm circumference) placed in locations away from the perches to prevent hens from defecating in the bell drinker or feeding trough. Hens were fed a pre-lay feed for two weeks after arrival, from 19 weeks of age onwards a standard layers ration (as finely ground meal) was fed during the production cycle. Feed and water were provided *ad libitum*. A 2x2 design, with Housing (no ramps (NR) and ramps (R)) as the first factor and Hybrid (DW and IB) as the second factor resulted in 4 treatments; NR-DW, R-DW, NR-IB, R-IB. Erroneously Hybrid was not equally distributed over the Housing treatments, eventually leading to 3 R-IB pens, 3 NR-DW pens, 5 NR-IB pens and 5 R-DW pens.

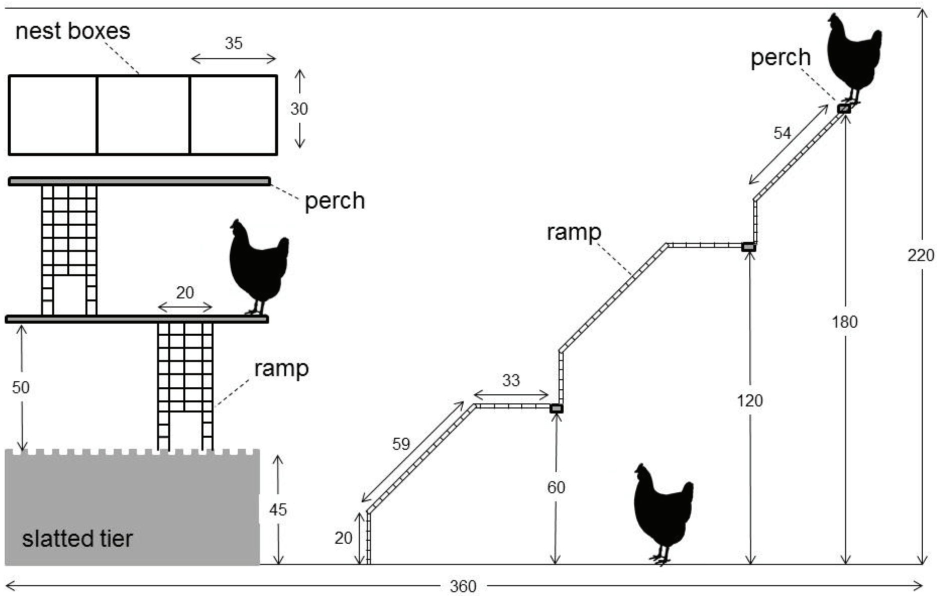

Figure 1. Simplified schematic side view and measurements (in cm) of experimental pen including ramps between floor or slatted tier towards perches and nest boxes.

5.2.2 DATA COLLECTION

During daily inspection from 20 weeks to 52 weeks of age, the number of first quality eggs and floor eggs, and mortality were recorded at pen level. Production rates (expressed as number of eggs laid per day per hen present) were calculated per pen per week.

KEEL BONE DISORDERS

From 19 weeks of age onwards, on six hens per pen keel bone injuries were scored on a 5-weekly interval, according to the method of Heerkens et al. (2016a) (chapter 4). Briefly, keel bones and their surrounding tissue were inspected for 1) skin hematomas, 2) wounds, 3) middle keel bone fractures, 4) caudal keel bone fractures, and 5) keel bone deviations. Hematomas of the skin surrounding the keel bone and wounds or scabs of the skin on the keel bone were scored binomially and recorded as either absent (0) or present (1). Palpation of the keel bones was performed by running two fingers along the bone (Wilkins et al. 2004) and was again scored for the presence (1) or absence (0) of fractures of the middle section (middle fracture) and fractures of the caudal end (last 1cm) (caudal fracture). The combined of both possible keel bone fractures was transformed into a binomial 'Total Fracture' score (i.e., any fracture present (1) or no fractures (0)). Deviations from theoretically perfect 2-dimensional straight plane in either the sagittal or transverse planes (Casey-Trott et al., 2015; Heerkens et al., 2016a) were scored visually as well as via palpation. Deviation was scored as a straight keel bone (straight or < 0.5 cm deviation), a keel with mild deviation (deviation between 0.5 and 1 cm) or a keel with severe deviation (> 1 cm deviation). All observations were conducted by the same experienced observer. At 53 weeks of age 115 hens were euthanized and taken to a dissection room in which the observer, blinded to the housing treatment, scored the keel bones for fractures and deviation. After dissection the keel bones were stored at -20^{o}C and examined a few weeks later for the presence or absence of (old) fractures and deviation to calculate the accuracy of the palpation method (Casey-Trott et al., 2015; Petrik et al., 2003; Wilkins et al., 2004). During the post-dissection keel bone assessment the observer was blinded to the pre-dissection score as well as blinded to both treatments (housing and hybrid).

Foot pad health was first scored at 29 weeks of age on the same six birds that had been assessed for keel bone disorders and thereafter at the 5-weekly interval as previously described for keel bone disorders. Foot pad health was scored by visual assessment of both feet for hyperkeratosis, foot pad dermatitis and bumble foot (Heerkens et al., 2016a). Hyperkeratosis, defined as proliferation of the corneus layer of toe- and metatarsal skin of the foot pads (Weitzenbürger et al. 2005), as present (1) or absent (0). Foot pad dermatitis was scored as (i) no dermatitis, (ii) mild dermatitis with a small lesion (< 0.2 cm) of the foot pad epithelium, or (iii) severe dermatitis with a large lesion (> 0.2 cm). Afterwards, the scores were transformed to a "Dermatitis present/absent" score, where no dermatitis was (0) and mild and/or severe dermatitis both counted as present (1). For both hyperkeratosis and dermatitis, no discrimination was made between the metatarsal pads and the toe pads. Bumble foot was defined as a high-grade swelling of the metatarsal foot pad visible from dorsal view and was again scored as present (1) or absent (1).

The observer was partially blinded to the housing treatment (R-NR) during the keel bone and foot pad assessments as the observations were executed in the walking corridor outside the pen. It was not possible to have the observer blinded for the hybrid treatment (DW-IB).

FOOT PAD DISORDERS

Foot pad health was first scored at 29 weeks of age on the same birds on which the keel bone damage assessment was performed and thereafter at the regular 5-weekly interval as previously described for keel bone injuries. Foot pad health was scored by visual assessment of both feet for hyperkeratosis, foot pad dermatitis and bumble foot (Heerkens et al., 2016a). Hyperkeratosis is defined as proliferation of the corneus layer of toe- and metatarsal skin of the foot pads (Weitzenbürger et al., 2005) and was scored on a binomial scale. Foot pad dermatitis was scored as (i) no dermatitis, (ii) mild dermatitis with a small lesion (< 0.2 cm) of the foot pad epithelium, or (iii) severe dermatitis with a large lesion (> 0.2 cm). The score for either a mild and/or severe was also transformed into a binomial 'Dermatitis Y/N' score. For both hyperkeratosis and dermatitis no discrimination was made between the metatarsal pads and the toe pads. Bumble foot was defined as a high-grade swelling of the metatarsal foot pad visible from dorsal view and scored on a dichotomous scale.

5.2.3 STATISTICAL ANALYSIS

To analyze the relationship between continuous dependent response variables (e.g. egg laying rate, percentage floor eggs) and the independent variables (age, housing, hybrid and their interactions) linear mixed models (LMM) were used. Housing design (R and NR) and Hybrid (DW and IB) were fixed effects in all models with pen as random factor. Non-significant interactions were removed from the final model (significance level of 0.05). The analyzed data were considered sufficiently normally distributed, based on the graphical evaluation (histogram and QQ-plot) of the residuals.

For the keel bone injury measurements initially six focal birds per pen were monitored according to identification of the leg rings (combination of color and number), however, due to the leg rings getting lost and numbers wearing off, hens could not unmistakably be identified anymore. For this reason repeated measures on individuals were not taken into account in the analyses. To analyze the relationship between the binomial response variables (keel bone and foot pad disorders) and independent variables (age, housing, hybrid and their interactions), similar generalized linear mixed models (GLMM) with the logit-link were used.

In case of posthoc pairwise testing, p-values were corrected with the Tukey-Kramer adjustment for multiple comparisons. In case of significant interactions with age, post-hoc tests were performed at 29, 39 and 49 weeks of age. All analyses were performed using the GLIMMIX procedure of SAS 9.4 software (SAS Institute Inc., Cary, NC).

5.3 RESULTS

5.3.1 PRODUCTION

Most floor eggs were laid in the NR-IB pens at 29 and 39 weeks of age and the lowest amount of floor eggs were laid in the NR-DW pens at 29 and 39 weeks of age, whereas at 49 weeks most floor eggs were laid in the R-DW pens (Table 1, Age*Housing*Hybrid, $P < 0.001$). The direction of the Housing*Hybrid interaction ($P < 0.001$) on the production of first quality eggs was not consistent over time between treatments (Table 1). Nonetheless, most first quality eggs were laid in the NR-DW pens at all ages, but the difference was not significant at all ages. The laying rate differences decreased with age for the four treatments (Age*Housing*Hybrid, $P = 0.002$).

Table 1. Least squares means (LSM) of egg production (% ± S.E.) at 29, 39 and 49 weeks of age for the four different treatments.

	Treatment			
	NR-DW	R-DW	NR-IB	R-IB
Age: 29 weeks				
Floor eggs	0.4 ± 0.2 ^{d}	1.8 ± 0.1 ^{c}	5.6 ± 0.1 ^{a}	3.3 ± 0.2 ^{b}
1st quality eggs	95.0 ± 0.4 ^{a}	94.1 ± 0.3 ^{a}	85.0 ± 0.3 ^{c}	86.9 ± 0.4 ^{b}
Egg rate	95.7 ± 0.4 ^{a}	96.0 ± 0.2 ^{a}	91.3 ± 0.2 ^{b}	90.9 ± 0.4 ^{b}
Age: 39 weeks				
Floor eggs	0.2* ± 0.1 ^{c}	1.1 ± 0.1 ^{b}	2.2 ± 0.1 ^{a}	1.2 ± 0.1 ^{b}
1st quality eggs	96.9 ± 0.3 ^{a}	95.5 ± 0.2 ^{b}	91.5 ± 0.2 ^{d}	92.8 ± 0.3 ^{c}
Egg rate	97.1 ± 0.2 ^{a}	96.8 ± 0.2 ^{a}	94.2 ± 0.2 ^{b}	94.6 ± 0.2 ^{b}
Age: 49 weeks				
Floor eggs	0.4 ± 0.2 ^{b}	1.5 ± 0.1 ^{a}	0.0* ± 0.0 ^{b}	0.2 ± 0.2 ^{b}
1st quality eggs	95.4 ± 0.4 ^{a}	93.4 ± 0.3 ^{b}	94.5 ± 0.3 ^{a}	95.3 ± 0.4 ^{a}
Egg rate	96.0 ± 0.4 ^{a}	95.1 ± 0.2 ^{ab}	94.6 ± 0.2 ^{b}	95.9 ± 0.4 ^{a}

Shared superscripts (a-d) within rows indicate no significant difference (P > 0.05)
Treatments: NR-DW=No ramps - Dekalb White; R-DW=Ramps - Dekalb; NR-IB=No ramps - ISA Brown; R-IB=Ramps - ISA Brown.
* LSM values are the result of the statistical analysis and resulted in small negative values for floor eggs for NR-DW at 39 weeks and NR-IB at 49 weeks. In reality this is not possible for those variables, therefore raw mean of floor eggs for NR-DW at 29 weeks and NR-IB at 49 weeks are given in Table 1.

5.3.2 KEEL BONE DISORDERS

All keel bone disorder prevalences increased with age (Table 2, Figure 2 and 3). Ramps reduced the prevalence of middle fractures at all ages. Caudal fractures increased more with age when no ramps were provided. Total fractures showed a lower prevalence in hens in pens with ramps at all ages. The presence of ramps resulted in a lower prevalence of mild deviations at all ages. Both keel hematomas and wounds showed a Hybrid effect with DW having a higher prevalence for both disorders at all ages. An Age*Hybrid interaction was found for middle fractures demonstrating a more rapid increase of fractures in DW with age compared to IB. Caudal fractures were less prevalent in DW at all ages and DW scored lower for total fractures at all ages.

For both mild deviation and severe deviation an Age*Hybrid interaction revealed increasing deviation over time for both hybrids, with DW having a significant higher mild and severe deviation prevalence after the continued laying period (49 weeks of age) than IB. There were no Housing*Hybrid interactions for any of the keel bone disorders. The accuracy of the palpation method was 69.9% for deviation, 70.4% for middle fractures, 70.4% for caudal fractures and 87.8% for fractures prevalence.

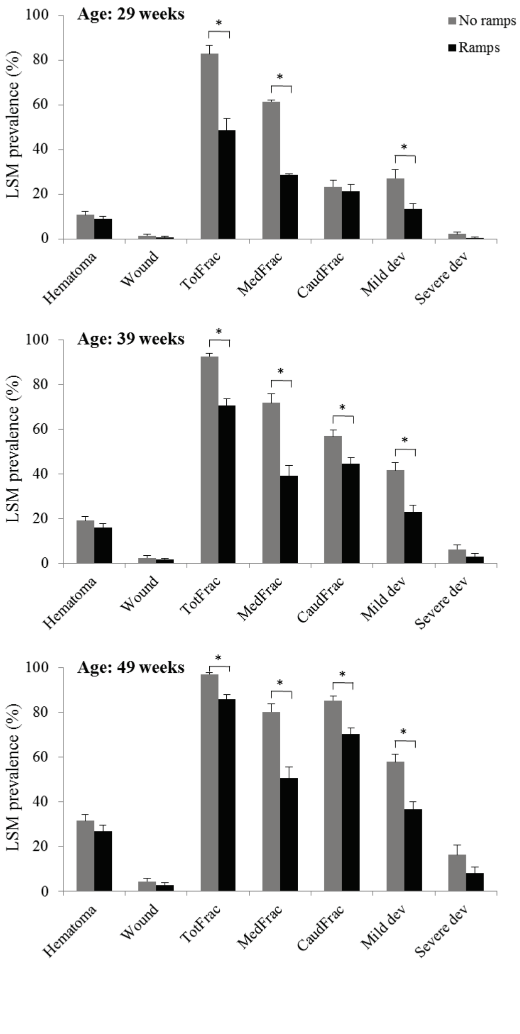

Figure 2. Least squares means percentages (± S.E.) for keel bone disorders (TotFrac=total fracture, MedFrac=middle fracture, CaudFrac=caudal fracture, Mild dev=mild deviation, Severe dev=severe deviation) in pens with ramps or without ramps. * indicates LSM differ significantly (P < 0.05) between the pens with or without ramps for the respective disorder.

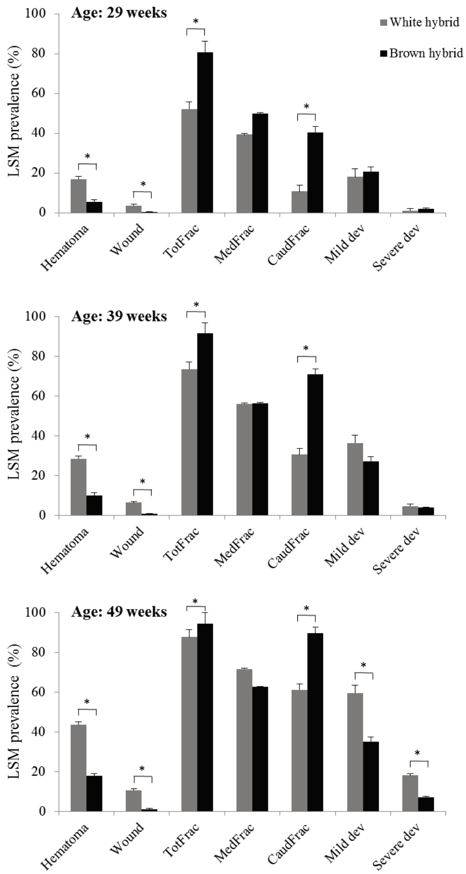

Figure 3. Least squares means percentages (± S.E.) for keel bone disorders (TotFrac= total fracture, MedFrac=middle fracture, CaudFrac=caudal fracture, Mild dev=mild deviation, Severe dev=severe deviation) Dekalb White and ISA Brown hens. * indicates LSM differ Significantly (P < 0.05) between the two hybrids for the respective disorder.

Table 2. Mean percentage of prevalence (± S.E.) and significance of effects on keel bone and footpad disorders at 29, 39 and 49 weeks of age

	Age			Significance of effects					
	29 wks	39 wks	49 wks	Age	Housing	Hybrid	Age *Housing	Age *Hybrid	Housing *Hybrid
Keel bone disorders									-
Hematoma	16.7 ± 3.8	21.9 ± 4.2	22.9 ± 4.3	<0.001	0.204	<0.001	-	-	-
Wounds	6.3 ± 2.5	1.0 ± 1.0	4.2 ± 2.1	<0.001	0.279	<0.001	-	-	-
Middle fracture	45.0 ± 6.5	49.0 ± 5.1	66.7 ± 4.8	<0.001	<0.001	0.017	-	0.006	-
Caudal fracture	28.3 ± 5.9	54.2 ± 5.1	75.0 ± 4.4	<0.001	0.112	<0.001	0.012	-	-
Total Fracture	60.0 ± 6.4	76.0 ± 4.4	86.5 ± 3.5	<0.001	<0.001	<0.001	-	-	-
Mild deviation	28.1 ± 4.6	26.0 ± 4.5	32.3 ± 4.8	<0.001	<0.001	0.030	-	0.002	-
Severe deviation	2.1 ± 1.5	5.2 ± 2.3	16.7 ± 3.8	<0.001	0.093	0.109	-	0.030	-
Footpad disorders									
Hyperkeratosis	25.0 ± 4.4	50.0 ± 5.1	46.9 ± 5.1	<0.001	0.001	<0.001	<0.001	-	-
Mild dermatitis	12.5 ± 3.3	7.3 ± 2.7	10.4 ± 3.1	<0.001	<0.001	0.623	-	0.012	0.001
Severe dermatitis	22.9 ± 4.3	24.0 ± 4.4	24.0 ± 4.4	0.305	0.028	0.720	-	0.052	0.003
Dermatitis Y/N	36.5 ± 4.9	35.4 ± 4.9	38.5 ± 5.0	0.060	<0.001	<0.001	0.002	-	-
Bumble foot	1.0 ± 1.0	4.2 ± 2.1	4.2 ± 2.1	0.554	0.004	-[1]	-	-[1]	-

[1] Bumble foot was not observed in ISA Brown. Housing effect was only tested within Dekalb White.

5.3.3 FOOT PAD DISORDERS

Dermatitis prevalence was higher in pens without ramps at all ages than in pens with ramps, but this difference decreased with age (Table 2, Figure 4). The presence of ramps resulted in a lower bumble foot prevalence in DW at all ages, whereas bumble foot was not observed in IB hens. Hyperkeratosis was more prevalent in IB at all ages and the prevalence of hyperkeratosis increased more with age in pens without ramps versus in pens with ramps. NR-DW had the highest dermatitis prevalence (mild as well as severe) at all ages compared to R-DW, NR-IB, and R-IB (Table 3). At 49 weeks R-DW had a higher severe dermatitis prevalence than R-IB, but did not differ from NR-IB. Severe dermatitis prevalence for NR-IB and R-IB was not significantly different. IB had a lower Dermatitis Y/N prevalence at all ages compared to DW.

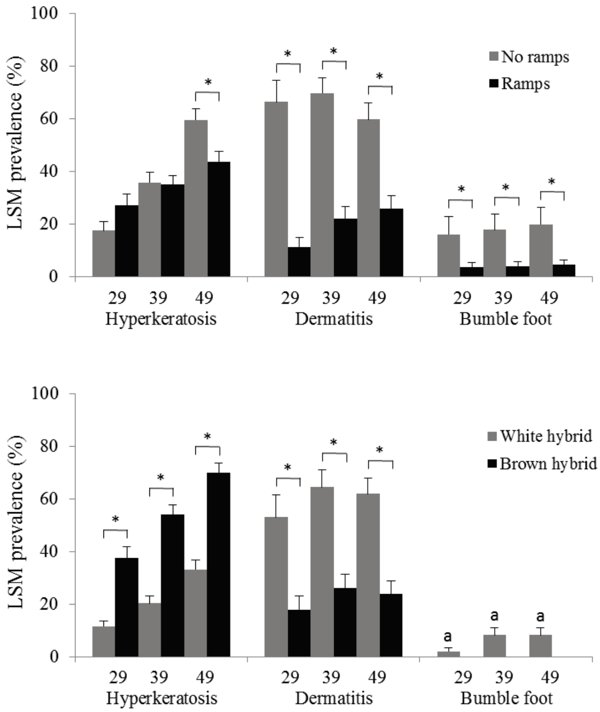

Figure 4. Least squares means percentages (± S.E.) of foot pad disorders at 29, 39 and 49 weeks of age for Housing (no ramps vs. ramps) and Hybrid (White Hybrid vs. Brown Hybrid). * indicate a significant ($P < 0.05$) effect of factor Housing or factor Hybrid at that age. [a] Bumble foot was not observed in ISA Brown.

Table 3. Least squares means (LSM) for mild dermatitis and severe dermatitis (% ± S.E.) at 29, 39 and 49 weeks of age for the four different treatments

	Treatment			
	NR-DW	R-DW	NR-IB	R-IB
Age: 29 wks				
Mild dermatitis	30.9 ± 4.3^{a}	6.2 ± 1.3^{b}	11.0 ± 1.9^{b}	5.9 ± 1.5^{b}
Severe dermatitis	71.7 ± 8.9^{a}	7.6 ± 3.2^{b}	14.4 ± 5.2^{b}	13.8 ± 6.9^{b}
Age: 39 wks				
Mild dermatitis	53.5 ± 4.7^{a}	14.6 ± 2.1^{b}	18.5 ± 2.4^{b}	10.3 ± 2.2^{b}
Severe dermatitis	65.2 ± 7.6^{a}	12.4 ± 3.3^{b}	12.0 ± 3.1^{b}	8.5 ± 3.2^{b}
Age: 49 wks				
Mild dermatitis	74.7 ± 4.0^{a}	30.5 ± 3.6^{b}	29.5 ± 3.6^{b}	17.4 ± 3.4^{b}
Severe dermatitis	58.0 ± 8.1^{a}	19.5 ± 4.4^{b}	9.9 ± 2.9^{bc}	5.1 ± 2.4^{c}

Shared superscripts (a-c) within rows indicate no significant difference ($P < 0.05$)
Treatments: NR-DW=No ramps - Dekalb White; R-DW=Ramps - Dekalb; NR-IB=No ramps - ISA Brown; R-IB=Ramps - ISA Brown.

5.4 DISCUSSION

The present study evaluated the influence of hybrid and the presence of ramps on keel bone and foot health disorders, and egg production. To our knowledge, this is the first study to report on the convincingly positive effects of ramps on reduction of foot pad disorders. Moreover, this study confirms the recent findings by Stratmann et al. (2015a) that ramps temporarily reduce keel bone fractures, keel bone deviations and floor eggs. The two selected hybrids showed various differences in keel bone and foot pad disorders, indicating scope for selective breeding against these disorders.

Age, housing and perch design, and hybrid have previously been shown to affect foot pad disorders (Abrahamsson and Tauson, 1995; Mahboub et al., 2004; Pickel et al., 2011; Rönchen et al., 2008; Tauson and Abrahamsson, 1994; Wang et al., 1998; Weitzenbürger et al., 2006). In a field-study on commercial aviaries (Heerkens et al., 2016a), 60-week-old laying hens showed a prevalence of 42% for hyperkeratosis (range 14 to 70%), 28% for foot pad dermatitis (range 0 to 70%) and 1% for bumble foot (range 0 to 16%) on flock-level.

Gunnarsson et al. (1995) found that hyperkeratosis ranged from 0 to 43% and bumble foot from 0 to 17% up to 77 weeks of age in loose housed laying hens at an experimental farm. Hence, our study is very much in line with both those studies, by demonstrating the prevalence of foot pad disorders to remain high throughout most of the production cycle. Severe foot pad lesions and bumble foot are most prevalent when hens are between 30 to 40 weeks of age after which the food pad normally heals by emptying of the pus (Tauson and Abrahamsson, 1994). We found that mild foot pad dermatitis kept increasing over time, and that severe foot pad dermatitis peaked at 29 weeks of age and decreased afterwards. This decrease could thus be the result of healing with the severe dermatitis developing back to mild dermatitis. However, both mild and severe dermatitis kept increasing in the Dekalb White with ramps treatment. Providing ramps considerably reduced foot pad dermatitis and bumble foot at all analyzed ages. Tauson and Abrahamsson (1994) reported a lower bumble foot incidence in hens that had wire platforms installed as perch space, compared to hens without such wire platforms. Prolonged and increased pressure load on the foot pads while standing, grabbing and perching on a wire floor causes proliferative hyperkeratosis (Rönchen et al., 2008; Tauson and Abrahamsson, 1994; Tauson et al., 1999; Weitzenbürger et al., 2005). Hyperkeratosis tended to be more present in pens with ramps at 29 weeks of age, but eventually at 49 weeks of age this disorder was reduced in those pens compared to pens without ramps. In our study the wire mesh ramps were only a small fraction of the available floor space. The prolonged pressure load on the toe and foot pads is therefore most likely less relevant in our study. The wire structure of the ramps may scrape stuck manure from the foot pads, thereby improving foot cleanliness and hygiene and reduce the detrimental effects of poor hygiene. Further investigation is needed to demonstrate whether the use of ramps leads to a better toe and foot pad hygiene because poor hygiene of the perch and foot pads does increase foot pad dermatitis and bumble foot (Rönchen et al., 2008; Tauson and Abrahamsson, 1994; Wang et al., 1998). The possible positive influence of better foot hygiene on hyperkeratosis also needs further investigation.

The hybrid effect on foot pad health in our study confirms the genetic predisposition of brown hybrids being more susceptible to hyperkeratosis, but less susceptible to dermatitis compared to white hybrids (Abrahamsson and Tauson, 1995; Abrahamsson et al., 1996; Mahboub et al., 2004; Tauson and Abrahamsson, 1996; Weitzenbürger et al., 2006). Ramps had a greater effect on foot pad health in the white hybrid compared to the brown hybrid.

Keel bone disorders were affected by hen age and the different treatments. The prevalence of all keel bone disorders increased with hen age and was similar to prevalences found in both experimental and field studies towards the end of the laying period (Freire et al., 2003; Heerkens et al., 2016a; Käpelli et al., 2011; Petrik et al., 2015; Rodenburg et al., 2008; Stratmann et al., 2015a; Wilkins et al., 2004). The accuracy of the palpation method in our study was similar to accuracy levels found in previous studies that used the palpation method to assess keel bone disorders on live animals in on-farm and experimental studies (Petrik et al., 2013; Stratmann et al., 2016; Wilkins et al., 2004). At 29 weeks of age hens without ramps already showed a much higher keel bone fracture prevalence, compared to hens with ramps. This difference in prevalence decreased as hens aged. The differences in fracture prevalence after the peak-of-lay in pens with versus without ramps are in line with the differences reported by Stratmann et al. (2015a) in an experimental aviary systems. The observations of the present study were obtained prior to the publication of Stratmann et al. (2015a), therefore, there was no expectancy bias based on the results of the latter study. It was, however, not possible having the observer blinded to the hybrid treatment in live birds, thereby, observer bias could have led to unconscious error in scoring (Tuyttens et al., 2014). Ramps also reduced the prevalence of hematomas and wounds of the skin covering the keel bone. The reduced keel bone disorders are very likely the result of fewer collisions when ramps are present due to WAIR and walking up- and down the system (Stratmann et al., 2015a). The effect of ramps on keel bone deviations was only observed later in the cycle. These keel bone deviations results from prolonged mechanical pressure during perching and is associated with perch shape (Pickel et al., 2011; Scholz et al., 2008; Tauson and Abrahamsson, 1994). Two of the five ramps per pen had a platform (Figure 1). These platforms possibly caused a lower mechanical pressure load on the keel bone compared the rectangular perches, which may have resulted in less keel bone deviation. However, it remains doubtful whether the lower level of deviation was indeed due to perching on the platforms, because these wire platforms only provided enough space for one or two hens per platform. The ramps facilitate WAIR and downward walking movements and reduce flights (Stratmann et al., 2015a). During flight, vigorous and forceful wing-flapping of the *pectoralis* muscles generates enormous forces on the keel bone. Such vigorous wing-flapping can be seen regularly in hens housed in aviaries (*personal observation*). Less flying due to the use of ramps may have reduced vigorous wing-flapping bouts. It has been proposed in the review by Harlander-Matauschek et al. (2015) that the enormous forces generated by the *pectoralis* muscles may contribute to deviation of the keel bone, although this suggestion of possible effects of such forces on keel bone disorders requires further investigation.

The substantial hybrid effect on keel bone disorders demonstrate the role of genetic predisposition. Already in 1955, Hyre demonstrated keel bone deformities were highly heritable. More recently, lines selected on high or low bone strength were shown to also differ in risk of keel bone fractures (Stratmann et al., 2016). Similar to our results, Vits et al. (2005) also found more keel bone deformities in a brown hybrid compared to a white hybrid. Selection on production traits in different hybrids may have resulted in trade-offs for traits, such as lower bone density and breaking strength, leading to higher keel bone disorder susceptibility (Fleming et al., 2007; Hocking et al., 2003; Vits et al., 2003). Differences in bone density and breaking strength between the two hybrids we used might thus explain the effect on keel bone disorder prevalence as the incidence of fractures increases as bone strength declines (Bishop et al., 2000). The hybrid effect on keel bone disorders could also relate to differences in behaviour or bodyweight. White hybrids are better capable of moving through a complex environment and have a lower bodyweight, compared to brown hybrids. Thus inferior navigation skills of brown hybrids could lead to more collisions and the peak-force on impact is also higher due to the heavier bodyweight of the brown hybrids (Wilkins et al., 2011). However, white hens are generally more fearful and flighty than brown hybrids (Heerkens et al., 2015b; Uitdehaag et al., 2009). These behavioural traits and behavioural responses could lead to more panic reactions, e.g. when a caretaker enters the pen, ultimately leading to collisions. Our results support the assumption that brown laying hens are more susceptible for sustaining keel bone fractures due to the impaired flight capabilities and increased force on impact. However, the heavier brown hens would also be expected to show more keel bone deviation due to increased pressure load on the keel bone during perching (Pickel et al., 2011), but our findings contradict this assumption. Behavioural observations could have demonstrated different perching behaviour between the hybrids, such as white hens perching more or longer compared to the brown hens (Faure and Jones,1982; Schrader and Müller, 2009), but these were not included in the present study. Susceptibility to keel bone deviation or keel bone fractures can also be caused by genetic predisposition for certain bone quality traits, such as bone elasticity and brittleness. Measuring such bone quality parameters (e.g. breaking strength, ash content, pyrrolic cross-link constant, radiographic density) may expose differences of bone quality traits between the two hybrids (Fleming et al., 2004; Rath et al., 2000; Riczu et al., 2004).

Egg production traits (floor eggs, first quality eggs, egg rate) were affected by age, ramps and hybrid. We could not demonstrate an association between keel bone or foot pad disorders with production traits, most likely because age, ramps and hybrid also affected keel bone and foot pad disorders and thus these factors may have been confounded. Ramps had beneficial effects on the production traits in the brown hybrid, whereas the white hens showed more beneficial production traits when no ramps were provided. Tauson et al. (1999) found that a brown hybrid laid more floor eggs compared to a white hybrid. Brown hens may less efficiently use and navigate through the complex environment (Donaldson et al., 2012) or are in more pain due to the higher prevalence of keel bone fractures and therefore prefer to stay on the floor (Nasr et al., 2012b). Nasr et al. (2012a; 2013) found that keel bone fractures were associated with reduced egg production. It is known that white hybrids produce more eggs than brown hybrids, but whether the better egg production of the white hybrid is related to the lower prevalence of keel bone fractures needs further investigation. In contrast, the higher producing white hens may have weaker keel bones, thereby progressively weakening the keel bone and making it more vulnerable for deviation by prolonged pressure load during perching.

5.5 CONCLUSIONS

We conclude that providing ramps is an effective measure to reduce keel bone and foot pad disorders in non-cage systems for laying hens. The welfare benefits and relative low-cost investments could make extra ramps valuable and feasible improvements of non-cage systems for laying hens. Further testing on commercial aviaries seems warranted. The demonstrated hybrid effects on the investigated disorders offer opportunities to improve laying hen welfare by selective breeding for favorable traits.

ACKNOWLEDGEMENTS

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CHAPTER 6

GENERAL DISCUSSION

INTRODUCTION

Non-cage housing systems for laying hens, such as aviary systems, have the potential to be more animal-friendly as compared with cage systems. Mainly due to the EU Directive 1999/74/EC on the protection of laying hens, approximately 160 million laying hens are nowadays kept in aviary systems in the EU. Aviaries is generally considered the best non-cage substitute for commercial large scale egg production. Worldwide, the number of hens kept in aviaries is expected to increase drastically in the coming years as even more countries and egg producers are implementing similar rules and regulations, a trend that is mainly driven by societal demands for better animal welfare. However, housing laying hens in aviary systems can be challenging because of certain welfare problems due to characteristics of the housing system as well as due to genetic predisposition. These welfare problems include damaging pecking behaviours, keel bone disorders, impaired foot health, and increased mortality. Furthermore, farm economics can be suboptimal in aviary systems due to lower stocking density (fewer hens per m^2 floor space compared to cage systems), high flock mortality, floor eggs, lower egg production rate and higher feed intake. The aim of this thesis was to examine the magnitude of the welfare problems in aviary systems, and contribute to improved welfare by identifying welfare related risk factors of aviary housing system characteristics, management procedures and hybrids. Furthermore, we aimed to reduce keel bone and foot pad disorders in two commercial hybrids by testing an innovative remedial measure that is applicable in commercial aviary systems. For the first aim we were the first to conduct a cross-sectional observational field study specifically aimed at commercial henhouses with aviary systems, whereas we performed an experimental study with a 2x2 factorial design for the second aim. Improved animal welfare in turn can contribute to better farm profitability, thereby making improvements beneficial for both the laying hen and the farmer.

The observational field study on commercial aviaries demonstrated that the welfare problems concerning plumage condition and wounds (chapter 3), and keel bone disorders and foot pad health (chapter 4) could reach high prevalences with large variation within and between farms. Several characteristics of the aviary housing systems were identified as risk factors for those problems, and also genetic predisposition due to hybrid was found to affect the prevalence of several of the investigated welfare problems (chapters 3 and 4). The field study also identified several risk factors that affect the farm profitability of egg production and mortality (chapter 3 and 4). The experimental study in the modified aviaries demonstrated how providing ramps improved laying hen welfare by effectively reducing the prevalence of keel bone and foot pad disorders. This study also showed that those disorders were affected by genetic predisposition as we found differences to varying degrees between two hybrids, indicating scope for selective breeding (chapter 5).

6.1 FIELD STUDY - COMMERCIAL AVIARIES

Cross-sectional observational field studies, like our field study (chapter 3 and 4), have the advantage that results provide valuable and relevant information concerning the current conditions found on commercial laying hen farms and allow identification of risk factors that are related to the welfare of laying hens on those farms (Nicol et al., 2013). Such field studies, on the other hand, have the limitation that comparing welfare between farms can be difficult, because of the difficulty to control for a multitude of factors that affect and interact with the outcomes of the measured welfare issues (Dawkins, 2012). Therefore, we considered it desirable to investigate a representative sample of Belgian farms with aviary housing at a certain time point during the production cycle. Investigating a representative sample would allow us to reveal the true magnitude of the welfare problems for laying hens housed in Belgian commercial aviary systems (at that certain time point in the production cycle). For the statistical analyses we also needed a sample size with replications of aviary system characteristics for the identification of risk factors in relation to the welfare problems. The observational field study described in chapters 3 and 4 embodied the majority of all Belgian egg production farms with aviary housing, therefore the results are likely to be fairly representative.

When laying hens arrive from the rearing farm the flock is rather uniform and their plumage, keel bones and foot pads generally are in good and healthy condition and the hens have not started to lay eggs (Petrik et al., 2015; Wang et al., 1998). Nonetheless, feather pecking may already occur during the rearing phase (De Haas et al., 2014b), whereas keel bone and foot pad disorders generally develop after the onset of lay (Petrik et al., 2015; Stratmann et al., 2015a; Wang et al., 1998). These welfare issues may develop to varying degrees as the production cycle proceeds. Furthermore, the study was dependent on the farmers' consent, and many farmers prefer to avoid disturbances towards and during the peak of lay. To be able to observe the welfare problems in varying degrees when the hens were at a comparable and sufficiently advanced stage of the production cycle, and to respect the farmers' preferences, it was decided to investigate 60-weeks-old flocks. Many other field studies have used the same approximate age of 60 weeks, thereby allowing comparisons between studies (e.g. Bestman and Wagenaar 2003; Donaldson et al., 2015; Gregory et al., 1990; Gunnarsson et al., 1995; Leenstra et al., 2012; Nicol et al., 2003; Odén et al., 2002; Petrik et al., 2015; Richards et al., 2015; Rodenburg et al., 2008; Sherwin et al., 2010).

The vast majority of the aviaries investigated in this thesis (85%) have been installed during the last three to four years prior to the study, indicating that the benchmark set by these results applies to rather new henhouses in Belgium. This could mean that farmers with older aviary systems were less willing to participate in this study, although this is probably not the case as aviary housing was not a very common housing system in Belgium prior to the ban on conventional cages in 2012 (Tuyttens et al., 2011). Belgian farmers acted late with regard to the transition to new housing systems in response to the EU Directive 1999/74/EC that banned the conventional cages (Rodenburg et al., 2008; Tuyttens et al., 2011). Consequently, many farmers were relatively unexperienced with aviaries and still learning how to operate the new system and manage flocks in an aviary. For 34% of the flocks in our study, the investigated flock was the first flock in their new housing system, and for another 36% it was only the second flock in their 'new' aviary. Because most farmers were still learning how to manage laying hens in aviary housing and how to operate the system, it could be argued that the results from our field study were not representative for farms that already had an aviary for several years, as farmers with more aviary experience may encounter fewer welfare problems in their flocks by recognizing problems earlier and adequately responding to problematic situations (Bestman and Wagenaar, 2003; Lambton et al., 2013). However, we aimed to report on the situation in 2012 and 2013, and caution is required when extrapolating findings to other times. If the same farms would have been assessed again several flocks and production rounds later, it might be expected that some of the welfare problems either may have decreased due to the learning process, or may have increased due to persistent red mite infestations. This was, however, not supported by our results, because age of housing system (which is a good proxy for farmer experience) could not be associated with any of the investigated welfare problems.

As more countries and large egg producers worldwide are converting, or considering to convert, to aviary housing systems the learning process of managing hens in an aviary becomes very important. An adapted or bespoke management that encourages farmers to be more aware to follow suggested management strategies has already proven to be effective in preventing damaging pecking behaviours (De Haas et al., 2014b; Lambton et al., 2013; Zimmerman et al., 2006). The management strategies applied in those studies were based on results from both field and experimental studies. Therefore, observational field studies as described in this thesis, as well as experimental studies, are of great value for formulating management strategies and specifying aviary housing characteristics in the near future. Farmers, advisors, poultry veterinarians and other stakeholders should be made aware of both the old and the new risk factors and should be encouraged to adopt specific management strategies. This approach may lead to reduced flock mortality, improved laying hen welfare and realization of the full potential of aviary systems as animal-friendly housing system for egg production (Lambton et al., 2013; Zimmerman et al., 2013).

6.1.1 MORTALITY

Reducing mortality should always be a priority when improving animal welfare as death is most often preceded by morbidity and suffering. The reported wide variety of causes of mortality was provided by the farmers during the interview, but no autopsies or inspection reports were consulted in order to obtain more specific information of the causes of mortality. The farmers indicated that feather pecking and cannibalism, salpingitis, *Escherichia coli* infections and smothering (resulting in death by suffocation) were the main causes of mortality. Chapter 3 describes that flock mortality in our sample of Belgian aviaries at 60 weeks of age was 4.1%, which is as low as has been reported in cage systems (Tactacan et al., 2009; Weitzenbürger et al., 2005). Previously, the higher mortality in non-cage systems was commonly considered as a particular disadvantage compared to cage systems (Fossum et al., 2009; Lay et al., 2011; Nicol et al., 2006; Sherwin et al, 2010; Tauson, 2002; 2005a), whereas the recent results from our field study (chapter 3) and a Canadian study of Petrik et al. (2015) indicate that important progress has been made in reducing mortality levels in non-cage systems, and thus also can be concluded that laying hen welfare in non-cage systems has improved.

The progress in reducing mortality levels in Belgian flocks is profound when we compare our field study with the field study by Rodenburg et al. (2008). The latter small-scale field study on commercial laying hen farms compared welfare and mortality between furnished cages, floor housing and aviaries in Belgium, Germany and The Netherlands in 2005 and 2006. Mortality levels (also at 60 weeks of age) in both types of non-cage housing systems were almost twice as high as the mortality levels of our field study conducted in 2012 and 2013. Mortality thus seems to have decreased since the other field study was conducted. High flock mortality in non-cage systems still occur occasionally as the risks for mortality are higher in those systems, and may have interfered with the outcomes of experience with aviary systems as factor that may influence laying hen welfare issues, such as mortality. A possible explanation of the decreased mortality is that over the last few years also advisors of feed companies, breeding companies, housing system companies, and poultry veterinarians have gained more knowledge and experience concerning the management of flocks in aviaries (e.g. water management at placement, timely disease detection, prevention of smothering), and this knowledge is forwarded to the ‘new’ farmers.

6.1.2 RISK FACTORS FOR PLUMAGE, WOUNDS, MORTALITY

The range of plumage damage prevalence from the field study in chapter 3 is similar to that reported in previous field studies (Bestman and Wagenaar, 2003; Green et al., 2000; Lambton et al., 2010, 2013). The causes of damaging pecking behaviours in laying hens are multifactorial and some known risk factors (Lambton et al., 2010, 2013; Nicol et al., 2013; Rodenburg et al., 2013) have been confirmed and new risk factors have been identified in chapter 3. Only 22 (1.0%) out of 2,150 inspected hens had perfect undamaged plumage according to our scoring system, which gives a good indication of the magnitude of this welfare problem. Several studies have demonstrated that plumage condition deteriorates with age due to both feather pecking and damage caused by wear and abrasion (Bilčík and Keeling, 1999; Nicol et al., 1999; McAdie and Keeling, 2000). In our field study we found damaged plumage in every flock investigated at 60 weeks of age with considerable variation within flocks as well as between flocks. The decision of investigating flocks at 60 weeks of age therefore seems to be appropriate to investigate potential risk factors for plumage condition of laying hens housed in commercial aviary systems.

In chapter 3 we showed that a worse plumage condition was associated with increased mortality, a finding that matches previous studies performed in the UK, and that provides evidence that damaging pecking behaviours may result in mortality (Green et al., 2000; Nicol et al., 2006; Whay et al., 2007). Hens that had access to a free-range had a better total plumage score, mostly explained by the better plumage score on the back, compared to hens that had no access to a free-range (chapter 3). The back is a known target area for feather pecking (Bilčík and Keeling, 1999, Savory, 1995). Therefore it can be concluded that the presence of a free-range likely reduced feather pecking behaviour. Feather pecking is derived from foraging behaviours and the free-range offers hens more opportunities to perform these foraging behaviours (Rodenburg et al., 2013). The free-range also reduces the stocking density in the henhouse (Nicol et al., 1999), and makes it easier for victims to avoid and escape peckers. Fossum et al. (2009) associated the presence of a free-range with increased mortality due to predation, and higher occurrence of bacterial and parasitic diseases, and cannibalism or in worst case avian influenza. Our field study did not confirm such an association between mortality and the presence of a free-range. A farmer can only assign predation as cause of mortality or include a dead hen in the mortality results if the carcass of the victimized hen is found in the free-range, whereas if the hen is taken by the predator it will not be accounted for in the mortality results unless records from the slaughterhouse as consulted. Our field study did not consult these records from the slaughterhouse to account for hens that were missing due to predation.

<u>RED MITE INFESTATIONS</u>

Red mite infestations are known to impair laying hen welfare by negatively affecting the plumage (Kilpinen et al., 2005), and causing disease and mortality (Chauve, 1998; Fossum et al., 2009). These negative effects on laying hen welfare were confirmed in chapter 3 by demonstrating that hens in red mite infested aviaries had poorer plumage condition and increased mortality. Due to the complex environment with many cracks, crevices, perches, nest boxes and side-beams in aviaries, aviary systems are more difficult to clean and disinfect between production cycles compared with cage systems. For this reason it is almost impossible for farmers to keep their aviaries free of red mites after several production cycles, and this contributes to red mite infestations becoming endemic (Sparagano et al., 2009, 2014). To further stress the problem of red mites in relation to the results presented in chapter 3, we may expect this problem to increase in Belgian aviaries because of the large proportion of new aviaries involved in our study that were free of red mites in their first production cycle but inevitably will encounter this problem in the coming years. Aviaries with tiers constructed with wire mesh flooring had fewer red mite infestations compared to aviaries with plastic slatted flooring. This could indicate that wire mesh offers fewer hiding places for red mites and can be cleaned more effectively between production cycles compared to plastic slatted flooring (Sander et al., 2003). Flooring materials that are even less attractive to red mites, e.g. wire flooring coated with impregnated red mite repelling substances (e.g. essential thyme or lavender oils) that are not harmful for the laying hen or food safety concerning the eggs should be developed and tested. Alternative strategies against red mites are being developed, such as CO_2-traps, traps with acaricidal properties, natural predators (*Hypoaspis miles, Hypoaspis aculeifer, Amblyseius degenerans* and *Phytoseiulus persimilis*), and the use of proteases as reviewed by Pritchard et al. (2015).

TIER FLOORING MATERIAL: WIRE MESH *VERSUS* PLASTIC SLATS

Birds in wire mesh aviaries had a better plumage condition and less severe wounds compared to the birds housed on plastic slats. This is in line with the field study on free-range farms by Whay et al. (2007), in which more aggression, arousal and feather loss was found when hens were held on plastic slats compared to on wire mesh. In the risk factor analysis field study by Nicol et al. (2003) this difference between tier material could not be identified as risk factor for the plumage, which could be due to the low number of henhouses with plastic slats in that study.

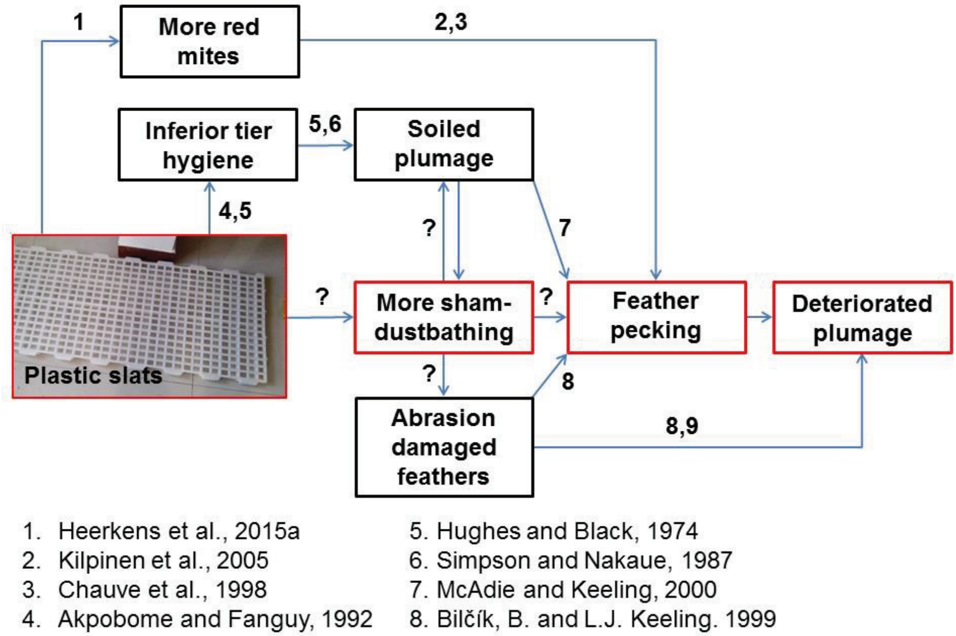

Figure 1. Schematic representation of the various negative effects of plastic slats on the plumage condition as found by previous studies. The question marks indicate that more research is needed to investigate the effect of tier floor material on sham-dustbathing and its consequent soiling and damaging effects on the plumage

Differences in plumage damage may also be related to dustbathing behaviours performed on the tiers, so-called sham-dustbathing, as well as a consequence of tier hygiene (Figure 1). The litter floor provides space and substrate to perform dustbathing behaviours. However, sham-dustbathing still occurs in the presence of litter and may cause conspecifics to peck and scratch at the sham-dustbathing hen (*personal observations*; Colson et al., 2007; Lindberg and Nicol, Merril and Nicol, 2005; 1997; Olsson et al., 2002). Plastic slats are most likely perceived to give more comfort, support and warmth when walking and laying on the tier and could therefore encourage more sham-dustbathing compared to wire mesh. This sham-dustbathing on the tiers causes abrasion and wear of the feathers thereby damaging the plumage. This possibility is supported by the observation that, besides the back area, also the vent and wings areas showed more damage on plastic slats compared to wire mesh. The vent and wings are body parts that will endure vigorous abrasion during sham-dustbathing. Tier hygiene may also have affected the mortality due to bacterial infections as plastic slats have an inferior hygiene compared to wire mesh due to manure being less effectively trampled through onto the manure belt (Akpobome and Fanguy 1992; Fossum et al., 2009; Hughes and Black 1974). Manure on the slats also provides more substrate for dustbathing compared to a lower amount of manure on the wire mesh. In previous studies chickens on plastic slatted flooring had a dirtier and more soiled plumage compared to chickens on wire flooring (Akpabome and Fanguy, 1992; Merril and Nicol, 2005; Simpson and Nakaue, 1987). Hens on plastic slats may thus have a more damaged and soiled plumage due to sham-dustbathing, and both may cause feather pecking (Appleby and Hughes, 1991; McAdie and Keeling, 2000; Savory, 1995) (Figure 1). Furthermore, particles in the plumage of conspecifics stimulate allopreening behaviour and lead to gentle pecks at the feathers (Zimmerman et al., 2006). Unfortunately, the observations that were performed during the field-study did not allow us to clarify the suggestions of sham-dustbathing, bird and system hygiene or discriminate between feather damage due to wear ad abrasion or pecking.

NEST ENTRANCE: PERCH *VERSUS* PLATFORM

Hens in aviaries with a platform or walkway in front of the nest box entrance had a better tail plumage score compared to hens in aviaries with a perch in front of the nest box (chapter 3). Prior to laying their egg, hens are highly motivated to perform pre-laying behaviours, such as nest seeking and nest exploration. These explorative behaviours are characterized by increased locomotion in front of the nest boxes and searching and exploration of the nest site and are performed prior to every oviposition (Sherwin and Nicol, 1993; Freire et al., 1996). In front of nest boxes a drinking line is often placed to attract hens to the specific tier to stimulate egg laying in the nest boxes (Lentfer et al., 2013). In commercial aviaries, this drinking line can be placed above a platform or a perch. Hens that are not able to adequately perform nest searching behaviours may express frustration and agonistic behaviours, which may be detrimental to their welfare (Hunniford et al., 2014; Lentfer et al., 2013; Weeks and Nicol, 2006; Zimmerman et al., 2000). Platforms offer more space for hens to surpass each other during searching behaviours and drinking, and this may have resulted in less frustration and agonistic behaviour towards conspecifics in front of the nest boxes. Many studies have investigated the effect of nest box characteristics (e.g. nest box position, design, size, lining, seclusion) on behaviours associated with nesting and egg laying (e.g. Hunniford et al., 2014; Kruschwitz et al., 2008; Lentfer et al., 2013; Ringgenberg et al., 2015; Struelens et al., 2005; 2008). The effect of the pathway or space in front of the nest box on nest searching behaviour, and possible associated frustration and agonistic behaviours, has not yet been studied though. Lentfer et al. (2011) did mention that space and design in front of nest boxes need to be taken into account when studying laying hen nest choice behaviour in aviaries.

AVIARY TYPE: ROW *VERSUS* PORTAL

Aviaries can be categorized into row-type and portal-type aviaries, as described in chapter 1. Farmers report that working conditions in a portal-type aviary are poorer as it comprises walking and working on tiers with less stable footing, working on heights and experiencing more discomfort from high fine dust and ammonia levels, compared to working in row-type aviaries (*personal communication during the interview*). The observers who performed the observations during our field study confirmed that all these negative aspects seemed to occur more often in portal-type than in row-type aviaries (*personal observation*). Farmers or stockpersons with less willingness or motivation to work in a particular system may develop a working attitude and human-animal relationship that may negatively affect the welfare of the animals kept in that system (Hemsworth, 2004). In the case of portal-type compared to row-type aviaries this could lead to a more negative attitude of the person working in the portal-type aviaries, ultimately resulting in a poorer welfare of the laying hens in those systems. However, the absence of relevant significant effect of aviary type on welfare outcomes in our field study (chapter 3 and 4) does not support this hypothesis.

The increased neck feather damage in row-type versus portal-type aviaries is not caused by more abrasion during feeding as can be seen in cage systems (Bilčík and Keeling, 1999; Blatchford et al., 2016; Hughes, 1980; Weitzenbürger et al., 2006), because in both aviary types nearly all hens are fed from chain-feeders. Damage from trough feeding would typically affect feathers at the front of the neck, whereas we scored the back of the neck.

MANAGEMENT PROCEDURES

Our results confirm that **beak treatment** does not eliminate pecking behaviours or the consequences of these behaviours (Nicol et al., 2013). In contrast, only one organic flock from our field study had intact beaks, and that particular flock had the best total plumage score (TPS=19.3) of all investigated flocks. One other organic flock with treated beaks had a TPS of 12.6, which is near the overall TPS average of 13.4. No other organic flocks were included, therefore we were not able to demonstrate if flocks in organic systems scored better or worse than conventional flocks.

The **number of changes in the feed** during a production round was associated with plumage condition (chapter 3). Hens are generally fed different diets during their life cycle to better fulfill needs at certain time points in their life, so-called phase feeding (MacLeod, 2004). In our field study, a better plumage of the neck was associated with a higher number of feed changes and could indicate that hens that were fed more different phase feeds received a more balanced diet. This finding is strengthened by the outcome of better neck feather scores being associated with higher egg laying rate at 60 weeks of age. Feather loss in the neck could also have resulted from natural moulting of the neck feathers (Rodenburg and Koene, 2004), a process which may have been suppressed by feeding more different phase feeds. A better (neck) plumage with more phase feeds is in contrast with other studies that found that three or more phase feeds increased the risk of feather pecking (Bestman et al., *in press*; Green et al., 2000; Pötzsch et al., 2001). Changing diets may cause stress due to temporary aversion for the new diet or food neophobia, ultimately leading to hungry birds with a greater tendency to peck at each other (Green et al., 2000). Alternatively, competition for the new feed may lead to increased feather pecking behaviours (Lindberg and Nicol, 1994). Hence, feed changes may lead to more aggressive pecking behaviours, which could lead to more neck plumage damage (Bilčík and Keeling, 1999; De Haas et al., 2014b). These assumptions are, however, not supported by our results of better neck (neck) plumage with more phase feeds. Diet compositions may have changed to better fulfill the nutritional needs of flocks kept in non-cages systems and the transition between diets may be better managed nowadays. For a more gradual transition between phase feeds farmers may have adapted their feeding management by mixing the former with the new phase feed to get the hens accustomed with the taste and texture of the new feed, thereby reducing the possible harmful effects of diet changes.

All flocks were fed fine mash diets, thereby avoiding an increased risk for feather pecking when feeding pellets, as demonstrated by Lambton et al. (2010) and El-Lethey et al. (2000). We were not able to find any effect of how often the hens were fed per day on plumage condition.

Furthermore, we found a positive effect of the daily time spent in the henhouse by the farmer or stockperson on the vent plumage score. Graml et al. (2007) demonstrated that non-cage housed hens are less stressed and less fearful towards humans if the hens encounter more **human-animal contact**. Flocks from our field study that encountered more human-animal contact, may thus have been less stressed and less fearful, ultimately resulting in less undesired vent pecking behaviour.

A limitation of our field study is that we could not demonstrate the beneficial effect of additional litter substrate provisioning or roughage provision in order to reduce feather pecking. Most farmers provided litter or roughage (e.g. alfalfa, straw) at placement, but did not supply more litter substrates throughout the production cycle. Very few farmers provided pecking blocks or other occupational materials throughout the production cycle to stimulate foraging and scratching behaviours (data not presented). Hens are highly motivated to perform foraging behaviours and spend the majority (40% to 60%) of their time during daytime performing these behaviours, even when feed is available *ad libitum* (Dawkins, 1981). Providing roughage, additional litter substrates, scattered grain, pecking blocks or other measures to stimulate and increase foraging behaviours may reduce damaging pecking behaviours (De Haas et al., 2014a; Rodenburg et al., 2013; Van Krimpen et al., 2005). These measures should already start at the rearing farm (De Haas et al., 2014a).

Altogether, the findings from our field study regarding plumage damage, wounds and mortality (chapter 3) confirmed that these issues currently still affect laying hen welfare in aviary systems. Multiple identified risk factors demonstrate how these welfare problems are multifactorial and offer opportunities to select the most appropriate aviary housing characteristics and management procedures to improve laying hen welfare. Moreover, the results regarding free-range availability and nest entrance design may be extrapolated to floor housing systems. Our findings regarding red mite infestations, human-animal contact, phase feeding and tier flooring material are all factors that are also present in other housing systems for laying hens (e.g. furnished cages and floor housing). By demonstrating that those factors affect laying hen welfare, our results can, to some extent, be extrapolated to both floor housing and cage systems.

6.1.3 RISK FACTORS FOR KEEL BONE DISORDERS

The high prevalence of several keel bone disorders (KBD) in aviaries reported in chapter 4 supports the growing concerns regarding KBD in laying hens (FAWC, 2010; 2013; EFSA, 2005; 2015; Harlander-Matauschek et al., 2015; Sandilands et al., 2009). The prevalence of keel bone fractures (ranging from 36 to 92% for middle fractures, 6 to 78% for caudal fractures, and 60 to 100% for either middle and/or caudal fracture) in the field study on aviaries matched the findings by previous field studies. Most of those field studies only quantified the KBD prevalences in non-cage housing systems, with the majority investigating flocks in floor housing systems (Freire et al., 2003; Nicol et al., 2006; Rodenburg et al., 2008; Wilkins et al., 2004). Our field study was the first to focus only on commercial aviary systems and we were also the first to study four different KBD, respectively hematomas, wounds, deviations and fractures. Furthermore, we did not just quantify KBD, but another novelty of our study was the extent to which we attempted to identify numerous potential risk factors of aviary housing characteristics for KBD (chapter 4). Most of those studies assessed KBD without distinguishing between fractures and deviations, even though keel bone fractures and keel bone deviations are believed to have different causes. This emphasizes the discrimination we made between keel bone fractures and keel bone deviations (Casey-Trott et al., 2015) to identify and explain effects on the different forms of KBD. However, due to the complex interactions between the different aviary types and configurations, perch arrangements, management, feed, hybrids and many other factors robust conclusions of the identified risk factors remain subject to some ambiguity. Research under controlled experimental conditions, such as our experimental study described in chapter 5, can eliminate such complex interactions, thereby allowing better explanation of the impact of the different factors. The presence of perches has many times been associated with keel bone disorders (as reviewed by Sandilands et al. 2009). Similarity in fracture prevalence between environments with and without perches (Donaldson et al., 2012; Petrik et al., 2015) is evidence for other risk factors for KBD than the mere presence of perches in non-cages systems.

Chapter 4 describes how results from our field study provide evidence that could confirm known risk factors (e.g. flight distance, hybrid, free-range) and the identification of new risk factors (e.g. aviary type, tier flooring material) for KBD. Especially perches have received a lot of attention in previous studies and reviews that reported on the role of housing system characteristics on KBD (EFSA, 2015; Käpelli et al., 2011b; Pickel et al., 2010;2011; Sandilands et al., 2009; Scholz et al., 2014; Stratmann et al., 2015a; Struelens and Tuyttens, 2009; Wilkins et al., 2011). In aviary housing, at least 15 cm perch per hen is required by the EU Directive and therefore perches were present in all aviaries in the field study. Effects of perch shape or material could not be identified as all aviaries provided round steel perches. However, round steel perches are systematically regarded as the least suitable shape for perches for laying hens regarding foot stability, KBD, FPD, and safe landings (Pickel et al., 2010; Rönchen et al., 2008; Scholz et al., 2014; Stratmann et al., 2015b; Tauson and Abrahamsson, 1994, 1996). Round steel perches have the advantage of being cheaper, more easily installed, more easily cleaned, provide better thermoregulation and are more durable compared to most other perch shapes and materials. Some aviaries in our field study were, besides the round steel perches, also equipped with mushroom-shaped plastic perches or curved metal profiles at the edges of the tiers. These other perch-types, however, comprised only a minority of the total number of perches in those systems. Fewer or less severe KBD would probably have been found in our field study if more aviaries would have been equipped with perches of softer materials (e.g. wood, plastic polyurethane) or with more flattened surfaces (e.g. mushroom-shaped, square, oval, rectangular), due to reduced high impact pressure, increased keel bone contact area and more safe landings (Chen et al., 2014; Duncan et al., 1992; Käpelli et al., 2011a; Pickel et al., 2010, 2011; Scholz et al., 2014; Stratmann et al., 2015b; Tauson and Abrahamsson., 1996). Apart from covering perches with soft polyurethane, KBD may also be reduced by using softer, and shock absorbing materials elsewhere in the aviary systems (Stratmann et al., 2015b). In the field study we found, for example, that hens on plastic tiers had fewer keel bone fractures as compared to hens in aviaries with wire mesh tiers. The plastic slats may have had a similar effect as the soft polyurethane perches, by having more shock absorbing capacity.

In aviary systems hens, move around freely within the system and on the litter floor. As laying hens are terrestrial birds and not the most elegant flyers, they prefer ground-based movements, such as walking, running and jumping, whereas flight is more exclusively used for escape behaviours (Dial and Jackson, 2011). The distances (either horizontally, vertically of diagonally) and angles to be covered during jumps and flights in aviary systems may vary from only a few centimeters at an angle of 30° (e.g. perch to nearest tier) up to several meters at 70° (e.g. highest perch to floor). The distance and angle to cover when jumping or flying is related to the rate of successful landings. Distances further than 60 cm and angles beyond 45° significantly increase the likelihood of failed landings (Moinard et al., 2004b,c; Scott et al., 1994, 1997, 1999). In commercial aviaries the distances and angles are determined by the aviary configuration. The space needed for proper take-offs and landings is likely to be insufficient in commercial aviaries due to the high stocking densities and the enclosed environment (Stratmann et al., 2015a).

In our field study (chapter 4), the corridor width between two stacks in row-type aviaries, and aviary type (row-type or portal-type) affected the prevalence of KBD. An increasing distance between two aviary stacks was associated with a lower prevalence of hematomas of the keel bone covering skin. This is in contrast with increasing flight or jump distance being associated with an increased likelihood of failed landings (Moinard et al., 2004b, c; Scott et al., 1994, 1997). However, if the distance becomes too far for a hen to even try or dare to jump (e.g. more than 150 cm), the number of failed landings may decrease as hens step down tier by tier or descend in a more controlled way. Hence, both a distance of 0 to 60 cm and a distance of more than 150 cm may result in a lower number of failed landings.

In row-type aviaries, more middle keel bone fractures and fewer wounds on the skin covering the keel bone were found compared to portal-type aviaries. We tentatively suggest that the portal-type configuration facilitates shorter jumps and distances to reach all facilities compared to the row-type configuration (chapter 1, Figure 2). This is likely the cause of the lower keel fracture prevalence in the portal-type aviaries. Admittedly, it was a missed opportunity to not have measured the (maximum) height for jumps and flights in the different aviary systems as a potential risk factor for KBD.

The field study included aviaries from six different producers (Jansen Poultry Equipment, BigDutchman, Vencomatic, Specht, Farmer Automatic and Meller) with 11 different models in total, with each model having a different configuration with even slight differences within the same models across farms. Due to the small number of replicates of each system and the large variation between the different models we were not able to identify any other effects of specific configurations of tiers, perches and other structural elements of the aviary.

Aviaries can be equipped with ramps that connect tiers to enable hens to walk – instead of jumping or flying - to higher or lower levels in the aviary. A limitation of our field study is that availability of ramps was not recorded and consequently not analyzed as an aviary characteristic to affect KBD or FPD. A study under controlled conditions in modified aviary systems (control aviary vs. additional perches vs. platforms vs. ramps) already showed a reduced prevalence of keel bone fractures in systems where fewer falls and collisions occur (Stratmann et al., 2015a). Chapter 5 describes how ramps affected both KBD and FPD in small modified aviaries under controlled experimental settings. This indicates that more appropriate aviary and perch configurations may improve the hens' ability to maneuver in the aviary, ultimately leading to fewer keel bone fractures. These assumptions may also be extrapolated to floor housing systems.

Altogether, our results confirm the concerns regarding the high prevalence of KBD in laying hens housed in aviary systems. Housing system characteristics and hen movements through the systems are likely causing falls and collisions that lead to keel bone fractures, wounds and hematomas.

6.1.4 RISK FACTORS FOR FOOT PAD DISORDERS

The high prevalences for foot pad hyperkeratosis (42%), dermatitis (28%) and to a lesser extent also bumble foot (1.2%) prove that FPD also were major welfare problem of laying hens housed in Belgian aviary systems (chapter 4). Non-cage housing characteristics, such as contact with poor quality litter and perch availability, have been associated with those FPD (Lay et al., 2011). Similar as for KBD, perch shape and material are known to affect FPD (Duncan et al., 1992; Pickel et al., 2011; Rönchen et al., 2008; Tauson and Abrahamsson, 1994, 1996; Wang et al., 1998). As discussed in the previous section, all aviaries were equipped with round steel perches and sometimes some mushroom-shaped perches or metal profiles at the edges of the tiers were provided alongside the round metal perches. Due to the lack of variation and replicates of different perches, no perch design effects on FPD could be identified. Peak force on foot pads in standing hens is higher on smaller supporting surfaces (e.g. wire mesh tiers), which could lead to FPD (Alvey and Tucker, 1994). On the other hand, plastic slatted tiers are believed to have inferior hygiene leading to more contact between foot pads and manure, ultimately leading to more foot pad dermatitis.

Contact with litter, and more specifically wet litter, is also considered a risk factor for foot pad dermatitis and bumble foot (Wang et al., 1998). Hens in portal-type aviaries had less severe foot pad dermatitis (chapter 4), which could indicate that the litter quality was better than in row-type aviaries. Ventilation, air flow and heating affect litter quality, indicating that there might have been environmental and climatic differences between row- and portal-type aviaries. In our field study litter quality and ventilation method were part of the questionnaire, but were eventually excluded from the statistical analyses. Litter moisture content was not assessed. We were, therefore, not able to determine litter quality or climatic conditions as risk factors for foot pad dermatitis nor could we determine risk factors for poor litter quality. Hens in portal-type aviaries possibly spent less time on the litter floor and more time on the tiers and therefore were less in contact with the litter compared to hens in row-type aviaries.

Foot pad dermatitis and bumble foot incidence and prevalence peak when hens are between 30 and 40 weeks of age, after which these disorders are less severe and less prevalent (chapter 5; Gunnarsson et al., 1995; Tauson and Abrahamsson, 1994; Wang et al., 1998). In our field study we observed FPD at 60 weeks of age, during which bumble foot showed a prevalence of a mere 1%. Hence, the age of 60 weeks to determine bumble foot was probably less optimal, and observations between 30 to 40 weeks of age may have resulted in higher prevalences and more variation. Higher prevalences and more variation between flocks could have resulted in the identification of more risk factors for this severe FPD.

Although not as prevalent as the plumage damage and KBD, FPD of laying hens in aviaries still compromises the welfare of laying hens in aviaries. To improve laying hen welfare regarding FPD, further research under controlled experimental conditions is needed to reveal the most appropriate housing system characteristics.

6.1.5 HYBRID AS RISK FACTOR

In the field study (chapters 3 and 4) a total of seven different hybrids, of which six were brown feathered hybrids (44 flocks) and one white feathered hybrid (three flocks), were observed. Therefore it was not possible to compare the welfare of brown hybrids with the welfare of white hybrids. At the time of the farm visits (2012-2013) not many farmers chose to house white flocks, because there was less demand for white eggs from non-cage systems. Farmers were also more reluctant to house white hybrids in non-cage systems at the time, as it was believed that white hybrids were more fearful than the more docile brown hybrid, and therefore more difficult to manage in aviary systems. This last assumption is confirmed by studies that demonstrated that white hybrids show more fearful behaviours towards humans compared to brown hybrids (De Haas et al., 2013, 2014a; Heerkens et al., 2015b; Uitdehaag et al., 2009).

The majority of the flocks investigated in the field study were either Lohmann Brown Classic (45%) or ISA Brown (28%) hens and only these two hybrids were compared statistically. The prevalence of fractures of the keel bone caudal tip and foot pad hyperkeratosis differed between both hybrids. These differences may be caused by various differences in physiology (e.g. bone quality) or behaviour (e.g. perching behaviour) (Abrahamsson and Tauson, 1995; Fleming et al., 2007; Kjaer, 2000; Schrader and Müller, 2009). Many studies found differences in KBD between hybrids, but results were inconsistent between studies (e.g. Abrahamsson et al., 1996; Käpelli et al., 2011a; Kjaer et al., 2000; Vits et al., 2005; Wahlström et al., 2001). The differences found in our field study, therefore, should be treated cautiously before concluding that one hybrid is better than the other.

For the integument scoring, the ISA Brown hens had a better wing plumage score. Although the wings are not the main target area for feather pecking, our findings contradict the results from Nicol et al. (2003) who found that flocks that show increased feather pecking behaviours were more likely to be ISA Brown hens than Lohmann Brown hens. Although these results are contradictive, they do imply the existence of genetic predisposition for feather pecking behaviour or other behavioural differences that result in plumage damage and allow for opportunities to breed for more favorable traits for improved welfare in commercial laying hen hybrids (De Haas et al., 2013, 2014a). A limitation of our field study is that no behavioural observations or physiological measurements were performed that could explain the differences we found between the two hybrids.

Although only two brown hybrids could be compared, the differences found between those two hybrids offer a scope for further research on selective breeding and research on genetic predisposition for welfare issues in laying hens housed in aviary systems.

6.1.6 PRODUCTION

Suboptimal performance in aviary systems due to large number of floor eggs, high feed intake and high mortality rates may affect farm profitability (Matthews and Sumner, 2015; Tauson 2002, 2005a). Mortality has already been discussed in a previous section. Alterations in egg production and feed intake are often a consequence of impaired welfare rather than a cause of impaired welfare. Feed intake information was asked in the questionnaire, but eventually omitted from the analyses due to inconsistent answers. Egg production parameters were associated with four aspects investigated in the field study (chapters 3 and 4).

First, increasing barn age was associated with a higher percentage of downgraded eggs. New purpose-built barns are designed more properly to accommodate an aviary system than older barns, which generally housed conventional cages previously. The older barns may have had less appropriate ventilation or lighting in the henhouse, factors known to affect the risk of floor and system eggs.

Second, flocks with a higher proportion of hens with a straight (non-deviated) keel bone produced fewer downgraded eggs. Although similar results were found by Nasr et al. (2012a), the mechanism or causation of this result remains uncertain. In our field study we did not find an association, at flock level, between keel bone fracture prevalence and production rate, therefore we could not confirm results that were obtained on experimental scale in which hens with keel bone fractures produced fewer eggs (Nasr et al., 2012a, 2013; Toscano et al., 2015).

Third, egg production rate was 3% higher, which is a substantial difference in large scale flocks regarding profitability, in aviaries with wire mesh tiers compared to in plastic slatted aviaries. The lower production performance may be caused by higher levels of damaging pecking behaviours and associated stress on the plastic tiers, and warrants further research.

Fourth, Lohmann Brown Classic hens had a higher production rate at 60 weeks of age than ISA Brown hens. These production results are in line with the information provided by the breeding companies' product sheets for those two hybrids and are the result of the selective breeding for productivity and performance.

6.2 EXPERIMENTAL STUDY – RAMPS & HYBRIDS

In our experimental study (chapter 5) we aimed to improve laying hen welfare by modification of the housing configuration that would result in a lower prevalence of KBD and FPD as well as improved production parameters (mainly fewer floor eggs). Furthermore, we aimed to demonstrate the effect of genetic predisposition for KBD and FPD by comparing two commercial hybrids, respectively Dekalb White (DW) and ISA Brown (ISA). The knowledge generated in the experimental study needed to be transferable to commercial farms, therefore the modification of the housing system needed to be applicable in already existing aviaries without any major modifications of the system or unreasonable costs. The ramps that were installed between the perches and to approach the nest boxes were standard ramps that are used in aviary systems and were purchased from a commercial aviary housing producer (Jansen Poultry Equipment, Barneveld, The Netherlands). The ramps were constructed of galvanized wire mesh, were easily installed and relatively cheap. In general, provision of ramps proved to be a very effective measure to improve laying hen welfare in non-cage systems as the prevalence of FPD and KBD in pens with ramps was reduced. The hybrid differences demonstrated that genetic predisposition affects susceptibility for these disorders.

6.2.1 RAMP EFFECTS ON KEEL BONE DISORDERS

Despite that providing ramps improved laying hen welfare by the reduction of KBD at all analyzed ages, prevalences of KBD remained high in the groups with ramps, especially with increasing age. Petrik et al. (2015) found the highest incidence of keel bone fractures between 30 to 35 weeks of age and the prevalence continued to increase up to 50 weeks of age, after which further increase was negligible. This justifies our measurements and analyses for KBD when the hens were 29, 39, and 49 weeks of age.

Compared to the pens without ramps, the prevalence of keel bone fractures in pens with ramps was lower (49% vs. 83%, 71% vs. 93, 86% vs. 97%) at 29, 39, and 49 weeks of age, respectively. Stratmann et al. (2015a) found a reduction of 55% for falls and 42% fewer collisions in a modified aviary in which ramps were installed. Thus, the reduced keel fracture prevalence in our pens with ramps likely resulted from fewer falls and collisions. Ramps facilitate the opportunity for preferred natural movement behaviours of hens, such as wing assisted incline running (WAIR) and walking, in order to move between levels rather than the necessity to jump or fly to other levels. Explorative data from behavioural observations showed that in pens with ramps more hens used the perches and more movements occurred between perches, compared to pens without ramps. Furthermore, hens in pens with ramps used the ramps in 60% of all movements between the perches and towards the nest boxes (*unpublished data*). The ramps may not only prevent falls and collisions of the moving hen, but also of the hens present in the landing area. This double effect of ramps may explain the efficiency of ramp provision on keel bone fracture reduction. As hens can perform more preferred natural movements, it was hypothesized that hens in the ramp treatments may have been less stressed compared to hens without the access to ramps. Behavioural tests of stress and fearfulness demonstrated no effects of ramps on these behaviours, though (Heerkens et al., 2015b).

The prevalence of fractures remained, nevertheless, high in both treatments, which could be related to the low stocking density. The stocking density of 3.2 hens/m^2 (3,080 cm^2/hen) in our experiment was far below commercial standards of 9 hens/m^2 for non-cage housing (1,111 cm^2/hen). The low stocking density provided the hens more freedom of space per hen which may have led to more wing flapping. Although no study so far has proved that wing flapping itself may be a source of keel fractures, this has been suggested as a possible cause (Harlander-Matauschek et al., 2015; Sandilands et al., 2009). Despite the low stocking density, space to glide through the air and to land is often limited under experimental conditions (Christman and Leone, 2007), as it also was in our experimental pens. This limited space may have prevented controlled descending flights and controlled landings. The hens were housed in groups of 25 birds. Such relatively small groups of hens will form social hierarchies, which may have caused group social disturbance on the perches (Keeling et al., 2003), ultimately leading to falls and collisions during those disturbances.

Fractures in the middle part of the keel bone were already highly prevalent at 29 weeks of age, whereas caudal fractures increased after this age (chapter 5, Figure 2). This increase of fractures after 29 weeks of age is in line with previous studies that demonstrated that most fractures occur between 30 and 40 weeks of age (Donaldson et al., 2012; Gregory and Wilkins, 1995; Gebhardt-Henrich et al., 2015; Petrik et al., 2015). Laying hen movements and activities in aviaries decrease over time, which could result from reduced mobility due to keel bone fractures (Campbell et al., 2016b; Stratmann et al., 2015a). Further research is needed to demonstrate the relationship between keel bone fractures and bird activity and could extend the studies of Nasr et al. (2012a, b) who demonstrated that laying hens with fractures showed decreased mobility and most likely suffered from pain.

Ramps also reduced keel bone deviation prevalence (24% vs. 29%, 26% vs. 48%, 45% vs. 74%) at 29, 39, and 49 weeks of age, respectively (chapter 5). All pens were equipped with the same number of identical rectangular wooden perches, therefore the lower prevalence of keel bone deviation can be attributed to the presence of ramps that likely caused a difference of pressure load on the keel bone during perching (Pickel et al., 2011; Scholz et al., 2008a; Tauson and Abrahamsson, 1994). As discussed in chapter 5, two of the five ramps had a small platform on which one or two hens could sit comfortably. Furthermore hens were observed sitting on the slope of the ramps during daytime (*unpublished data*). Peak pressure on the keel bone may be lower when hens sit on the wire ramps instead of on perches, ultimately leading to less severe or fewer deviations. More physical exercise improves bone strength in laying hens (Leyendecker et al., 2005; Rodenburg et al., 2008). Explorative data from behavioural observations showed that ramps increased activity between perches (*unpublished data*). The increased activity may have increased bone strength, ultimately leading to fewer keel bone fractures and/or fewer keel bone deviations. At the end of the experiment hens were euthanized humanely, after which the keel bones were dissected and stored. This allows us to, in a later stage, measure bone quality and to test for possible effects of ramps on keel bone traits.

6.2.2 RAMP EFFECTS ON FOOT PAD DISORDERS

To our knowledge this is the first report investigating the welfare enhancing effects of ramps in a non-cage system on foot pad health in laying hens. A limitation of the field study (chapter 4) was that flocks were assessed at 60 weeks of age, whereas foot pad dermatitis and bumble foot are most prevalent between 30 and 40 weeks of age (Tauson and Abrahamsson, 1994; Wang et al., 1998). Therefore, the longitudinal experimental design of our experimental study (chapter 5) in which KBD was measured and analysed at 29, 39, and 49 weeks of age was in the appropriate time frame.

To exclude the detrimental effects of poor litter quality on FPD (Keutgen et al., 1999; Tauson and Abrahamsson, 1994; Rönchen et al., 2008; Tauson et al., 1999; Wang et al., 1998), litter quality was maintained in good condition throughout the experiment by monthly replacing litter with fresh wood shavings. All pens were equipped with rectangular wooden perches, which are preferred over round perches (Duncan et al., 1992). This could have resulted in less FPD compared to the prevalences found in our field study. However, the hygiene of rectangular wooden perches is poorer than that of round steel perches, which could have resulted in increased foot pad dermatitis and bumble foot (Tauson and Abrahamsson, 1994). Our findings are in line with Tauson and Abrahamsson (1994) who found a lower prevalence of bumble foot when wire platforms were offered compared to only flat wooden perches and concluded that wire flooring kept foot pads clean and dry. Gripping of perches, wire mesh or other structures has been associated with reduced dirt on the structural surface and, consequently, an improvement of foot pad hygiene (Tauson and Abrahamsson, 1996). We suggest that the occasional use of ramps may have scraped manure from foot pads, thereby improving foot and perch hygiene. A limitation of our experimental study was that perch and foot pad cleanliness or hygiene were not assessed so our assumption that the FPD reducing effect of ramps was related to foot pad hygiene and cleanliness could not be confirmed.

The mechanical compression load on the foot pads when birds are housed on wire mesh can cause foot pad hyperkeratosis (Abrahamsson and Tauson, 1995; Rönchen et al., 2008; Tauson and Abrahamsson, 1994; Tauson et al., 1999; Weitzenbürger et al., 2006). The occasional use of the wire mesh ramps in non-cage systems was probably less harmful than permanently being housed on wire flooring (e.g. in cage systems). Moreover, at the end of the experiment the hens in pens with ramps had a lower hyperkeratosis prevalence. The underlying cause of this ramp effect on hyperkeratosis remains unknown and needs further investigation.

6.2.3 HYBRID EFFECTS ON KEEL BONE AND FOOT PAD DISORDERS

The many large differences between the two commercial hybrids demonstrate how genetic predisposition affects laying hen welfare regarding KBD and FPD in non-cage systems. An increasing number of white flocks are kept in aviary systems nowadays as compared to 2012-2013 when the field study took place (*personal communication with rearing and breeding companies VEPYMO and ISA-Hendrix Genetics*). Therefore comparing a commonly used white hybrid (Dekalb White) with a commonly used brown hybrid (ISA Brown) in this experimental study was a justified decision.

Stratmann et al. (2015a) demonstrated that fewer falls and collisions were associated with a lower keel fracture prevalence, whereas Scholz et al. (2014) demonstrated that hens of a white hybrid encountered fewer failed landings compared to hens from a brown hybrid. Furthermore, hybrids show differences in activity and perching behaviours (e.g. time budget, perching frequency, perching duration) (Faure and Jones, 1983, 2004; Schrader and Müller, 2009). It is likely that the hybrid effects in the experimental study concerning KBD are caused by the landing capabilities and behavioural differences of both hybrids. Video observations of the behaviour of perch and ramp use have been made during the final phase of the experiment. The data of the video observations will be used to provide evidence that differences in failed landing rate and/or behavioural differences are underlying causes of the difference in KBD between both hybrids.

Hybrids may also differ in bone quality parameters (Knowles et al., 1993; Leyendecker et al., 2005; Riczu et al, 2004; Silversides et al., 2012; Vits et al., 2005). As have we dissected and stored keel bones and tibias of both hybrids, bone quality measurements may confirm that the genetic predisposition effects on bone parameters between both hybrids partially determined the susceptibility for KBD. It already has been demonstrated that selection on bone characteristics can result in better bone quality, which is a promising avenue to improve laying hen welfare (Bishop et al., 2000; Stratmann et al., 2016).

Many studies, including our experimental study, demonstrated that white hens have more foot pad dermatitis and bumble foot, but less foot pad hyperkeratosis than brown hens (Abrahamsson and Tauson, 1995; Mahboub et al., 2004; Tauson and Abrahamson 1994, 1995, 1996; Weitzenbürger et al 2006). Perches and litter were not present in most of those studies, therefore factors other than perching behaviour and contact with litter affect FPD susceptibility in laying hens too. There still seems to be a lack of knowledge on the underlying causes of the differences in FPD between brown and white hybrids. Pickel et al. (2011) found an effect of hybrid on peak forces applied on the foot pads during sitting or standing on a perch, with the brown hybrid having a smaller foot pad peak force and larger contact area during sitting and standing on perches compared to the white hybrid. Rönchen et al. (2008) found that body weight affected foot pad lesions with heavier hens being more affected. Hybrids show behavioural differences in perch use (e.g. duration and frequency) and the amount of time spent on the litter (Faure and Jones, 1982; Tauson and Abrahamsson, 1994; Wall and Tauson, 2007). A limitation of the experimental study is that foot pad pressure and foot pad hygiene were not assessed. Analyses of the video recordings that have been made may provide evidence of the behavioural differences concerning perch use and time spent on the litter.

6.2.4 PRODUCTION

The Dekalb White hens had in general a better egg production performance as could be expected. We could not confirm the results of Stratmann et al. (2015a) who found a higher egg production in the ramp treatment compared to the control group.

Hens are highly motivated to lay their eggs in nest boxes and sleep on high perches (as reviewed by Weeks and Nicol, 2006). The percentage of floor eggs was low in our study, suggesting that hens easily found their way to the nest boxes despite the high prevalence of KBD, FPD or absence of ramps in the control treatment. Both, our experiment as the study of Stratmann et al. (2015a), could not demonstrate that the provision of ramps could result in fewer floor eggs. This assumption was based on the reasoning that hens with severe KBD and FPD are in pain and discomfort (Nasr et al., 2012b, Tauson, 2002) and would use the ramps to reach the nest box for egg laying, whereas hens without ramps would choose to lay their eggs on the floor over taking the risk of flying or jumping to the higher positioned nest boxes. This hypothesis was also based on Nasr et al. (2012a), who found that hens with fractures spent more time sleeping on the floor, which may lead to more eggs being laid on the floor during daytime (Nasr et al., 2012a).

6.3 IMPLICATIONS AND FURTHER RESEARCH

Identification of housing characteristics and management strategies to affect hen welfare indicate that selecting appropriate housing system characteristics and management strategies may improve the welfare of laying hens housed in aviary systems. The provision of ramps proved to be a remedial measure to reduce KBD and FPD. Future research should focus on investigating some of the identified aviary housing characteristics and management procedures in more depth under more controlled conditions. Farmers, advisors, poultry veterinarians and other stakeholders need to be aware of all known (old and new) risk factors to be able to select or advice appropriate aviary housing and management strategies. Differences between hybrids indicate that some hybrids, due to genetic predisposition, may be more susceptible for certain welfare issues than other hybrids. This offers opportunities for breeding companies to further select on desirable hybrid characteristics.

Systematically recording mortality on commercial farms for several years or production cycles could clarify if mortality levels in aviaries are decreasing. Also registration of the causes of mortality could clarify what affects mortality the most. Data collection on mortality (and the possible causes) could be part of an obligatory monitoring programme to protect the welfare of laying hens.

Several causes have been associated in chapter 3 with outcomes all in favor of wire mesh tiers and thus emphasize the importance to conduct further research on the most appropriate tier flooring material and the underlying causes of the beneficial characteristics of the flooring material. It is needed to demonstrate whether hens on plastic slatted tiers have a more soiled plumage or show more sham-dustbathing on the tiers compared to hens on wire mesh tiers. We also suggest that the effect of inferior hygiene of the bird and system on flock mortality needs further investigation.

The field study (chapters 3 and 4) comprised no behavioural observations of pecking behaviour in the henhouse which could have demonstrated whether or not feather pecking or other agonistic and frustration behaviours occurred more frequently in portal-type or row-type aviaries, in front of nest boxes with perches versus platforms, in absence or presence of a free range, on plastic slatted tiers compared to wire mesh tiers, and the effects of human-animal contact.

Further research is warranted to test the appropriate shape, material and dimensions of aviary housing equipment to reduce KBD and FPD. Behavioural observations could demonstrate associations between laying hen behaviour (e.g. general activity, perch use, flying capabilities, bird movement through the aviary system) and KBD or FPD. Also physiological properties of the laying hen (e.g. bone quality, foot pad anatomy) need to be investigated to unravel underlying causes of KBD and FPD susceptibility of the different hybrids.

CHAPTER 7

CONCLUSIONS

This thesis on laying hen welfare in aviaries shows that it remains challenging to house laying hens in aviary systems while safeguarding good welfare. An interplay of aviary housing characteristics, management, as well as genetic predisposition affects aspects of laying hen welfare. Identification of appropriate housing system characteristics, management and hybrid provides opportunities to improve laying hen welfare and profitability in aviary systems.

From the field study we conclude that damaging pecking behaviours, keel bone disorders and foot pad disorders are still highly prevalent in many Belgian egg production farms with aviary housing (Objective 1 and 2). Risk factors we identified that affect laying hen welfare, mortality, and production include aspects of the aviary housing design (e.g. aviary type, nest design, free-range access, corridor width), flooring materials, human-animal contact, feeding regime, and hybrid (Objective 3 and 4). The identification of these risk factors offers opportunities to develop improved aviary systems, adapt laying hen management, and perform genetic selection that may result in improved welfare of laying hens housed in aviaries. Further research on the underlying causes of the effects of aviary design and material, and research on laying hen behaviour and movements in aviary housing systems are strongly recommended.

From the experimental study can be concluded that the provision of ramps proves to be very effective in improving laying hen welfare by reducing KBD and FPD (Objective 5). The ramps likely contribute to easier bird movement through a complex environment, thereby reducing painful falls and collisions that may cause KBD. The use of ramps possibly reduce FPD by improved foot pad hygiene. The large differences in KBD and FPD between the white and brown hybrid are likely relate to both behavioural (e.g. activity levels, perching behaviour, fearfulness) and physiological (e.g. bone quality, body weight, anatomy) differences (Objective 6). This genetic predisposition offers opportunities to select for favorable behavioural traits in commercial hybrids, as well as for better physical ability of flight and navigation skills, and improved bone quality.

Many of the results obtained in this thesis can be extrapolated to other non-cage systems and some even to furnished cage systems. The take home message is that it is most important to create a better match between the hen’s behaviour and physiology and its housing environment.

SUMMARY

Recent major changes in EU legislation resulted in the situation that nowadays millions of laying hens are kept in aviary housing systems. This number will increase drastically in the coming years. The multi-tiered aviary housing system is a non-cage housing system that offers hens plenty of opportunities to perform highly-motivated behaviours. Hens can move freely through the henhouse and use a complex environment in which feed, water, perches, and nest boxes are provided at several levels. Hens in aviaries, however, are at risk for certain welfare problems due to characteristics of the housing system and management procedures. The major animal welfare challenges include damaging pecking behaviours, mortality levels, keel bone and foot pad disorders. Identification of strong and weak points of the housing offers opportunities to improve the housing system, with the ultimate goal to improve laying hen welfare. Furthermore, some hybrids may be better suited for aviary systems than others (chapter 1).

This doctoral thesis reports on a cross-sectional field study in commercial henhouses with aviary systems aimed at identifying aviary system and management characteristics that are associated with aspects of laying hen welfare (damaging pecking behaviours, mortality levels, keel bone disorders and impaired foot health) and egg production. Furthermore we report on an experimental study with a 2x2 factorial design aimed to test how providing ramps could be a remedial measure to reduce keel bone and foot pad disorders in two commercial hybrids (chapter 2).

The cross-sectional field study was conducted in 47 henhouses to examine the severity and variation of the welfare issues investigated between commercial farms and flocks (chapter 3 and 4). This sample represented the majority of all Belgian aviaries and, although possibly confounded by the effect of farmer consent, sets a reasonably representative benchmark for laying hen welfare in Belgian aviaries in 2013 and 2014. As Belgian farmers acted late on the conventional cage ban per January 1st, 2012, many farmers in our field study were only housing their first (34%) or second flock (36%) in their new aviary at the time of our farm visit. Farmer experience, however, could not be associated with the different aspects of laying hen welfare that we investigated. The only age-related effect we found was that the percentage of downgraded eggs increased with increasing age of the barn.

Damaging pecking behaviours (feather pecking, vent pecking, and cannibalism), and keel bone and foot pad disorders were highly prevalent on many Belgian farms with

aviary systems. The large variation between farms in the prevalence and severity of the investigated aspects of laying hen welfare were associated with several housing system characteristics, farm management and flock specific hybrid (chapter 3 and 4). Identification of the strong and weak points of the housing system and management is useful to be able to improve laying hen welfare in aviary systems.

Damaging pecking behaviours were reported by the farmers as the main causes of mortality. This was confirmed by the association we found of a lower mortality in flocks with a better plumage condition. Plumage damage to the back, vent, tail and upper neck was likely the result of damaging pecking behaviours, whereas plumage damage to the wings more likely resulted from abrasion against the system. Red mites, that cause stress and damaging pecking behaviours, were found on many farms. The detrimental effects of red mite infestations were demonstrated by the negative effects on the plumage condition and increased flock mortality. On a more positive note, our field study found that mortality in aviaries appeared to have decreased and was as low (4.1%) as levels reported to be found in cage systems.

Aviaries can roughly be divided into two types, namely portal-type and row-type aviaries. Hens in row-type aviaries had a better neck plumage score, fewer keel wounds, more middle keel bone fractures and more severe foot pad dermatitis, compared to hens in portal-type aviaries. Corridor width in row-type aviaries may affect successful landing rate, which could explain the finding of fewer hematomas in systems with wider corridors. Although not investigated in the present study, we suggest that hen movement in aviary systems can very well be an underlying cause of the difference in keel bone and foot pad disorders between aviaries.

Tier flooring material affected several aspects of laying hen welfare. Aviaries with wire mesh tiers were associated with hens having a better plumage condition, fewer wounds, lower mortality, and fewer red mite infestations compared with aviaries with plastic slatted tiers. Flocks that were housed on plastic slatted tiers, however, had fewer keel bone fractures. Sham-dustbathing on the tiers, aviary hygiene, red mite infestations, and laying hen movement and landing behaviours are potential underlying causes of the tier flooring material effects. Further research is highly recommended to investigate these underlying causes of tier flooring material effect on the various aspects of laying hen welfare. Additionally, hens on wire mesh had a 3% higher egg

laying rate compared to hens on plastic slats, thereby demonstrating that housing characteristics can also affect farm profitability.

Highly-motivated behaviours were possibly affected by housing system characteristics as well. Pre-laying behaviours may be affected by the design in front of the nest box, as we could associate plumage scores and keel wounds with having either a platform or a perch in front of the nest box entrance. Free-range availability offered hens more foraging and active behaviour opportunities, as well as possibilities to escape feather peckers, explaining the positive effects we found of free-range access on plumage condition. Moreover, foot pad hyperkeratosis was less prevalent in flocks that had access to a free-range, which could be related to less walking on tiers and less perching on round steel perches.

For identifying the most suitable hybrid for aviary systems, only Lohmann Brown Classic and ISA Brown could be compared due to the small number of replicate flocks from the other hybrids. Lohmann Brown Classic hens had more wing feathers damage, but fewer caudal tip fractures, a lower prevalence of foot pad hyperkeratosis, and a higher egg laying rate compared to ISA Brown hens. Further research under controlled conditions could elucidate the underlying causes of these differences between these two common hybrids.

More human-animal contact and feeding more different phase feeds during the production cycle were associated with a better plumage condition. The neck plumage score was associated to positively affect the egg laying rate.

The findings from our field study implied that selecting appropriate housing system characteristics and hybrid may improve laying hen welfare. Therefore, an experimental study with a 2x2 factorial design was performed to test if providing ramps in the housing system could reduce keel bone and foot pad disorders in two commercial hybrids (chapter 5). In this experiment, 16 pens were equipped either with or without ramps and housed either 25 Dekalb White or 25 ISA Brown hens. In our field study, it was not possible to compare white and brown hybrids. The experimental study, however, allowed us to compare two hybrids of a different colour for their susceptibility to certain welfare problems. Keel bone and foot pad disorders were assessed repeatedly between 17 and 52 weeks of age via visual assessment and/or palpation of the keel bone and foot pads. The ramps created a continuous pathway to enable hens to use

more controlled movements, such as walking and wing-assisted-incline-running (WAIR), to reach lower or higher levels of their housing without the necessity to jump or fly. These controllable movements lead to fewer falls and collisions, which very well could explain the differences in keel bone fractures we found. Although all keel bone disorders increased with age, ramps reduced fracture prevalence at all ages. Furthermore, mild keel bone deviations were less prevalent if ramps were present. This latter outcome may have resulted from a lower pressure load on the keel bone during perching on the ramps. In the field study, we found that in flocks with fewer deviated keel bones, also fewer downgraded eggs were produced. The experimental study could not confirm this result. The wire structure of the ramps may contribute to better food pad hygiene by scraping of manure. This may explain why hens in pens with ramps had less foot pad dermatitis and fewer bumble feet (in the case of Dekalb White hens). Hyperkeratosis increased more with age in pens without ramps compared to pens with ramps, although further research is needed to reveal the underlying cause of the latter result.

White hybrids are more flighty and show more fearful and panic reactions than brown hybrids. White hybrids, however, are also known to possess better navigation and flight skills than brown hybrids. These skills may lead to more successful landings and fewer falls and collisions, ultimately leading to fewer keel bone fractures in white hybrids. This hypothesis is supported by our findings that, even though Dekalb White hens had more keel hematomas and wounds, they had fewer fractures at all ages compared to ISA Brown hens. Differences in bone quality and/or perching behaviour between both hybrids may be underlying causes of Dekalb White hens having more keel bone deviations with increasing age compared to ISA Brown hens. Dekalb White hens had more foot pad dermatitis at all ages compared to ISA Brown hens. In contrast, foot pad hyperkeratosis was more prevalent in ISA Brown at all ages, whereas bumble foot was not observed in ISA Brown hens. Further research is needed to explain if and how physiological, anatomical, and/or behavioural differences between both hybrids are the underlying cause(s) of their susceptibility to keel bone and foot pad disorders and how this information can be used for breeding purposes.

The large variation between commercial flocks in prevalence and severity of the various welfare problems, and the association with characteristics of aviary housing system and management procedures with those welfare problems, opens

opportunities to improve laying hen welfare in commercial aviaries. This prospect is further strengthened by the experiment that demonstrated how providing ramps in modified aviaries very effectively reduced keel bone and foot pad disorders in two commercial hybrids. The differences found between hybrids demonstrated the possibility to select for more suitable behavioural and/or physiological characteristics of hybrids to make them more compatible for housing in aviary systems.

To conclude, selection of appropriate aviary system characteristics, management procedures and laying hen hybrid may create a better match between the laying hen and its environment. Ultimately this may result in improved health and welfare of laying hens in commercial aviary systems, as well as better egg production and farm profitability.

SAMENVATTING

Recente veranderingen in de EU-wetgeving hebben ertoe geleid dat miljoenen legkippen in volièresystemen worden gehuisvest. Dit aantal zal wereldwijd de komende jaren drastisch toenemen. Het volièresysteem is een niet-kooi systeem, ofwel een scharrelsysteem, met meerdere etages en biedt hennen tal van mogelijkheden tot het uitvoeren van natuurlijk gedrag. De kippen kunnen zich vrij door de stal bewegen waarbij ze gebruik maken van een complexe omgeving waarin voer, water, zitstokken en legnesten aangeboden worden op de verschillende etages. Kippen in volières lopen echter wel een verhoogd risico op bepaalde welzijnsproblemen als gevolg van specifieke kenmerken gerelateerd aan dit huisvestingssysteem. De meest voorname uitdagingen wat betreft dierenwelzijn in volièresystemen zijn schadelijk pikgedrag, verhoogde sterfte, en borstbeen- en voetzoolaandoeningen. Het identificeren van de zwakke en sterke punten van het huisvestingssysteem biedt mogelijkheden om systeem en management te verbeteren met als uiteindelijk doel een verbeterd dierenwelzijn. Bovendien kunnen bepaalde genetische kruisingen beter geschikt zijn voor het volièresysteem dan andere (hoofdstuk 1).

Dit proefschrift rapporteert over een cross-sectionele studie in commerciële volières met als doel kenmerken van het volièresysteem en management te identificeren, die van invloed zijn op aspecten van dierenwelzijn (schadelijk pikgedrag, verhoogde sterfte, borstbeen- en voetzoolaandoeningen) en eierproductie. Bovendien werd een 2x2 factorieel experiment uitgevoerd om het effect van loopplanken op het voorkomen van borstbeen- en voetzoolaandoeningen bij twee kruisingen te testen (hoofdstuk 2).

De veldstudie werd uitgevoerd in 47 stallen met volièrehuisvesting om de variatie en ernst van de welzijnsproblemen te onderzoeken (hoofdstuk 3 en 4). Alhoewel de studie afhankelijk was van de vrijwillige deelname van pluimveehouders, beschouwen wij de door ons onderzochte stallen met de daaruit verkregen resultaten als een representatieve weergave betreffende het welzijn van leghennen in Belgische volières in 2013 en 2014. Doordat veel Belgische pluimveehouders pas laat actie hebben ondernomen naar aanleiding van het EU-verbod per 1 januari 2012 op conventionele kooien, ofwel ‘batterijkooien’, hielden 34% en 36% van de pluimveehouders respectievelijk pas hun eerste of tweede koppel in hun nieuwe volièresysteem tijdens het bedrijfsbezoek. Het aantal jaar werkervaring met een volièresysteem kon echter niet worden geassocieerd met de onderzochte aspecten van welzijn. Het enige tijds gerelateerde effect dat we hebben aangetoond was dat er meer grondeieren werden aangetroffen in oudere stallen.

Schadelijk pikgedrag (verenpikken, cloacapikken, kannibalisme), borstbeen- (breuken, krommingen) en voetzool- (hyperkeratose, dermatitis, bumble foot) aandoeningen kwamen frequent voor in nagenoeg alle onderzochte koppels. De grote variatie die werd aangetroffen in de prevalentie en ernst van de onderzochte aspecten van dierenwelzijn konden worden geassocieerd met een aantal specifieke volière-, management-, en koppel-gerelateerde factoren (hoofdstuk 3 en 4). Identificatie van de sterke en zwakke punten van het huisvestingssysteem en management is nuttig om het welzijn van leghennen in volièresystemen te kunnen verbeteren.

Verenpikkerij en kannibalisme waren de belangrijkste doodsoorzaken volgens de pluimveehouders en dit werd gedeeltelijk bevestigd door onze bevinding dat mortaliteit lager is bij koppels met een beter verenkleed. Schade aan het verenkleed op de rug, staart, nek en rond de cloaca was waarschijnlijk het gevolg van pikkerij. Schade aan vleugelveren is echter waarschijnlijk het gevolg van slijtage door wrijving met het systeem. Op veel bedrijven werden ook rode bloedluizen aangetroffen. Bloedluizen veroorzaken stress en schadelijk pikgedrag, en de schadelijke gevolgen werden bevestigd doordat de met bloedluizen besmette koppels een slechter verenkleed en hoger sterftepercentage hadden. Een uiterst positieve bevinding uit onze veldstudie is dat de sterfte in volières niet hoger was dan waardes die zijn gerapporteerd voor kooisystemen.

Leghennen in rij-type volières hadden meer nekveren, minder borstbeenwonden, meer midden borstbeenbreuken en ernstigere voetzooldermatitis in vergelijking met kippen in portaal-type volières. De gangpadbreedte in rij-systemen was mogelijk van invloed op succesvolle landingen van de hennen wat zou kunnen verklaren waarom er minder hematomen worden aangetroffen bij bredere gangpaden. In de huidige studie is niet onderzocht hoe de kippen zich in de verschillende volièresystemen bewegen, maar wij stellen wel dat daar deels de oorzaak ligt van het verschil in borstbeen- en voetzoolafwijkingen tussen volièresystemen.

Het materiaal van etagevloeren was geassocieerd met meerdere welzijnsproblemen bij leghennen. Koppels in volières met ijzeren draadroosters hadden een beter verenkleed, minder wonden, een lager sterftepercentage en minder bloedluisbesmettingen in vergelijking met volières met kunststof roosters. Daarentegen hadden koppels op kunststof roosters minder borstbeenbreuken. Pseudo-stofbaden op de etagevloer, volièrehygiëne, bloedluizen, en mislukte landingen zijn mogelijke onderliggende oorzaken van de etagevloereffecten. Verder onderzoek is sterk aanbevolen voor het achterhalen van deze onderliggende oorzaken. Bovendien werd aangetoond dat het legpercentage mogelijk 3% hoger lag bij koppels op draadroosters ten opzichte van kunststof roosters, waarmee aangetoond werd dat de huisvesting ook invloed kan hebben op de rendabiliteit.

Sterk gemotiveerde natuurlijke gedragingen werden mogelijk ook beïnvloed door verschillende kenmerken van de huisvesting. Het nestgedrag werd mogelijk beïnvloed door de inrichting voor de ingang van het legnest, zoals aangetoond door de verschillen in verenkleed en borstbeenwonden bij kippen met legnesten met een zitstok of een platform bij de nestingang. De positieve effecten van een vrije uitloop op het verenkleed komen mogelijk doordat deze hennen beter kunnen foerageren, meer actief gedrag uitvoeren, alsook meer mogelijkheden hebben om te ontsnappen aan verenpikkende kippen. De lagere prevalentie van hyperkeratose in koppels met een vrije uitloop kan gerelateerd zijn aan het eventueel minder gebruik maken van de zitstokken en/of minder op verharde ondergronden lopen.

Voor het identificeren van geschikte kruising voor volièresystemen kon alleen de Lohmann Brown Classic met de ISA Brown vergeleken worden, omwille van het kleine aantal herhalingen van koppels met andere kruisingen. Lohmann Brown Classic kippen hadden meer schade aan de vleugelveren, maar minder caudale borstbeenbreuken, minder vaak voetzoolhyperkeratose, en een hoger legpercentage ten opzichte van ISA Brown kippen. Verder onderzoek onder gecontroleerde omstandigheden kan meer opheldering geven over de onderliggende oorzaken van de verschillen tussen deze twee hybrides.

Meer mens-dier contact en meer verschillende fase voeders waren twee managementprocedures die konden worden geassocieerd met een betere verenscore.

De veldstudie impliceerde dat bepaalde kenmerken van volièrehuisvesting beter kunnen zijn voor het welzijn van leghennen. Hiertoe hebben we een experimentele studie uitgevoerd met een 2x2 factorieel ontwerp, waarbij getest werd of het verstrekken van loopplanken in het systeem kon resulteren in minder borstbeen- en/of voetzoolaandoeningen in twee commerciële kruisingen (hoofdstuk 5). In dit experiment waren 8 hokken met en 8 hokken zonder loopplanken uitgerust en werden 25 Dekalb White of 25 ISA Brown hennen per hok gehuisvest. Deze twee kruisingen werden mede gekozen omdat witte kruisingen dermate ondervertegenwoordigd waren in de veldstudie (n=3), dat een vergelijking van witte met bruine kruisingen niet mogelijk was. Borstbeen- en voetzoolaandoeningen werden herhaaldelijk beoordeeld van 17 tot 52 weken leeftijd via visuele beoordeling en palpatie. Door de loopplanken kunnen de hennen meer gecontroleerde bewegingen maken, zoals lopen en vleugel-geassisteerde-loopbewegingen, om lagere of hogere niveaus in hun leefomgeving te bereiken zonder te moeten springen of vliegen. Dit zal vermoedelijk leiden tot minder mislukte landingen en botsingen, met als gevolg minder kans op borstbeenbreuken. Uit onze experimentele studie bleek inderdaad dat het verstrekken van loopplanken leidt tot minder borstbeenbreuken. De kleinere kans op borstbeenkrommingen bij hennen met een loopplank ter beschikking, heeft mogelijk te maken met een lagere belasting op het borstbeen tijdens het zitten op de loopplanken. In de veldstudie vonden we dat in koppels met minder borstbeenkrommingen er ook minder tweede keus eieren werden geproduceerd. Deze bevinding konden we niet bevestigen in de experimentele studie. De draadrooster structuur van de loopplanken heeft mogelijk bijgedragen aan een betere voetzoolhygiëne doordat deze mest van de voetzolen afschraapt. Dit kan verklaren waarom kippen in hokken met loopplanken minder voetzooldermatitis hadden en minder bumble foot (enkel bij Dekalb White kippen). In hokken zonder loopplank werd op het einde van het experiment meer hyperkeratose aangetroffen. Verder onderzoek is nodig om de onderliggende oorzaken aan te tonen.

Ondanks dat witte kruisingen angstiger zijn en meer vlucht- en paniekgedrag vertonen dan bruine kruisingen, beschikken witte kruisingen over betere navigatie en vliegvaardigheid dan bruine kruisingen. Deze vaardigheden leiden mogelijk tot meer succesvolle landingen en minder botsingen, met als gevolg minder borstbeen breuken in de witte kruisingen. Deze hypothese wordt ondersteund door onze bevindingen dat, hoewel Dekalb White kippen wel meer hematomen en wonden hadden, ze uiteindelijk minder breuken hadden in vergelijking met ISA Brown kippen. Verschil in botkwaliteit en/of zitstokgebruik kunnen onderliggende oorzaken zijn van dat Dekalb White kippen op latere leeftijd meer borstbeenkrommingen vertoonden dan ISA Brown kippen. De Dekalb White kippen hadden meer en ernstigere voetzool dermatitis in vergelijking met

ISA Brown kippen. Bumble foot werd helemaal niet aangetroffen bij ISA Brown kippen, daarentegen kwam voetzoolhyperkeratosis meer voor bij ISA Brown kippen in vergelijking met Dekalb White kippen. Verder onderzoek moet aantonen hoe eventuele verschillen in fysiologie, anatomie en/of gedrag tussen beide kruisingen de onderliggende oorzaken zijn van hun predispositie voor de verschillende borstbeen- en voetzool aandoeningen en hoe deze gegevens toegepast kunnen worden in de fokkerij.

De grote variatie tussen commerciële koppels betreffende de verschillende welzijnsproblemen, en de gevonden associaties met specifieke systeem- en managementkenmerken bieden mogelijkheden om het welzijn van leghennen in commerciële volièresystemen te verbeteren. Deze bewering wordt verder versterkt door de 2x2 factoriële proef die aantoonde dat het aantal borstbeen- en voetzoolaandoeningen aanzienlijk verlaagd kan worden door het verstrekken van loopplanken en door de keuze van (en verdere selectie naar) kruisingen met gedrags- en/of fysiologische eigenschappen die geschikt zijn voor huisvesting in volièresystemen

Selectie van de kenmerken van het volièresysteem, management en type leghen kan leiden tot een betere afstemming tussen de leghen en haar leefomgeving. Uiteindelijk zal dit kunnen leiden tot een verbetering van de gezondheid en welzijn van de leghennen in commerciële volièresystemen, alsook tot een betere eierproductie en dus tot betere bedrijfsresultaten.

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Appendix I

Question in farmer questionnaire in the cross-sectional field study	
Age manager	Cleaning dry or wet
Years experience as poultry farmer	Waterline disinfection + method
N employees	N visits veterinarian
Flock size at placement	Aviary type (portal/row)
Current flock size	Aviary brand, version + N tiers
Age flock at placement	Egg production parameters
Age flock at time of visit	Floor egg prevention
Hybrid	Data management
Reason for choosing hybrid	Cumulative mortality
Rearing system	Last weeks' mortality
Visited rearing farm	N culls
Feed supplier	Main cause(s) of mortality
Labels / certification body	Perch orientation
Age of barn	Nest lining material
Age of aviary	Nests closure + times
New barn or renovation	Litter at placement
Dimensions barn	Litter quality
N compartments + size compartments	Phase feeds
Presence covered veranda + size	Feed enrichments
Litter in covered veranda	Water source + treatment
Free-range presence + size	Light source(s)
Feed and/or water in free-range	Light scheme
Access covered veranda / free-range	Familiarization phase at placement
Annual N days free-range	Moulting + method
N hens per compartment	Hygiene gate + usage
Planned slaughter-age	Disinfection mats at entrance
Presence of cockerels (+ N)	Preventive veterinarian screening
Beak treatment + method	Red mite prevention
Body weight abnormalities	Detected diseases, bacteria, parasites
Feather pecking + cannibalism	Vaccination scheme
Feather pecking type	Blood sampling
Estimated % loss by feather pecking	Method of cleaning between rounds
Perch material + shape	Compartment equipment
Nest perch / platform	Ventilation type
N nests per compartment	Fine dust reduction
Type of nest	Pests + pest control
N feed turns/day	Reason for aviary housing
Feed texture	Satisfaction aviary housing
Feeder type	Corridor width
Drinker type + N	Henhouse climate (Temp, RH, CO_2)
Water consumption	Stockperson barn rounds + duration
Litter replacement + material	Music or radio playing
Manure removal system + aerated	Empty period between rounds

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Heerkens, J. The influence of aviary housing characteristics, management and genetics on health, welfare and production in laying hens. Presentation at Welfare Quality Network Workshop, 11 December 2013, Lille, France.

Heerkens, J. Examining the latest challenges in aviary housing. *Invited speaker.* 12th Annual Conference World Poultry, 22 May 2013, Brussels, Belgium.

AWARDS AND TRAVEL GRANTS

Student Travel Grant for participation in the 104th Annual Poultry Science Meeting, 27-30 July, 2015, Louisville, US.

Avialter Award for participation in the ISAE: 48th conference of the International Society of Applied ethology. July 29 – August 2, 2014, Victoria-Gasteiz, Spain.

Curriculum Vitae

Jasper L.T. Heerkens werd geboren op 28 augustus 1981 te Maasbree (Limburg, Nederland) en behaalde in 1999 zijn HAVO diploma aan het Thomascollege in Venlo. Na een verkort traject behaalde hij in 2002 zijn diploma aan het Middelbaar Laboratorium Onderwijs, afstudeerrichting Biotechnologie, waarna hij in Utrecht aan de Hoger Laboratorium Opleiding aan de Hogeschool Utrecht begon. Al gauw bleek dat Jasper graag met dieren werkte en koos hij de afstudeerrichting Zoölogie. Na zijn BSc-afstudeerstage van 12 maanden in Bristol (Engeland) behaalde hij in 2006 zijn BSc-diploma. Direct na zijn opleiding kon hij gaan werken aan het Academisch Medisch Centrum Amsterdam als research biotechnicus, waarbij hij onderzoek deed naar de invloed van synthetische epo op celformatie in humane en muizen bloedvaten. Het werken met dieren beviel hem ten zeerste, echter, verkoos hij liever met dieren te werken ten behoeve van de dieren zelf. In 2008 werd hij toegelaten tot de Master Dierwetenschappen aan de Wageningen Universiteit. Hier kwam hij voor het eerst in aanraking met leghennenonderzoek tijdens zijn Major afstudeeropdracht bij de faculteit Diergeneeskunde in Utrecht. In 2010 behaalde Jasper zijn MSc diploma, waarna hij een jaar bij het Nederlands Vaccin Instituut heeft gewerkt als senior biotechnicus. Het was Dr.Ir. Bas Rodenburg (Wageningen Universiteit), die Jasper attendeerde over een interessante praktijkgerichte dierenwelzijnsstudie bij het Instituut voor Landbouw en Visserij Onderzoek (ILVO). Gefinancierd door de FOD Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu en het ILVO begon hij in december 2011 aan zijn doctoraatstudie omtrent het identificeren van risicofactoren die van invloed zijn op het welzijn van leghennen in volièresystemen, alsook het testen van innovatieve remediërende maatregelen ter verbetering van het welzijn. Tijdens zijn doctoraatstudie heeft Jasper veel praktijkbedrijven met volièresystemen bezocht, een experiment uitgevoerd in de proefstallen van het ILVO en heeft hij op meerdere internationale symposiums gesproken en een wereldwijd netwerk opgebouwd. Op dit moment is Jasper werkzaam als pluimvee specialist bij Jansen Poultry Equipment in Barneveld, waarbij hij met informatie uit de praktijk bijdraagt tot optimalisatie van de huisvesting en management van pluimvee.

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