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Data-driven housing designs to improve bone health and welfare in laying hens

Nikki Mackie

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of PhD in the Faculty of Health Sciences, School of Veterinary Sciences, November 2018.

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Abstract

This study aims to improve laying hen welfare in multi-tier systems, particularly with respect to keel bone fractures, by understanding how hens move around the system, identifying impact hazards, and investigating modifications directed at mitigating identified hazards. A variety of techniques were used to investigate housing hazards, including the use of accelerometers, behavioural observation, keel bone palpation and foot pad scoring. Although the overriding aim of this thesis was to identify hazardous areas in a multi-tier system and modify these areas to reduce keel bone fractures, the effect of modifications on the overall health of the hens was also investigated. The main findings of this study indicated, through accelerometry data, that falls resulted in higher loads at the keel compared to non-falls, and that collisions had higher loads compared to non-collisions. Movements around the nest box and top tier region resulted in a higher percentage of falls compared to other regions in the system. Dusk and dawn were more hazardous compared with day and night times, considering both the number of movements within the time point and the percentage of falls. Changes made to a multi-tier system aimed at reducing keel bone fracture prevalence either showed no clear benefit or increased prevalence. The only modification that made an improvement in health was the provision of ramps reducing the prevalence of foot pad dermatitis. On-farm studies showed a reduced keel bone fracture prevalence and bumblefoot prevalence with ramp access compared to those with no ramp access. This work has shown that it is possible to identify hazards associated with certain types of hen movement, specific regions within the system, and times of the day. Modifications to housing systems can influence hazards, though not necessarily as expected, with the provision of ramps showing the most promise at improving welfare.

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Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: .... DATE:..........................

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Chapter 1

General Introduction

1.1. Thesis Aims

This thesis will focus on how to improve multi-tier housing system design, to reduce keel bone fracture prevalence, by examining hazardous keel threatening transitions within multi-tier systems. When researching this topic, it is important to consider other factors that can influence laying hen health. Creating an optimal housing system with the aim to reduce keel bone fractures and hazardous behaviours would not be suitable unless other welfare detriments did not occur. Throughout this thesis, as well as keel bone damage (deviations and fractures), foot pad health and feather damage are referenced to give an overall health assessment of a flock with different internal structures.

Chapter 2 will look at the physical characteristics in a multi-tier system for laying hens and will aim to look at the height and movement behaviour of hens using video observation and accelerometers. Chapter 3 will look at the navigation paths in a multi-tier system and where falls and collisions are most likely to occur and at what time of day falls are more likely to occur. Chapter 4 will use the results obtained from Chapter 2 and 3 to look at the effects of modifications in a multi-tier system, designed to reduce keel bone fracture prevalence, on laying hen health and behaviour. Chapter 5 will discuss the findings from an on-farm study looking at the influence of genetic hybrid and ramp provision on keel bone fracture prevalence and other health parameters.

1.2. Background of the commercial layer

Studies have shown that the domestic laying hen is genetically of little difference to her wild ancestor, the red jungle fowl (Sawai *et al.*, 2010). Laying hens have kept most of the behavioural characteristics of their wild counterparts but time budgets for some behaviours have altered (Garnham and Løvlie, 2018). Indigenous communities began to breed red jungle fowl with other poultry stock, referred to in the literature as “village chickens” (GuÈye, 1998). However, selection pressure from genetic companies did not begin until the 1900’s (Appleby, 1992) and general production rates of all farm animals have increased substantially since then (Rauw *et al.*, 1998).

Commercial layers now produce approximately 300 eggs per year, with their wild counterparts (red jungle fowl) laying approximately 10-15 eggs per year (GuÈye, 1998; Romanov and Weigend, 2001; Besbes *et al.*, 2007). Traditional breeds have produced fewer eggs in comparison to commercial breeds, but they have stronger, stiffer and more radio-dense bones (Hocking *et al.*, 2003). Traditional breeds also have a higher volume of cortical bone present in tibiotarsus compared to commercial breeds (Hocking *et al.*, 2003). This increase in egg production has resulted in a reduced feed conversion ratio (Schütz *et al.*, 2001) and possibly due to this major change in the production ability, laying hens’ skeletal health is affected. Furthermore, genetics have been shown to play a role in skeletal integrity, with bone strength being a heritable trait (Bishop *et al.*, 2000).

1.3. Skeletal health in the commercial layer

Cortical and trabecular (or cancellous) bone provide the structural component of the skeleton, they are both forms of lamellar bone (Whitehead and Fleming, 2000) and are formed during growth and maturity. When hens reach sexual maturity, medullary bone (a type of non-structural woven bone) is formed (Whitehead and Fleming, 2000). Once formed, bone continuously remodels, mineralised areas of the bone are resorbed by osteoclasts and then the osteoblasts provide the new bone. Remodelling takes place due to changes in the mechanical need of the bone as well as to repair small damages (Hadjidakis and Androulakis, 2007). Multiple bone signalling techniques are used to trigger bone remodelling involving hormones and cytokines. If a problem occurs along these signalling pathways then diseases of the bone can develop, such as osteoporosis (Proff and Römer, 2009). Woven bone deposits quickly during ossification and during fracture healing, this bone has loosely packed collagen fibres and a lower mineral density compared with lamellar bone (Gorski, 1998; Rath *et al.*, 2000). The main purpose of medullary bone is to provide a source of calcium for egg shell formation (Whitehead and Fleming, 2000).

At the onset of lay; production of structural bone is arrested and production is changed to medullary bone in order to provide the eggshell with calcium (Whitehead, 2004). Medullary bone is a calcified woven bone laid down in the medullary cavity of long bones, and is capable of rapid deposition and resorption, but provides little mechanical strength. Naturally (as would be seen in red jungle fowl), once a hen is out of the laying cycle, production of medullary bone is replaced by deposition of structural bone until the hen is ready to produce another clutch of eggs (Whitehead, 2004). Domestic laying hens, have been bred to have a high egg laying capability, meaning they stay in lay for a longer period of time (Hocking *et al.*, 2003). Osteoclasts, the cells specialised for resorption of bone, release calcium into the circulation when reserves are needed for correction of blood calcium concentration, particularly during egg shell production (Whitehead, 2004). It is thought that resorption of medullary bone and structural bone during this process leads to some collateral damage to the bone. This loss of structural bone can lead to weak, osteoporotic bones that have a heightened vulnerability to fracture and in extreme cases lead to “cage layer fatigue” which may result in paralysis (Whitehead, 2004). Cage layer fatigue is an extreme form of osteoporosis and involves the loss of endogenous calcium in the skeleton and causes vertebrae weakness and collapse (Bell and Siller, 1962; Whitehead and Fleming, 2000). However, weakened bones are common in hens housed in cage systems where lack of skeletal loading exercise results greater loss of bone strength (Whitehead and Fleming, 2000).

One of the most widespread skeletal problems in the laying hen industry is the prevalence of keel bone fractures (FAWC, 2010). The keel bone protrudes from the sternum and is the anchoring point for the flight muscles; due to the prominent position on the body, it is at the most vulnerable position for becoming damaged during falls and collisions (Figure 1.1). Keel bone fracture prevalence at the end of lay in cage systems is between 24-62% (Rodenburg *et al.*, 2008; Wilkins *et al.*, 2011; Petrik *et al.*, 2015), the prevalence in single-tiered systems is 48-82% and in multi-tiered systems the prevalence is highest at 60-100% (Rodenburg *et al.*, 2008; Wilkins *et al.*, 2011; Petrik *et al.*, 2015; Heerkens *et al.*, 2016b). Prevalence of keel bone fractures increase in a system as the cumulative height of the perching structures increase (Wilkins *et al.*, 2011). This suggests that increased complexity and height of a system increases prevalence of keel bone fractures (Gregory and Wilkins, 1996; Moinard *et al.*, 2004a). It is assumed that as the height of a collision increases the energy absorbed by the keel would be greater, resulting in an increased likelihood of fracture. In experimental settings, it was shown that as the energy of collision at the keel increases (using a drop-weight), the probability of keel bone fracture increases (Toscano *et al.*, 2018).

As well as fractures due to trauma, aberrant wing flapping may be a cause. The keel bone could be pulled, resulting in a stress fracture or a hen can have an asymmetrical flight causing strain on the keel bone (Harlander-Matauschek *et al.*, 2015). Although data relating to stress fractures in laying hens are not available, stress fracture development has been studied in humans (Knapp and Garrett, 1997; Warden *et al.*, 2006). Stress fractures are common in athletes that run or take part in high weight-bearing activities (Knapp and Garrett, 1997) and are caused by a repetitive mechanical load causing strain on the bone (Warden *et al.*, 2006).

The causes of keel bone damage are multi-factorial, meaning there are many other underlying causes that influence keel bone damage (Harlander-Matauschek *et al.*, 2015). Causes included genetics, nutrition and housing design and each will be discussed in detail later in this review.

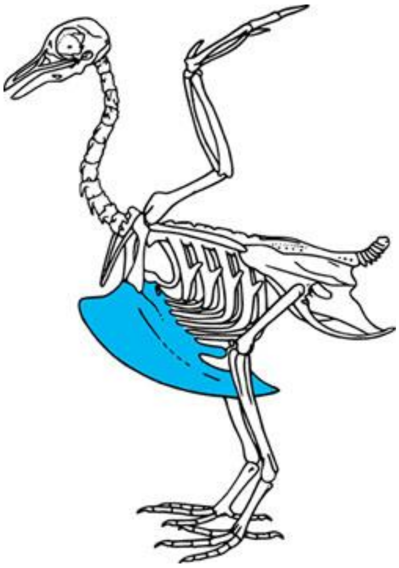

Figure 1.1: *Location of the keel bone in blue*

[*Image:(Toony, 2018)*]

Fracture development and the pain experienced from the fracture may be different across individuals and be a result of different fracture severities (Richards *et al.*, 2011). In humans with fractures, 6 out of 27 reported that they felt no pain when the fracture occurred (Melzack *et al.*, 1982). Although it is unknown whether a similar phenomenon occurs in hens with keel bone fractures it is worth noting that there may be a difference in pain perception between individuals. Hens with healed keel bone fractures preferentially consume food containing Butorphanol, an opioid analgesic often used to treat severe pain, in comparison to hens that did not have keel bone fractures (Nasr *et al.*, 2013a). Hens with healed keel bone fractures had a reduced latency to move from a perch when given the same opioid analgesic (Butorphanol) compared to saline (Nasr *et al.*, 2012b). However, when hens were given two different types of NSAIDS (non-steroidal anti-inflammatory drugs), used to treat moderate pain, there was no difference in the latency to land from perches when compared to those not given NSAIDS (Nasr *et al.*, 2015). Keel bone fractures may cause pain, but the results were not conclusive across all studies. One factor that may have affected the results was that although all hens had keel bone fractures, the severity of the fracture may have been different, suggesting that the level of pain experience may have been different across individuals (Richards *et al.*, 2011). The focal hens were aged between 35-40 weeks of age (Nasr *et al.*, 2012b; Nasr *et al.*, 2013a; Nasr *et al.*, 2015). Due to the keel bone being flexible at about 30 weeks of age and continuing to ossify as the hen ages (Richards *et al.*, 2011), and that ossification continuing until approximately 40 weeks of age (Buckner *et al.*, 1948), it may be that older hens experience pain from keel bone fractures differently.

Due to some sections of the keel bone potentially being more ossified than others, the location of a break may affect the level of pain experienced by the hen. Figure 1.2 shows several types of fractured keel bone, ranging from no fracture (0) to extremely severe fractures (5). The fracture at stage 1 may be linked to the cartilaginous region of the keel compared to the area in the central region of the keel that develops at stage 4. The fracture at stage 4 may be a more ossified region of the keel and therefore, contains more nociceptors, resulting in a greater pain sensation (Mannion and Woolf, 2000).

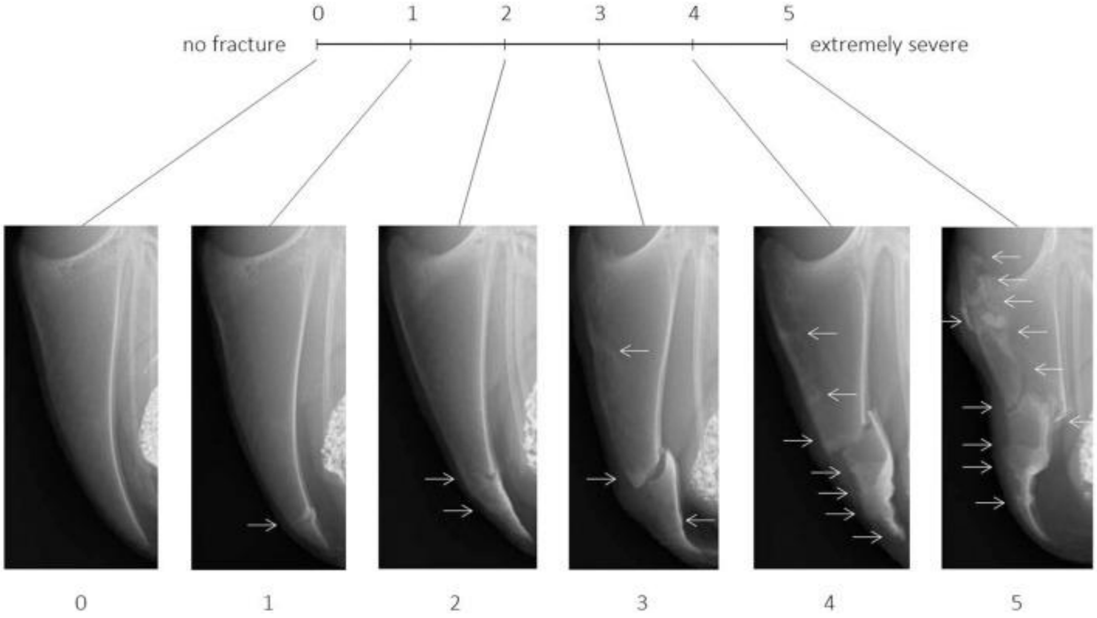

Figure 1.2: *Keel bone fractures at different stages of development. Arrows denote areas where keel bone fractures have occurred.*

Image from: (Rufener *et al.*, 2018) 0-5 on the graph are stages of fracture status

Another form of keel bone damage discussed in the literature are keel bone deviations (Harlander-Matauschek *et al.*, 2015; Riber *et al.*, 2018). Few studies have looked at the causes of keel bone deviations, but many aetiologies have been theorised. Keel bone deviations are thought to be linked to roosting behaviour in young pullets as well as the diet composition (Warren, 1937). They are thought to be caused by excessive perching and is corroborated with the incidence of keel bone deviations being higher in systems with access to perches (Tauson and Abrahamsson, 1994; Harlander-Matauschek *et al.*, 2015). Pressure load and surface area on the keel bone when roosting is higher than that experienced on foot pads, this corroborates speculation that perching may be a cause of keel bone deviations (Pickel *et al.*, 2011). Hens with access to standard, round metal perches are almost twice as likely to develop keel bone deviations compared to hens with access to soft perches (Stratmann *et al.*, 2015b). The authors suggest that it is a combination of the increased diameter (due to a greater surface area to spread pressure throughout the keel instead of localised pressure) of the softer perches and the material that resulted in the reduction in keel bone deviation prevalence (Pickel *et al.*, 2011; Stratmann *et al.*, 2015b). There may be a link between keel bone fractures and deviations because keel bone deviations increase during peak lay, like keel bone fractures (Gebhardt-Henrich and Fröhlich, 2015). At dissection, 50% of keel bone deviations were found with callus formation, suggesting that those keels were either deviations with fractures or the fracture process caused a deviation in the bone (Stratmann *et al.*, 2015b).

Looking back at fracture development, it is not only keel bone fractures that pose a problem for the laying hen industry, fractures of the furculum, pubis, tibia and humerus are also issues (Gregory and Wilkins, 1989; Knowles and Wilkins, 1998). However, these bone fractures tend to be more prominent and obvious to farmers and veterinarians and are of lower prevalence. The keel bone is both structural bone and cartilage, whereas other bones in the body (particularly the long bones) are composed of structural bone exclusively (Buckner *et al.*, 1948; Buckner *et al.*, 1954). This suggests that the pain and healing process associated with long bones may be different than that of the keel bone.

1.4. Factors contributing to keel bone damage

1.4.1. Genetics

1.4.1.1. Predisposition to keel bone fractures and bone properties

Many studies have found that genetics are crucial in the prevalence of keel bone fractures, with some commercial hybrids having higher rates of keel bone fractures in comparison to others. The link between genetic hybrid (including pure lines) and keel bone damage will be discussed below.

In general, brown hybrids (brown-feathered layers) have higher levels of keel bone fractures and stronger bones in comparison to white hybrids (white-feathered layers) (Habig and Distl, 2013; Riber and Hinrichsen, 2016; Eusemann *et al.*, 2018). However, this may be attributed to differences in body mass because bone strength is related to body mass (Knowles and Broom, 1990). Lohmann browns also have greater humerus and tibia breaking strength compared to Lohmann Selected Leghorn hens (white hens) (Habig and Distl, 2013) combined with greater radius and tibia wet, dry and ash masses compared with white hybrids (Silversides *et al.*, 2012). However, bone mineral density (BMD) can be inconsistent, with white hybrids having greater BMD of the tibia compared to Lohmann browns (Silversides *et al.*, 2012). In general, long bones of brown hybrids tend to be stronger than white hybrids. Due to long bone (humerus and tibia) radiographic densities correlating with keel bone radiographic density, brown hybrids may also have denser keel bone than white hybrids (Hocking *et al.*, 2003). This would suggest that brown hybrids should have an overall lower keel bone prevalence than white hybrids.

Conversely, ISA brown hens have a higher percentage of keel bone fractures compared to white hybrids at 51 and 72 weeks of age, irrespective of low or high-performance capability (Eusemann *et al.*, 2018). Previous research has shown that ISA brown hens have also shown a higher prevalence of keel bone fractures compared to Delkalb white hens but have fewer keel bone deviations (Heerkens *et al.*, 2016a). The effect of hybrid is age dependent; Stratmann *et al.* (2016) found an interaction effect between the occurrence of keel bone damage in ISA brown hybrids and Delkalb white hybrids. Whilst ISA brown hens had a higher prevalence of keel bone damage at the onset of the laying period, Delkalb white hens had a higher prevalence of keel bone damage at the end of the laying period (Stratmann *et al.*, 2016).

An experimental white hybrid and a Dekalb white hybrid had the highest prevalence of fractures compared to all brown hybrids, whether pure lines or commercial hybrids (Candelotto *et al.*, 2017). However, when impact tested it was shown that the keel from the purebred was less likely to fracture compared to the commercial hybrids, with the white hybrids in each instance having weaker bones (Candelotto *et al.*, 2017). A brown-feathered low performing hybrid (bred for reduced egg production) had a lower prevalence of deviations and fractures compared to the high performing hybrid (bred for increased egg production) (Eusemann *et al.*, 2018). This would suggest that production of eggs has a detrimental effect on the skeleton of the laying hen, causing weaker bones and an increased fracture and deviation risk.

Lohmann brown lite hybrids had a higher prevalence of keel bone fractures compared with ISA brown (Riber and Hinrichsen, 2016). However, the mentioned studied had extremely low levels of keel bone damage in all groups (up to 11.6% at end of lay) (Riber and Hinrichsen, 2016), which suggests there may have been false negatives in the dataset or that the farms visited were not a true representation of other farms where keel bone fracture prevalence is as high at 97% (Rodenburg *et al.*, 2008). One study, conducted on different farms, found no difference between hybrids with respect to keel bone damage prevalence (Käppeli *et al.*, 2011). As can be seen, a generalisation that brown-feathered layers have more keel bone fractures compared with white-feathered layers is true most of the time but is not conclusive.

A high bone strength hybrid had greater tibia breaking strength and stiffness compared to the low bone strength hybrid (Fleming *et al.*, 2006). The hens that were used in the study were selected for “bone index” (to increase the strength and radiographic density of bones) over 7 generations (Bishop *et al.*, 2000). The high bone strength hybrid had higher radiographic densities of the keel and humerus but lower external and internal tibial area compared to the low bone strength hybrid (Fleming *et al.*, 2006). It was shown that hybrids selected for high bone strength had higher BMD and fewer keel bone fractures in comparison to hybrids selected for low bone strength (Stratmann *et al.*, 2016) and was based on the same genetic lines used in the previous study (Fleming *et al.*, 2006). A commercial hybrid (LSL) was used as a comparison and keel bone fracture prevalence was higher in the LSL compared to the high bone strength hybrid but no difference was found when compared with the low bone strength hybrid (Stratmann *et al.*, 2016). The same pattern was also found for BMD. However, increased bone index was associated with lower egg production (Stratmann *et al.*, 2016).

In conclusion, it is possible to improve bone strength through genetic selection, but this selection process can have drawbacks such as a potential reduction in egg production. Commercial lines also differ in bone strength and keel bone damage, but it is not as clear as all white hybrids have lower keel bone fracture prevalence compared to brown hybrids.

1.4.1.2. Behavioural differences

When trying to understand keel bone fracture occurrence; it is important to look at laying hen behaviour due to the link between falls, perching and keel bone damage (Gregory and Wilkins, 1996; Moinard *et al.*, 2004a). Behaviour of laying hens is influenced from as early as the rearing phase. Providing a similar environment during rear to that applied during lay is important so that hens' have knowledge of their environment to allow them to use the behavioural repertoire they are familiar with (Colson *et al.*, 2008). Hens that have not developed their spatial abilities and in particular those that have never experienced perches, will find it more difficult to navigate complex systems with elevated perches and tiers (Colson *et al.*, 2008).

Genetic hybrids differ in their use of a 3-dimensional space as chicks (Kozak *et al.*, 2016a), which could affect their ability to navigate 3D environments as adult hens. Lohmann selected leghorn lite (LSL-lite) hens were observed on raised surfaces more frequently and performed a greater number of transitions compared to the other hybrids studied (Delkalb White, Lohmann Brown and Hyline Brown) (Kozak *et al.*, 2016a). Behavioural measurements using tri-axial accelerometers have shown that brown hybrids (Lohmann Brown and Hyline Brown) performed fewer low intensity movements (perching and sleeping as examples) compared to White hybrids (Delkalb White and LSL-lite) from 10-57 weeks of age (Kozak *et al.*, 2016b). This suggests further that there may be a genetic component of behaviour in laying hens.

1.4.2. Nutrition

As well as genetics, nutrition can influence bone health and the development of keel bone damage (Harlander-Matauschek *et al.*, 2015). It is estimated that 2g of calcium (10% of a hen's total calcium reserves) is needed to produce one egg (Taylor, 1970). Calcium is reabsorbed from the skeletal system, which weakens the keel bone and other bones in the body due to reduced skeletal strength. One way to counteract this loss in calcium is through feed supplementation.

Bone ash concentration and bone ash per unit volume increases positively with calcium uptake, meaning that as calcium provision increases bone mineral content increases (Cheng and Coon, 1990). This result was further supported by other bone measurements (bone breaking force, bone bending moment, bone stress and bone breaking force/100g of body weight) showing the same relationship with calcium provision (Cheng and Coon, 1990). No differences in tibia properties (including breaking strength) at 70 weeks of age between different calcium diets were identified, but egg shell weights, thickness and density at 70 weeks of age was improved as calcium levels in the diet increased (Safaa *et al.*, 2008). This shows a complex relationship between dietary calcium, calcium up-take and bone mineralisation. This could potentially affect availability of calcium for use in the skeleton, e.g. in the keel bone.

Although it was previously discussed that there was no difference in tibia properties between diets with/without calcium, the addition of calcium into the diet may still have the potential to increase bone strength.. Fleming *et al.* (1998) examined the differences in caged birds and the effects of four different feed treatments (1. Control (with powdered calcium), 2. Control (with particle calcium), 3. Control (with added ascorbic acid) and 4. Control (with added Vitamin K). Trabecular bone volume decreased with age in all treatments in the tarsometatarsus and the thoracic vertebra, whereas medullary bone increased with age (Fleming *et al.*, 1998). In the same study, breaking strength of the tibia increased with age in the control with powdered calcium and the control with particle calcium (Fleming *et al.*, 1998).

Differences persisted when comparing the other feed additive groups. In general, ascorbic acid (a co-factor required in collagen synthesis, an important stage in bone development (Rath *et al.*, 2000)), Vitamin K and the use of particle calcium compared to powdered calcium all had an overall beneficial effect on bone health (Fleming *et al.*, 1998). However, there are confounding factors in this study, as it was carried out in caged birds where there is limited space to perform complex behaviours, making the hens in these systems more vulnerable to skeletal loss (Fleming *et al.*, 1994). Another factor is that the keel bone progressively ossifies and even at 70 weeks of age the keel bone will mainly be cartilage (personal communication, John Tarlton).

Omega-3 supplementation has been shown in many human and animal studies to improve bone health (Salari *et al.*, 2008). By altering the diet of laying hens to include more omega-3, keel bone damage was reduced by 40-60% (Tarlton *et al.*, 2013). The omega-3 diets also significantly reduced the severity of breaks and increased the breaking strength of the tibia (Tarlton *et al.*, 2013). These results show that alterations to the diet can significantly increase bone health in laying hens but do not eliminate fractures completely (Tarlton *et al.*, 2013), suggesting there are limits to what diet alterations can achieve. This suggests that there are other underlying causes in keel bone fracture development. Omega-3 in large quantities can result in production losses (increased food and water consumption, an increase in lower quality eggs, lower egg weight, decreased egg production and increased mortality rates) (Toscano *et al.*, 2015). The authors suggest that the source of the omega-3 diet is crucial and more work needs to be done to determine the best composition of omega-3 for laying hens.

However, there have also been studies that show the use of omega-3, and other dietary supplements, hinder laying hen bone health and welfare, with hens fed the omega-3 supplemented diets having more keel bone fractures and fewer hens approaching a test area in an approach test compared with control hens (Toscano *et al.*, 2015). When looking at the tibia, one study found that the only difference seen between omega-6 and omega-3 fatty acid diets was an increase in cortical thickness, which was higher at the intermediate levels of omega-6 compared to low and higher levels of omega-6 (Baird *et al.*, 2008).

Different diets and supplementation of the standard layer diet can influence keel bone fracture development. However, the outcome of the diet change and supplementations are not always clear and may vary depending on concentration, hybrid and housing system. Therefore, it is important to consider diet and nutrition when trying to improve skeletal health and reduce keel bone fractures in layers, but other factors must be altered to harmonise these effects.

1.4.3. Housing design

Housing design is a major factor in the development of keel bone fractures (Harlander-Matauschek *et al.*, 2015). Cage-free systems provide hens with the opportunity to express a greater range of natural behaviours (Rodenburg *et al.*, 2008), including osteogenic bone-loading activity, but they also expose hens to possibility of falls and collisions from potentially hazardous structures (Wilkins *et al.*, 2011; Stratmann *et al.*, 2015a). Cage-free systems increased in popularity after the EU ban on conventional cages (European Commision, 1999).

Cage-free systems can be single-tier or multi-tier. Single-tier, or “flat-deck” systems, as they are commonly known, consist of an open barn with a littered area, slats and nest boxes (usually located above the slats but can also be in a separate area of the system away from the slats) (Figure 1.3). Advantages to these types of housing systems compared to cages are that hens are provided with a large litter to dustbathe as a group and this provides foraging opportunities, they have enough room to wing flap and they can jump and fly around the system. Hens from these systems sometimes have access to a winter garden or free-range area. Single-tier systems contain perches, but they can be integrated into the slatted area or provided as A-frames (Figure 1.3).

Figure 1.3: *Single-tier housing system showing A-frame perches, nest box and feeders*

Multi-tier housing systems, like single-tier systems, provide hens with foraging and dustbathing substrate, allow hens to jump and fly around the system and hens may have access to an outdoor area (Figure 1.4). However, multi-tier systems are usually over 2m high but measure up to 3m. Nest boxes can be integrated as a tier in the middle of the multi-tier system or can be separate from the system and the hens must navigate the multi-tier system to reach the nest box area (Figure 1.4). Feeders and drinkers are distributed around multi-tier systems, usually with the feeders and drinkers present on the lower tier and then on the top tier, this provides feed and water access to hens wherever they are on the system. Feed and water are not provided on the litter because farmers want to encourage hens to use the system, providing the feed and water on the system motivates the hens to travel up. Perches are provided around feeders so that hens can both feed from perches and from the tiers.

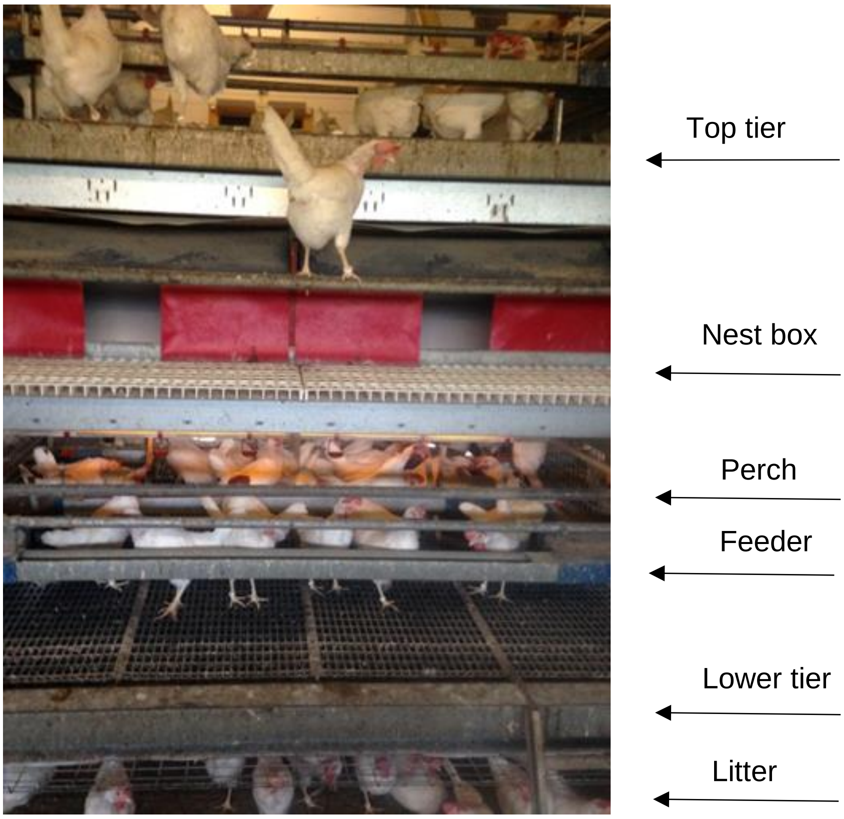

Figure 1.4: *Diagram of a multi-tier housing system for laying hens. The photograph shows the lower tier, nest box and upper tier regions.*

Keel bone damage exists in all laying hen housing systems (Rodenburg *et al.*, 2008; Sherwin *et al.*, 2010; Wilkins *et al.*, 2011). Conventional cages and enriched cages have a lower prevalence of keel bone fractures and multi-tiers have the highest (Rodenburg *et al.*, 2008; Sherwin *et al.*, 2010; Wilkins *et al.*, 2011). At the end of lay 17.7% of hens from conventional cages had keel bone fractures (Sherwin *et al.*, 2010). This makes it difficult to attribute keel bone fractures to one housing element because conventional cages do not contain perches and both conventional cages and enriched cages do not have tiers, yet keel bone fractures still occur.

1.4.3.1 Perches

The use of perches is important in relation to keel bone damage because if perches are more comfortable, and provide adequate grip, for the hens there may be fewer falls and collisions and a lower keel bone fracture prevalence. Perch comfort may also affect the amount of pressure on the keel bone, which can potentially lead to keel bone deviations because keel bone deviations are thought to be caused by prolonged roosting (Warren, 1937; Harlander-Matauschek *et al.*, 2015). The ancestral form of the laying hen (i.e., the red jungle fowl) and domestic chickens living in the wild roost on tree branches (Collias and Collias, 1967; Wood-Gush and Duncan, 1976). Some hens were seen perching as high as 10m into the trees, using intermediate branches as a way to gain height (Wood-Gush and Duncan, 1976). However, unlike tree branches, commercially available perches do not differ in width. In a single system, commercial perches are usually of identical length, width, diameter and material. This does not provide domestic laying hens with the same variety as their wild counterparts. It is important that research efforts focus on obtaining the optimal perching structure to suit the laying hens' physical and behavioural needs because this is the perch they will need to use throughout their life.

Laying hen housing systems can contain perches which are often made from metal or wood and are used as structures to rest during the day or roost at night. Legally, perches do not have to be elevated in England, meaning that slats can be included as perch space (RSPCA, 2017). To be considered a perch the surface must be easily grasped by the hen's foot with a recommended diameter of 1.5cm to allow hens to grasp comfortably and to prevent claws becoming trapped in the slats (RSPCA, 2017). If perches are raised they should be 20cm away from the wall and a 30cm horizontal distance away from other perches (RSPCA, 2017). However, the definition of a perch is different in Scotland; perches that are integrated into the flooring or slats do not count as perching space (Sandilands, 2014). This is based on a different interpretation of the EU regulation (European Commision, 1999) and not a new law in itself. Although perching standards have been specified in government documents, it is important that perching behaviour and perching features are still studied because there is not yet an optimal perch type and placement that is non-hazardous as keel bone damage occurs to a greater extent in systems with perches (Wilkins *et al.*, 2011).

1.4.3.1.1. Perch placement at rear

In humans, it has been shown that bone mass does not alter in athletes that no longer train as adults (Kontulainen *et al.*, 1999). This suggests that the process of training as a young adult increased peak bone mass and was not affected by individuals stopping exercising. Therefore, in humans it is important that exercise is conducted in the teenage and young adult years to prevent bone fragility and osteoporosis developing in later life (Kontulainen *et al.*, 1999). This links to studies in laying hens where access to a more physically challenging environment as chicks, one which contains perches and other structures, leads to stronger bones than those with access to caged systems without access to perches (Casey-Trott *et al.*, 2017).

Ability to navigate perches and other structures in the systems are important to prevent falls and collisions. It has been shown that providing perches at an early age (from one-day old) can affect the spatial awareness and cognitive ability of hens (Gunnarsson *et al.*, 2000). At 16 weeks of age, after being reared either with access or without access to perches, food was placed at variable heights to test whether prior access to perches affects an individual's ability to navigate to the food (Gunnarsson *et al.*, 2000). There was no difference in time or ability to reach the food at 40cm but as the height increased the hens that were reared with perch access were more readily able to reach the food (Gunnarsson *et al.*, 2000). The authors allude that it is the difference in spatial awareness and not physical ability that results in the differences seen because the hens were able to reach the 40cm height in a similar timeframe in both groups (Gunnarsson *et al.*, 2000).

Other early life experiences, such as having a broody hen present during rearing resulted in chicks accessing perches (20cm and 40cm in height) earlier in their life (9.8 days old) compared to those reared without broody hens (13.5 days old) (Riber *et al.*, 2007). One study that contradicts others is that of Habinski *et al.* (2017). They found that hens perched less on the highest perch (61cm). However, this could be because the observations focussed on pullets that were between 4-14 weeks of age and accessing a perch of 61cm may have been challenging for them (Habinski *et al.*, 2017). Studies on the rearing system of laying hens once again showed that the system birds are exposed to as pullets can affect their behaviour during lay (Colson *et al.*, 2008). Pullets that were reared in floor pens showed lower usage of upper perches and levels during the laying period compared to pullets that were reared in aviaries (Colson *et al.*, 2008). Additionally, those raised in floor systems had more failed transitions compared to those reared in aviaries (Colson *et al.*, 2008). During the first two weeks upon transfer they also laid fewer eggs in the nest box and had a higher number of floor eggs throughout the observation period (Colson *et al.*, 2008).

This would suggest that hens reared in floor housing find it more challenging to navigate an aviary system compared with hens that had previous experience of an aviary system during rear.

1.4.3.1.2. Perch placement during the laying cycle

The placement of perches within housing systems is also vital to prevent both falls and collisions. Providing easy to navigate movements paths by placing structures at angles or supplying structures made of materials that are easily accessed by laying hens has the potential to reduce falls (Scott *et al.*, 1997). However, it is first important to understand how laying hens move through these systems to make educated alterations.

Placement of perches around feeders has shown to reduce aggression, jostling and mortality compared to hens that were feeding from a platform (Sirovnik *et al.*, 2018). There was no difference in keel bone damage between the groups but the result shows that perch placement can affect other aspects of laying hen welfare and behaviour (Sirovnik *et al.*, 2018). Therefore, there is a lot to consider when choosing optimal furnishings and placement of those furnishings in commercial laying hen systems.

It was shown that small horizontal distances between perches <25cm result in reduced perching frequency and a different pattern of perching. Specifically, hens would rest on neighbouring perches, in a sequential pattern across both perches (Liu and Xin, 2017). However, there was little difference in the number of times hens would visit perches depending on the horizontal distances, except when horizontal distances reached 60cm when the frequency of trips was low (Liu and Xin, 2017).

One study has looked at perching behaviour in broiler breeders showing that, similar to hens, they use perches, particularly during lights out (Gebhardt-Henrich *et al.*, 2018). Broiler breeders also showed a preference for the way perches were arranged. Birds perched more on aviary style perches, when perches were integrated into the tiered structure, compared to A-frames (Gebhardt-Henrich *et al.*, 2018). Also, in the control pens, birds rested on the elevated tiers, which indicates that in the absence of perches the birds will still try and find a way to elevated themselves (Gebhardt-Henrich *et al.*, 2018). Relating back to keel bone damage; birds that had access to elevated perches (in the A-frame and aviary systems) had increased levels of keel bone fractures compared to the control groups (Gebhardt-Henrich *et al.*, 2018). However, broiler breeders are different from laying hens, in that they do not lay an egg every day, so keel bone strength may be different in broiler breeders than laying hens. Nevertheless, this shows further evidence that providing raised perches increases the likelihood of keel bone fracture development.

The study by Wilkins *et al.* (2011) showed this relationship more prominently, as cumulative perch height of farm increased, the prevalence of keel bone fractures increased. Not all studies found a connection between keel bone fractures and perch height (Donaldson *et al.*, 2012). There was also no difference in keel bone fractures detected when hens were housed with or without perches in cages but this study was done in cages so cannot directly be compared to that of aviary systems (Abrahamsson and Tauson, 1993).

1.4.3.1.3. Perch Material and Shape

The diameter of perches may be important to allow hens to use their tendon-locking mechanism for roosting (Quinn and Baumel, 1990; Backus *et al.*, 2015). As a result of this, perch diameter may influence hen health and behaviour, as well as keel bone fracture prevalence making perch diameter a relevant avenue to consider in perch design. It has been shown that balance movements decreased as the diameter of the perch increased (2.7cm, 3.4cm and 4.5cm) and there was no difference in the time spent on each of these perches (Pickel *et al.*, 2010). However, hens have been shown to prefer perches of 3.0cm in diameter compared to 5.0cm in diameter when housed individually and as groups of four (Chen *et al.*, 2014). Perch diameters of 1.5cm were preferred the least when compared with other perch diameters (3.0, 4.5, 6.0, 7.5, 9.0 and 10.5 cm) (Struelens *et al.*, 2009). There was no preference for perch width when birds were roosting at night (Struelens *et al.*, 2009). The number of failed landings on metal perches were not affected by different perch diameters (2.7cm, 4.2cm and 6.0cm) (Scholz *et al.*, 2014).

Distinguishing the effect of perch shape alone on laying hen health and behaviour is difficult because perch shape is often dependant on perch material. Metal perches can be round or mushroom-shaped, wooden perches can be round, rectangular, square or mushroom-shaped, and plastic perches can be round or mushroom-shaped. However, perches can be made of any combination of material and shape, but the combinations mentioned are the most popular commercially. Perch material is widely studied and can be important for laying hen health and behaviour. Due to the confounding nature of perch shape and material, they will be discussed separately in the next section. Perch characteristics, relating to keel bone damage, have been studied since the 90’s when it was shown that hard wood perches covered with soft rubber did not decrease keel bone damage when compared to hardwood perches alone (Tauson and Abrahamsson, 1996). Further studies since have highlighted that balance movements occur more often on wooden and metal perches in comparison to rubber perches and birds stood less on metal perches in comparison to wooden or rubber perches (Pickel *et al.*, 2010).

When considering plastic perches for welfare and comfort; two varieties of soft, round polyethylene perches were compared to three commercially used perches (round metal, plastic mushroom and plastic flat/round perches) (Pickel *et al.*, 2011). Pressure exerted on the keel bone and foot pads when perching was measured and in each case the polyethylene perches showed a reduced pressure load in comparison to the commercially available perches (Pickel *et al.*, 2011). This data can be used as a proxy to show that softer perches could potentially reduce keel bone deviations and foot pad lesions. When looking at differences in behaviour between different perch types (PVC and wooden perches), broiler chickens perched more on wooden perches (Hongchao *et al.*, 2014). This may potentially show that individuals preferred wooden perches compared with PVC perches. A similar preference for wooden perches was found, in this case compared with metal perches (Chen *et al.*, 2014). However, this data relates to broiler chickens and would need to be replicated in laying hen studies to determine whether they show the same preference behaviour.

Metal perches, coated in a softer material (like rubber), reduced the incidence of keel bone damage compared to standard, round, metal perches (Stratmann *et al.*, 2015b). When looking at how safely hens land on perches, one study examined the effects of a metal perch, a plastic mushroom perch and a metal perch that was coated in plastic (Scholz *et al.*, 2014). The plastic perch had the highest proportion of safe landings, followed by the mushroom perch, with the metal resulting in the highest proportion of unsafe/failed landings (Scholz *et al.*, 2014). Both these studies conclude that softer perches, like those made from plastic, have the potential to improve health and allow more controlled movements in laying hens. Although plastic perches have advantages, they are linked to health issues, e.g. foot pad health and bacterial contamination of newly arrived flocks (Sandilands *et al.*, 2009; Stratmann *et al.*, 2015b). One reasoning is that plastic is difficult to clean in comparison with metal (Sandilands *et al.*, 2009; Stratmann *et al.*, 2015b).

As discussed previously, only wooden and metal perches were compared, wooden perches were chosen by the hens more and birds performed a higher proportion of comfort behaviours on the wooden perches in comparison to the metal perches (Chen *et al.*, 2014). Looking once more at the study by Pickel *et al.* (2011) but focussing on the perch shape aspect of their study; square perches resulted in reduced pressure on the keel bone and a larger area where pressure was applied compared to round and oval perches, indicating these perches may be less likely to cause keel bone deviations. When studying differences between round, metal perches and hexagonal, metal perches; there were no differences between perch use and frequency of perching behaviour (Liu *et al.*, 2018).

Another potential welfare concern with perch material is that metal perches may lead to more keel bone deviations in comparison to wooden and plastic perches. Therefore, it would be reasonable to propose that perching on a harder structure would result in an increased likelihood of developing a deviation compared to roosting on softer structures. This creates greater complexity when designing a laying hen housing system. It is important to consider the health status, preference and ease of upkeep by the farmer when designing a perch for a system; making material an important aspect of perch design.

1.4.3.1.4. Perch Height

Perch height is important for both behaviour and welfare because hens prefer to roost high in the system at night (Schrader and Müller, 2009; Brendler and Schrader, 2016) but falls and collisions from perches may lead to keel bone fractures (Gregory and Wilkins, 1996; Moinard *et al.*, 2004a; Wilkins *et al.*, 2011; Stratmann *et al.*, 2015a). Due to the potential link between falls and collisions and keel bone fractures, it is extremely important to incorporate this into any decision on optimal perch heights.

In cages, hens preferred to perch on the highest, compared to lower perches (Struelens *et al.*, 2008). When cage height was lower, and thus removing the highest perches, time spent on perches both during the day and at night was reduced. This study suggests that hens prefer high cages and perches because they prefer to roost high in the system. Hens prefer perches to grids (slats) and high structures to low ones when roosting at night (Schrader and Müller, 2009). However, if grids were higher than perches, then hens' preferred the grids to roost at night (Schrader and Müller, 2009). This result has been repeated in commercial settings with a high density of birds roosting on the top level during night, leading to over-crowding on high perches and tiers, leaving lower perches and tiers empty (Campbell *et al.*, 2016d). This highlights that hens prefer to roost high in the system and will chose height over a preference for the material of the perching structure.

Another recent study, found the same pattern with more hens perching at night compared to perching when lights were on (Liu *et al.*, 2018). Once again in another study, a high proportion of hens used perches during the evening, when high perches were preferred over lower perches (Louton *et al.*, 2016). Low perches that were located underneath a drinking trough were more frequently used throughout the day, but this was mainly for accessing the drinking trough and not for roosting behaviour (Louton *et al.*, 2016).

It can be seen from the evidence presented above that hens prefer higher perches, particularly when roosting. However, one study using accelerometers determined that jumps from heights of 61cm resulted in higher forces upon landing compared to jumps from smaller heights of 41cm (Banerjee *et al.*, 2014). This shows that higher perches may be more likely to result in keel bone fractures because of the increased forces. Higher perches are used more frequently during the evening, this could potentially result in hens being more likely to be injured at those times of day.

Although perches have mainly been discussed in this section as a hazard for laying hens; the role they have in skeletal and cognitive development cannot be overlooked. It is important that perches are provided because they have shown to improve skeletal integrity (Whitehead, 2004). As discussed earlier systems which lack the opportunity for skeletal loading exercise result greater loss of bone strength (Whitehead and Fleming, 2000). The provision of perches is important in the development of the laying hen cognitive function and if provided during rear may increase their navigation capability as they age (Gunnarsson *et al.*, 2000).

1.4.3.2. Ramps

Ramps provide a walkway for hens to travel between different structures within a system. They aid in the transition between tiers, providing the opportunity for walking instead of flying and jumping potentially reducing the incidence of falls and collisions, which may lead to a decreased risk of keel bone fracture development. The influence of ramps on laying hen health and behaviour have been documented both in farm studies (Pettersson *et al.*, 2017a) and experimental conditions (Stratmann *et al.*, 2015a; Heerkens *et al.*, 2016a; Kozak *et al.*, 2016a; Kozak *et al.*, 2016b; LeBlanc *et al.*, 2017; Pettersson *et al.*, 2017b; Norman *et al.*, 2018). An on-farm study focussed on the behaviour of hens when they were approaching or leaving a ramped area. This was to understand whether ramps facilitate movement between the litter and the lower tier (Pettersson *et al.*, 2017a). Another researched area is the effect that the provision of ramps have during rearing in the process of bone development and behaviour (Kozak *et al.*, 2016a). Keel bone fracture prevalence throughout lay is lower in hens with ramp access compared to those without access to ramps (Stratmann *et al.*, 2015a; Heerkens *et al.*, 2016a).

Pullets use ramps as early as two weeks of age (Kozak *et al.*, 2016a) and providing ramps during rear may allow cognitive function to develop in a similar way to that seen with perches (Gunnarsson *et al.*, 2000). The provision of ramps at rear may have the potential to increase navigation ability during lay. This understanding of how hens learn to use their environment can help improve system design.

1.4.3.3. Overview of housing design

Research has suggested possible ways that laying hen housing structures might be improved to reduce keel bone fractures. However, in multi-tier system there has been no systematic analysis of the risks associated with elements within the system. This thesis will describe the use of accelerometers combined with visual observation to identify areas within a multi-tier laying hen housing system that are difficult for hens to navigate. Ramps, perch design and perch arrangement will be looked in at more detail through two on farm studies. One study will examine ramps specifically and another will examine modifications in a multi-tier housing system for laying hens using results obtained from Chapters 2 and 3 of this thesis.

1.4.4. Movement behaviour

Due to falls and collisions with structures potentially causing keel bone fractures, it is important than hen flight and movement ability is understood, and housing designs suit the needs of the hens raised in them. Hens are more likely to collide with conspecifics compared with slipping or colliding with other structures (Campbell *et al.*, 2016a). There were slightly different percentages of falls in the two different flocks observed; flock one having 9.1% and flock twon having 21% of all movements representing falls (Campbell *et al.*, 2016a), showing that falls can be much higher in some flocks compared with others. Stratmann *et al.* (2015a) showed that falls occur during the dusk phase in a commercial aviary system and hens do collide with perches and structures. This shows that hens do fall in aviary systems and it is important to understand how these falls can affect health parameters of the birds. It also shows that collisions with structures and perches do happen and this can show a legitimate pathway for keel bone fracture development.

1.5. Other health parameters to monitor when altering housing design

It is important when designing housing systems for hens that the overall health of the hens is considered and that changes are not made based on reducing keel bone fractures alone. One health problem is foot pad disorders. There are three main types of foot pad disorders; hyperkeratosis (Weitzenbürger *et al.*, 2006), foot pad dermatitis (Shepherd and Fairchild, 2010; Butterworth, 2013) and bumble foot (Tauson *et al.*, 2005). Systems design may improve foot pad problems; ramps have been shown to decrease the occurrence of foot pad problems (Heerkens *et al.*, 2016a).

Feather condition is a noticeable physical characteristic that can be monitored to determine whether hens are stressed in a housing system. When hens are stressed due to their housing system it can be manifested into feather pecking behaviour (El-Lethey *et al.*, 2000). Although this thesis will mainly focus on the determining hazardous zones within a multi-tier system for laying hens and altering these zones to reduce their hazard. These zones should be changed after monitoring behavioural and health parameters, including keel bone fracture prevalence in laying hens.

1.6. Accelerometers and body-worn sensing technology

Accelerometers were first used in the 1950's for assessment in gait analysis, with these studies increasing dramatically in the 1970's because of advances in monitoring technologies (Yang and Hsu, 2010). Newton's 2nd Law describes acceleration as being dependant on two factors; the force acting upon an object and the mass of that object (Yang and Hsu, 2010). Accelerometers are sensors that allow acceleration of objects, animals or people to be quantified along up to three axes; x, y or z axes (Yang and Hsu, 2010). The three most common types of accelerometers include; piezoresistive, piezoelectric and differential capacitive accelerometers (Yang and Hsu, 2010). Piezoresistive accelerometers work by measuring acceleration through a known load acting on a plate or beam. Piezoresistors inside the device are exposed to the same forces of the device. The electrical property changes and a difference in acceleration is then provided (Adams and Layton, 2010). Capacitive accelerometers measure the change in electrical charge due to a sensor or plate (Beliveau *et al.*, 1999). Piezoelectric accelerometers work by the bending of a sensing element under acceleration, this causes a displacement of the seismic mass, the mass attached to the deforming element, resulting in an output voltage that matches the applied acceleration (Yang and Hsu, 2010). Accelerometry research focusses mainly on human health and behaviour. Although, most of the research takes place in humans, the main areas of focus can be applied to animal behaviour studies. These include; posture and movement classification, energy expenditure, fall detection and balance analysis (Yang and Hsu, 2010).

Accelerometers are an increasingly popular method to monitor animal behaviour. The value of the body-mounted sensor industry is estimated to be worth £1.96 billion in the coming 10 years (Neethirajan, 2017). Body sensors can be used to monitor a range of different health issues. Some examples include sweat analysis, microfluidics and stress detection sensors to determine the health status of animals (Neethirajan, 2017).

Studies involving animals mainly focus on marine mammals and birds, with little focus on terrestrial mammals (Fehlmann *et al.*, 2017). Such research has led to improvements resulting in small, inexpensive, long-lasting devices leading to research being made possible on a wider variety of species and sample size increases (Fehlmann *et al.*, 2017). However, even though technology is advancing, an obstacle and deterrent to using accelerometers is the difficulty analysing collected data (Fehlmann *et al.*, 2017). When analysing accelerometer data, there are three main methods (expert interpretation, clustering and classification) and they all depend on the amount of direct observations that are available to match the accelerometer outputs (Shamoun-Baranes *et al.*, 2012). The first method is called expert interpretation and is used when there are no direct observation or video recordings of animal behaviour available. Another is clustering, which also does not require direct observations or video recordings and lastly; classification, which does require knowledge about the behaviour being performed (Shamoun-Baranes *et al.*, 2012). The difference between expert interpretation and clustering is that for the former, knowledge about specific behaviours must be known, then accelerometer outputs can be matched to these pre-determined behaviours. Whereas behaviours for clustering are determined after initial analysis of the accelerometry outputs (Shamoun-Baranes *et al.*, 2012). However, both these methods do not allow for sensitivity analysis because the true behaviour being performed is not known. Accuracy can be determined for classification analysis, but even if this method is used, only a small amount of behaviours may be correctly identified; with one study only able to correctly identify 5/24 behaviours (Shamoun-Baranes *et al.*, 2012). However, the same research group expressed the usefulness of video analysis, in that discrepancies between the algorithm and behaviour could be looked into with more detail to try and understand the intricacies of the behaviour being performed that may be skewing the accelerometry output (Shamoun-Baranes *et al.*, 2012). This method is excellent in retrospect but would require expert knowledge and may not be feasible economically or timewise.

The majority of uses of accelerometers in animal studies focus on the tracking of wild animals to identify mating behaviour (Whitney *et al.*, 2010) and migratory sites, allocation of resources and ranging (Kays *et al.*, 2015). One of the main uses of accelerometers in animal behaviour science has been to understand the behaviour and movements of wild, endangered species, to protect them (Wilson *et al.*, 2008; Pagano *et al.*, 2017). The field has been currently advancing in farm animal behaviour with multiple studies of farm animals (Martiskainen *et al.*, 2009; Pastell *et al.*, 2009; Vázquez Diosdado *et al.*, 2015; Zobel *et al.*, 2015; Alvarenga *et al.*, 2016), with some particularly focussing on chickens (Quwaider *et al.*, 2010; Daigle *et al.*, 2012; Banerjee *et al.*, 2014; Kozak *et al.*, 2016b; LeBlanc *et al.*, 2016). Accelerometers open a new area of animal behaviour science, for precision livestock farming (Berckmans, 2014) and disease detection (Thorup *et al.*, 2015). Focal observations and video analysis can be reduced with the use of accelerometers, especially when thresholds or algorithms to detect certain behaviours are used.

Falls in elderly patients is a primary use of accelerometers for human medicine. One study was successfully able to identify falls from normal movements using a threshold method, with falls occurring at greater accelerations compared with non-falls (Bourke *et al.*, 2007). Even though fall detection was not as reliable when falls were not simulated and where real-life scenarios (Bagalà *et al.*, 2012), accelerometers still appear to be an important tool to identify falls. Although falls and collisions are thought to cause keel bone fractures in laying hens (Gregory and Wilkins, 1996; Moinard *et al.*, 2004a; Wilkins *et al.*, 2011; Stratmann *et al.*, 2015a), no one has yet attempted to use accelerometers to determine where in laying hen housing systems falls and collisions occur with accelerometers. However, due to the need for the accelerometers to collect data on a potentially short event (a single fall) the sample rate must be high and as a result battery life is compromised. This contrasts with other devices such as pedometers, which have a much lower sampling rate (Lehman, 2013).

1.7 Conclusion

Understanding the characteristics of movements and locations of falls will be the main aim of this thesis and will be determined using accelerometers. Specific areas and structures of a multi-tier housing system will then be altered to determine whether changes reduced the prevalence of keel bone fractures.

Chapter 2

Physical characteristics of movement in a multi-tier system for laying hens

2.1. Introduction

2.1.1. Multi-tier systems for laying hens

Complex housing systems with multiple tiers can be a source of enrichment allowing birds to display natural behaviours such as vertical movement, long distance flight and perching (Appleby and Hughes, 1991). Multi-tier systems are those where resources are distributed on different levels of the system. There tends to be a littered area on the ground level and a lower tier equipped with feeders, drinkers and perches. Nest boxes can either be incorporated into the system as a second tier that can be reached from within the system by jumping or flying from platforms and perches, or nest boxes may be provided in a separate unit that is accessed through jumping or flying. The top tier is either above the nest box tier or above the lower tier and can contain feeders, drinkers and perches for roosting. Tier floors consist of metal or plastic grids with manure belts under each tier so that faeces can be removed automatically.

To facilitate movement in multi-tier systems other structures may be provided in addition, or instead of perches. Platforms or extensions of tiered structures are commonly used. A multi-tier system with platforms and tiers instead of perches had fewer synchronised movements, with hens tending not to change their position in the system throughout the day compared with a single-tier system with a structure containing perches on the slats (Odén *et al.*, 2002). This suggests that systems containing perches may facilitate movement more readily or that systems containing platforms are more comfortable for hens and result in fewer movements.

Perching behaviour is shown by the red jungle fowl (*Gallus gallus*), the ancestor of the domestic hen (*Gallus gallus domesticus*), and is thought to be an anti-predatory response (Newberry *et al.*, 2001). Laying hens have kept this innate behaviour and use perches for resting, preening and night time roosting (Olsson and Keeling, 2000; Schrader and Müller, 2009; Campbell *et al.*, 2016d), restricting perch access can cause frustration behaviours to develop (Olsson and Keeling, 2000). Previous studies have shown that hens will perch on the highest structure in the system, resulting in top perches becoming overcrowded and lower perches empty (Campbell *et al.*, 2016d). When hens are given the option of high areas with grids instead of perches or lower areas with perches, hens prefer the high area with the grid (Schrader and Müller 2009). This suggests that it is the height that is important, in terms of roosting, rather than the structure that the birds are roosting on.

The location of a perch relative to other structures influences how easily the hen can use it. The vertical distance between perches, or other structures in a system, are recommended to be no more than 29 cm (Scott *et al.*, 1997) to reduce the risk of falling when attempting to pass from one structure to another. To aid downward movements (which are more difficult for laying hens than upward movements) no angles should exceed 45^{o}. Others have reported that even more obtuse angles (over 30^{o}) are difficult for birds to successfully navigate compared with more acute angles (Scott *et al.*, 1997).

Overcrowding may be a factor that contributes to poor movement around a system. Multi-tier systems that adhere to overall stocking density requirements may still become crowded during specific time points (Campbell *et al.*, 2016c) and this can make movement through the system difficult. This may result in the displacement of hens from perches or falls when perches cannot be reached. Some research has previously shown that it is the subordinate hens that are displaced by the dominant hens in these situations (Cordiner and Savory, 2001).

Providing perches and high structures may allow hens to roost in high areas of the system and escape negative interactions from conspecifics. However, as the accumulated system height increases the prevalence of keel bone fractures within that system increases (Wilkins *et al.*, 2011); presumably due to falls and collisions from high in the system being linked to fractures. The theory that height itself represents a risk is demonstrated in experimental conditions using a drop-weight impact tester (Toscano *et al.*, 2018). Weights dropped onto the keel bone of hens from greater heights were more likely to result in keel bone fractures and those that were present were more severe. This was because as height increased, the energy when the drop-weight made an impact with the keel bone would also increase and lead to a greater likelihood of fracture (Toscano *et al.*, 2018).

It is important to determine areas and behaviours of hens in multi-tier systems that result in high impacts (increased forces during movements). Falls occur frequently in the dusk period and can be reduced through the addition of ramps between tiers (Stratmann *et al.*, 2015a). However, the number of falls and whether these falls have increased acceleration at the keel compared to controlled movements has not been determined. The number of falls was found to be greater in the highest tier compared to the lower and middle tier (Stratmann *et al.*, 2015a). However, no one has looked at whether these movements have the potential to cause more damage to the keel compared with other movements.

There are two possible causal relationships on the movement of hens with keel bone fractures; one is that hens with keel bone fractures are more likely to take part in dangerous activities and two, it is these hazardous movements that result in fractures. Another could be that having keel bone fractures make the individuals less able to perform an accurate transition due to the pain or lack of mobility experienced when trying to fly. This is highlighted in previous research where laying hens with fractures had taken longer moving between perch heights of 50 and 150cm compared to those without fractures (Nasr *et al.*, 2015).

Other health parameters may affect movement ability in laying hens. Individuals with poor foot health may find it more difficult or uncomfortable to move around a multi-tier system. It is thought that the poor foot condition would be painful due to ulceration and breaking of the skin, thus hindering movement due to swelling and pain (Greene *et al.*, 1985; Tauson *et al.*, 2005). Feather condition may also prevent accurate movement because reduced feather cover has shown to have an effect in other bird species, such as starlings where the flight performance is reduced during moult (Swaddle and Witter, 1997).

2.1.2. Quantifying movement using accelerometers

Body-worn sensors are a new and emerging field in animal behaviour science. Accelerometers, remote sensing equipment, gyroscopes, light monitors and many other smart technologies can give detailed information about animal movement and behaviour without the need for video analysis or direct observations (Kays *et al.*, 2015). Accelerometers have been used over the past decade to monitor animal movement (Siegford *et al.*, 2016; Williams *et al.*, 2017). There has been a large amount of research into placement and validation of accelerometers across a wide range of different species (Brown *et al.*, 2013). However, detailed individual tracking of movement and behaviour of animals is an area that needs to be studied in more detail. The current body of scientific studies have mainly focussed on conservation and knowledge gathering of animals in the wild (Kays *et al.*, 2015). It is also important to track animals that we keep in captive environments to understand more of how they interact with the environment we provide them with.

In the current study, we evaluated whether accelerometers could be used to discern between different movements in a multi-tier system that either were controlled (successful jumps or flights) or uncontrolled (falls or collisions), with the latter being expected to pose a greater risk of fracture. Fall detection using accelerometers is a well-developed field in human medicine and can be linked to a smartphone to send an alert when a fall has occurred (Thammasat and Chaicharn, 2012; El-Bendary *et al.*, 2013).

To access how hens are moving in a multi-tier system and how these movements can potentially impact keel bone health a variety of acceleration outputs will be analysed. Acceleration recorded using an accelerometer is influenced by gravitational acceleration (g) which is 9.8m/s^2 and the acceleration resulting from movement of an individual (Siegford *et al.*, 2016) totalled together in this these to represent “g”. The maximum summed acceleration vector (AV) at the keel of hens will be analysed. The maximum summed AV referred to in this study is the summed acceleration of all 3-axes of a tri-axial accelerometer. This considers a change in direction and change in speed. The 3 axes of an accelerometer are referred to as x, y and z. Each axis represents a different type of movements called surge, heave or sway (Williams *et al.*, 2017). Surge refers to the backwards and forward motion, heave is up and down, and sway is side to side. In the current study, the summed acceleration vector was used because it has been shown to be reliable as a proxy when the orientation of the sensor is not known (Qasem *et al.*, 2012). The orientation of the keel sensor could not be guaranteed because the sensor had the ability to move around within the vest. It is thought that the higher the maximum summed acceleration vector reading, potentially the more hazardous the behaviour. This may indicate that the hen is moving vertically or experiencing a collision, as was seen during the experiments using impact testers (Toscano *et al.*, 2018).

The readout duration was recorded because it is thought a behaviour that is long, in duration, may have a higher total energy and therefore a greater potential to cause injury than one that is shorter. As hens fly between different structures, they may wing flap to gain access. It has been shown that when landing areas are obstructed, hens flap their wings more, spend longer in the air and take longer to achieve balance (Moinard *et al.*, 2005). Hens that are in pain, potentially those with keel bone fractures, may take longer achieving balance as they may be less agile than hens without keel bone fractures (Nasr *et al.*, 2012a).Therefore, the readout duration obtained from the accelerometer may indicate the difficulty of a movement.

The average summed AV allows smoothing of the data and gives an indication of the continued summed AV experienced on the keel bone of the hen. To quantify the overall effect the acceleration may have, if the average summed AV is multiplied by the readout duration this will give an indication of how likely the behaviour that caused the acceleration readout is to be hazardous. Therefore, if the average AV x readout duration is high then this would indicate that the movement has the potential to be more hazardous compared with an acceleration readout that has a low value for average AV x readout duration.

2.1.3. Aims and predictions

The main aim of the current study was to quantify and distinguish the acceleration at the keel bone during controlled movements, falls and collisions in an aviary system for laying hens using tri-axial accelerometers. The main predictions of this study were;

1. Falls and collisions would result in higher maximum summed acceleration vectors (AV), longer readout durations, higher average summed AVs and greater values for readout duration x average summed AVs than controlled movements.

2. Greater total movement heights would result in higher maximum summed AV readings, longer readout durations, higher average summed AVs and greater values for readout duration x average summed AVs.

3. Individuals with keel bone fractures would have a higher maximum and average summed AV readings compared to those without keel bone fractures. Average maximum summed AVs x readout duration would be expected to be higher because both the average summed acceleration vector and the duration of the recorded movement are predicted to be higher and longer respectively. Hens with keel bone fractures may also have longer readout durations.

4. Individuals with poor feather cover would have a higher maximum and average summed AV readings compared to those with excellent or good feather cover. Hens with poor feather cover are predicted to have shorter readout durations.

5. Individuals with poor foot health would have higher maximum and average summed AV readings and longer readout durations compared to those with good foot health.

6. It was expected that heavier hens would have higher maximum summed and average AVs compared with lighter hens.

7. Collisions with perches and structures would have higher maximum summed AV at the keel compared with the litter or a conspecific.

8. When falls were caused by missed landings or a slip it is predicted that the maximum summed AV at the keel will have a higher reading.

2.2. Methods

2.2.1. Housing design

2.2.1.1. Rearing housing

All hens (Lohmann Selected Leghorn (LSL)) were reared with the same space allowance, lighting and feeding regime across 8 pens. The only difference during rear were that 4 pens contained a stacked, 2-tier system with chain feeders and round feeders on each tier (Natura 3, R. Inauen AG – Big Dutchman – Natura Company AG, Switzerland). The other 4 pens contained an offset 3-tier system with a chain feeder on the first and third tier (Harmony 3, Landmeco A/S, Denmark). However, all pens were 2.2 m high and provided ground-level access to a covered veranda from 5 weeks of age.

2.2.1.2. Laying housing

When transferred to the laying barn at 18 weeks of age, hens were distributed evenly from the 2 rearing systems into 20 pens, this random distribution meant that it was not known what rearing system each individual was moved from. Flock A was populated with 200 hens per pen (a total of 4,000 hens split across 20 pens). Flock B was populated with 200 hens per focal pen and 225 hens per non-focal pen (a total of 4,300 hens split across 20 pens). The numerical difference in flocks was out with the control of the study and was due to the hens being used in another study. The system was similar in design to a commercial unit but split into separate pens (Bolegg Terrace, Krieger AG, Ruswil, Switzerland, see Figure 2.1 and 2.2).

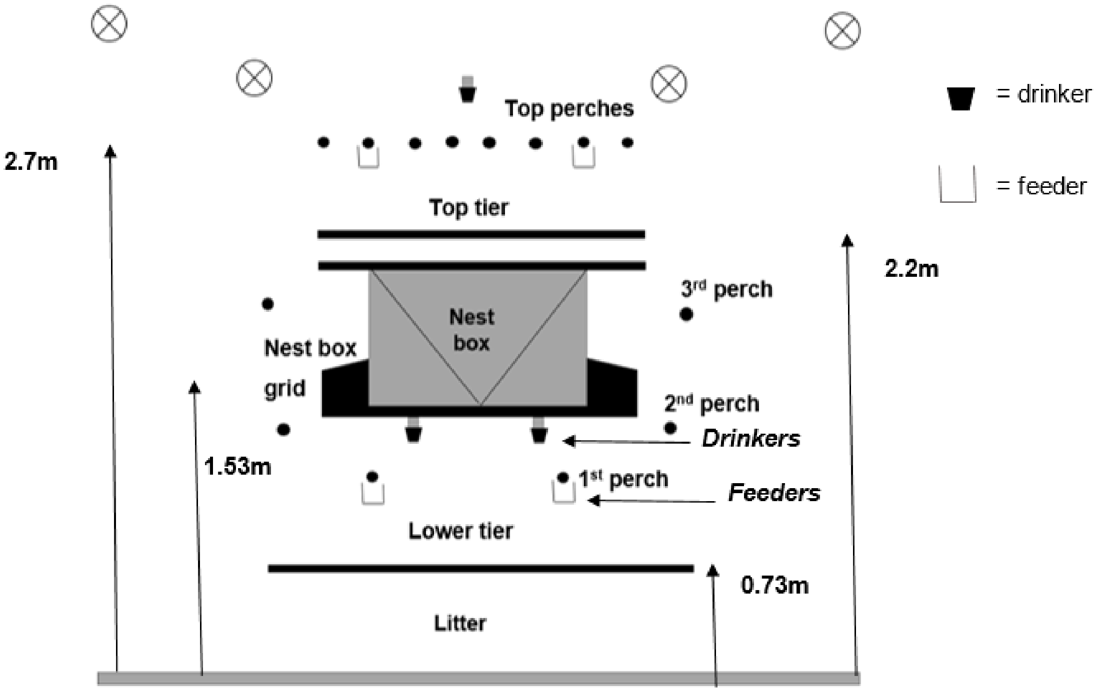

Figure 2.1: *Schematic of the transverse view the multi-tier system.*

All labels refer to areas of the system named later in the text. Location of feeders, drinkers and cameras (circles with crosses) have been indicated. Top perch and tier heights have been labelled within the system. Each perch is replicated at both sides of the system.

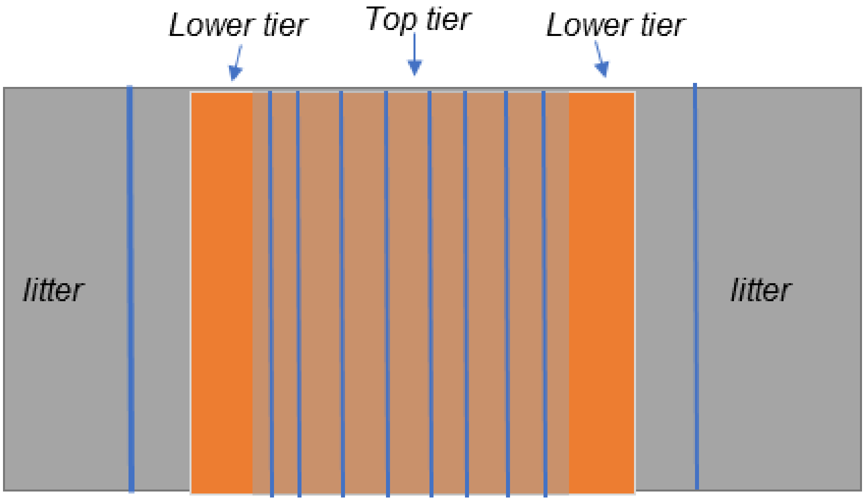

Figure 2.2: *Schematic of a plan view of the multi-tier system used. Blue lines show perches that can be seen from the top of the pen.*

The multi-tier unit ran continuously down the length of the barn and was in the centre of each pen. The floor of each pen was covered with wood shavings, approximately 3cm deep and was replenished every 2 weeks. Each pen measured (450cm (h) x 700cm (l) x 230cm (w)) and was separated from the neighbouring pens using wire mesh, meaning that conspecifics were visible through small holes (1cm x 2cm). Hens could not move between pens, but they could see, hear and smell the hens in other pens. The total height of the system was 2.7m. Birds had 12 cm of perch space each and a stocking density of 8.3 hens/m^2 of usable area; including grids and litter. In each focal pen, there were 12 perches, a manure belt, nipple drinkers, linear chain feeders and group nests. Nest boxes (located on the 2nd tier) were closed before lights off (16:00h – 02:00h) to prevent birds from sleeping in the nest box. The Bolegg Terrace system was modified slightly from what can be purchased: some perches on the top level were lowered so that all perches were on the same level and the nipple drinkers were removed from the top level. All perches were made from galvanised steel and measured 3.2 cm in diameter and were 230 cm long (as wide as the pen). Birds were given access to a veranda (area of 9.32 m^2) for approximately seven hours per day from twenty-one weeks of age onwards. The veranda contained nipple drinkers, perches, wood shavings and sand.

A combination of artificial and natural light resulted in an average light intensity of 8.8, 30.2, and 34.4 lux at 04:00h, 10:30hr and 15:00hr, respectively in the middle of the system at hen level. Artificial lights were turned on gradually for 10 minutes before lights were at full intensity (02:00h-02:10h) and were dimmed for 20 minutes at dusk (16:40-17:00) until light intensity reached 0 lux. The light intensity was measured in 6 directions and then averaged using a hand-held lux reader (Gossen, Mavolux 5032C). Daylight was provided via windows and the curtains opened automatically at 08:00h and closed at 16:30h.

2.2.3. Focal hen selection

In flock A and B, 8 out of the 20 pens were used as observation pens. Within those 8 observations pens approximately 7-8 focal hens per pen per week were selected for health assessment and equipped with accelerometers. Each week approximately two pens were recorded simultaneously, then the pens were alternated weekly. In one week, the focal hens were equipped with the accelerometers for an average of four days. If a focal hen's leg bands were missing and the hen could not be followed on to another age, another hen was selected in the pen, resulting in a new focal hen. In total, 62 focal hens were used from flock A (50-60 weeks of age) and 64 focal hens were used from flock B (21-36 weeks of age).

2.2.4. Recording acceleration

2.2.4.1. Accelerometer design

Custom made tri-axial accelerometers were used to record acceleration. They were programmed so that a predefined acceleration threshold could be created. This predefined threshold allowed only events registering at or above this threshold to be recorded, saving battery life and making behaviours of interest (falls and collisions) easier to find.

Predefined thresholds of 12-18g were chosen to make sure that behaviours of interest (falls and collisions) were being recorded. The output consisted of data for each of the 3 axes as well as the overall summed acceleration vector (AV) for the 3 axes, calculated as:

$$\textbf{\textit{Summed Acceleration Vector (AV)}} = \sqrt{(X^2+Y^2+Z^2)}$$

The numerical output of the summed acceleration vector (AV) is quantified using “g” and considers the gravitational acceleration (9.8m/s^2) and the individuals movement acceleration. When the predefined threshold was reached, the acceleration readings along all axes and the summed acceleration vector for one second of data was created (500ms before and 500ms after the first time point the thresholds were exceeded), for both the body and keel sensor. All data were recorded in an Excel and Access interface. Different predefined thresholds were trialled because it was originally unknown what the optimal threshold for detecting falls would be. A balance had to be made between picking up unwanted behaviours, such as preening and missing important behaviours, such as falls. At first, the higher threshold level was used (18g on both axis) then then over time this was reduced to 15g and then to 12g to make sure that no falls were being missed. The threshold refers to the summed AV and there was an individual sensor for both the keel and the body, both sensors had to be triggered for an output to be generated. Recording frequency was 500Hz per sensor, with the keel and body sensor alternating in recording capability. A time stamp was provided along with the acceleration output for the 3 axes and the summed AV. An output for the 500ms before the predefined threshold was first reached and after the predefined threshold was first reached was provided. The sensors remained inactive for 500ms following a recorded event. This data was stored on the device until it was removed from the hen at the end of the data collection period.

The two accelerometer sensors were placed on the back of the hen and the on the keel bone. The location of the dual sensors can be seen in Figure 2.3. In a pilot study (not discussed in this thesis but performed by our research group at Bristol) hens were found to peck the front of the vest (around the keel sensor), resulting in an increase in acceleration on the keel sensor. The addition of dual sensors (one at the keel and one on the body) was important to filter out unwanted behaviours like preening behaviour. Preening and pecking directed at the keel produced a low acceleration readout on the body sensor and a large acceleration readout on the keel sensor and thus, most of these behaviours would be effectively filtered out, when using the dual sensors, and not recorded as an impact of interest. By using dual sensors on the hens, it was found that unwanted behaviours were removed and previously these pecking and preening behaviours made the accelerometer extremely saturated with outputs. The use of the dual sensors eliminated this and allowed more data to be recorded because the battery-life was not being drained by these unwanted behaviours.

Figure 2.3: *Left: Custom made tri-axial accelerometer and vest. Middle: focal bird wearing numbered backpack. Right: Black arrows indicate regions of the hen that the accelerometer sensors were placed*

2.2.4.2. Attachment of the accelerometer

The focal hens were randomly selected from a total of 200 hens per pen, equally from the top tier, middle tier, lower tier and litter and the accelerometer was attached to the birds using a custom vest (Figure 2.3). These same focal hens were then followed through the whole length of the trial with pens alternating each week.

Birds were 50-60 weeks of age in Flock A and 21-36 weeks of age in Flock B. To identify individual birds with a specific (numbered) accelerometer, each bird had a numbered leg band and during accelerometer placement each hen and a numbered back tag (Figure 2.3).

Firstly, the accelerometer was placed inside the vest, ready for attachment to each focal hen. The body sensor was positioned on the back of the hen, in between the wings. The external keel sensor was threaded through the vest, passing over the shoulder region and down towards the centre of the keel. Depending on hen's size, some of the sensors were lower down at the keel (smaller birds) compared to other (larger birds) where the sensor was located closer to the centre of the keel. Care was taken to make sure that the keel sensor was pushed as far into the vest as possible so that the sensor was as close to the centre of the keel as possible. The slightly elastic vest (Figure 2.3) was fitted so that it was loose enough to be comfortable for the bird to encourage normal movement and behaviour, but also tight enough to limit movement and displacement of the accelerometer at the keel.

In total the equipment on each bird had a mass of 89g (back tag = 35g, accelerometer = 33g, fabric vest = 21g). The average body mass of the hens during this study was 1.71kg ± 0.16kg, therefore, the equipment weighed approximately 5.2% of the hens' body weight. It has been recommended that a body worn device should be no more than 5% of a hen's body weight to prevent changes in behaviour (Wilson and McMahon, 2006; Siegford *et al.*, 2016). Therefore, all the equipment together was slightly larger than recommended.

2.2.4.3. Acceleration data sorting

Accelerometer data analysed included; the maximum summed AV at the keel and body, the readout duration, the average summed AV at the keel and the average summed AV x readout duration at the keel. Each of the outputs analysed are presented in Table 2.1. The summed AV data used from the accelerometers was based on the first time 15g was recorded on either the keel or the body sensor and the last time 15g was recorded on either the keel or the body sensor during one event/readout.

Table 2.1: *Accelerometer outputs used in the analysis and their meaning.*

Accelerometer output	Description
Maximum summed acceleration vector (AV)	The highest reading on the summed acceleration vector (AV) accelerometer output, representing the greatest force/load of the movement.
Readout duration	The length of the accelerometer output for a given movement, from the first 15g to the last 15g of the summed AV. This represents the length of time the movement is above the threshold and potentially hazardous.
Average summed acceleration vector (AV)	The average of all the points from the summed AV accelerometer output that fall within the readout duration. It represents the acceleration experience over time.
Average summed AV x readout duration	Represents the average summed AV experienced, considering the length of time the hen is exposed to the potentially hazardous movement (readout duration).

The start and end of the readout duration (the first 15g to the last 15g) were found using R statistical software (R Core Team, 2017) with R studio (RStudio Team, 2016) as the interface. The average summed AV was calculated using a macro in excel and was calculated from the summed AV points that lay within the readout duration (Figure 2.4). If for the same behaviour; two or more data files overlapped, the total duration overlap was added to the readout duration. If there was any missing data, the missing time would be added to the readout duration and the average summed AV for the data available would be used. This would mean that the average summed AV would be inflated for these time-points. Including the missing data was important because it was usually fall movements that were affected by the long readout durations. In total 10.6% of the useable data files were affected by an overlap of some degree. This was done so that important behaviours, such as falls and collisions, were not removed from the dataset. Personal observations suggest that most of these behaviours were due to wing flapping, prolonging the readout duration but sometimes data files were not generated for every second of the behaviour. This is where the inflation comes from because if no file is generated this means that the threshold was not reached, suggesting that the average summed AV x readout duration was lower than the summed AVs within the files where a readout was generated.

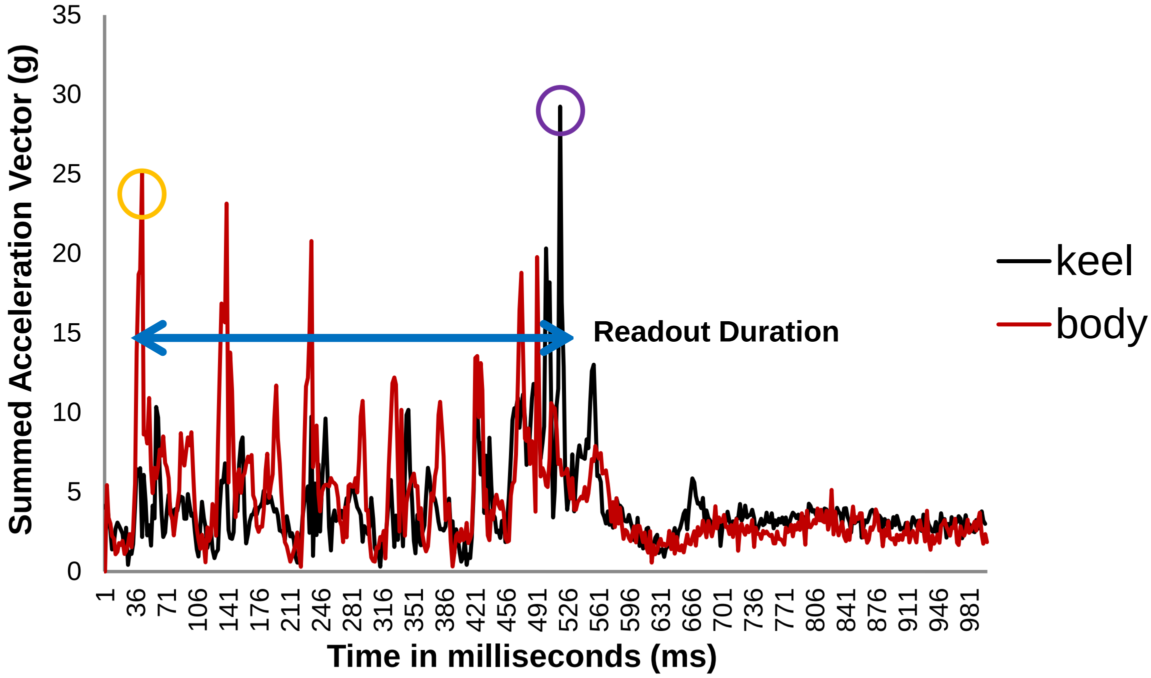

Figure 2.4: *Schematic showing classification of the maximum summed acceleration vector on both, the keel and the body accelerometer as well as the readout duration.*

Blue arrow represents the 15g threshold. The orange circle shows the maximum summed AV on the body and the purple circle shows the maximum summed AV on the keel. The average summed AV is the average of all the point on the keel (black line) that fall within the readout duration.

2.2.5. Behavioural Observations

To match the accelerometry readout with the behaviour of focal birds, five IP infrared cameras (Samsung SCO-2080R, IR, Samsung Techwin CO., Korea) were used, with two placed at either side of the aviary to view the whole pen. Another two cameras were placed so that the number on the bird's back tag could be seen more easily. A 5th camera was placed at the end of the veranda area so that most of veranda could be viewed. All data was stored on a recording unit

(Multieye Hybrid Recorder Version 2.3.1.8, Artec Technologies AG, Diepholz, Germany), videos were then downloaded to an external hard drive for analysis. The computer that was used to set-up the accelerometers was synced with the computer used for video recording. Once accelerometry data was downloaded, the time stamp on the accelerometry output could be matched to the timing on the videos. This allowed the behaviour of the hen to be paired with an accelerometry output.

For each accelerometry output and it’s time stamp, the associated behaviour was matched to the time of day, path (direction and area within the system in which the bird was moving), whether the movement was intentional and successful or a fall and/or a collision and what the bird collided with (Table 2.2 and Figure 2.5).

Table 2.2: *Classifications of different behaviours, movements and definition of terms*

Class of behaviour/ movement	Definition
Intentional movement	The head is orientated towards a destination area, sometimes corresponding with a pacing movement and head tilting. The hen jumps or flies towards this area.
Fall	The movement was not intentional; the focal bird was pushed or lost balance OR the movement was intentional, but the focal bird did not reach the desired area.
Collision	The focal bird crashed into a conspecific, perch or other furnishing in the system or the litter. Collisions can occur during falls or controlled movements.
Controlled	A movement from one area within the system to another (change in start and end location), not a fall.
Location of movement	The location of the beginning and the end of a movement. May be written vice versa, E.g. structure – perch may mean that a hen moved from a perch to a structure on the system but could also mean that a hen moved from a structure on the system to a perch.
Movement	A jump or flight to a different structure within the system.
Interaction	Contact with a conspecific that can constitute; scratching, grabbing, climbing onto and chasing. Also refers to a panic reaction in the system.
Push	Focal hen knocked from the area they are currently occupying by a conspecific and are displaced to another location.
Missed	Focal hen shows intentional movement, indicating movement to a specific location, but lands in another location.
Slip	Focal hen does not intend to change position but does change position

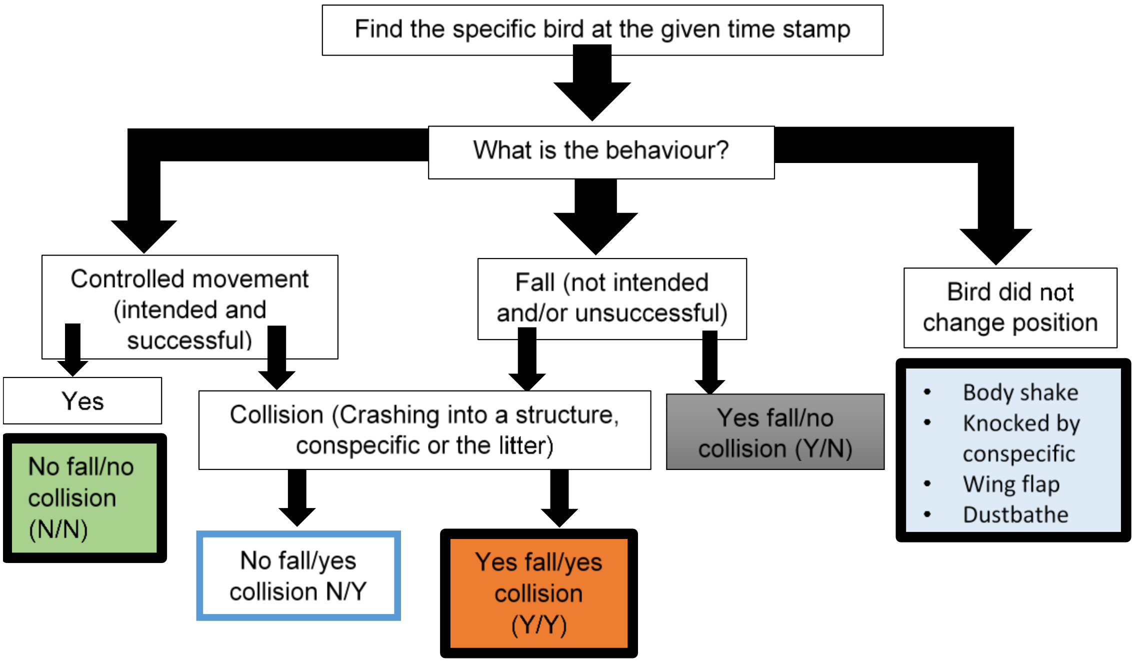

Figure 2.5: *Classification steps of behavioural analysis.*

2.2.6. Health recordings

Keel bones of the focal birds were assessed using palpation to determine the prevalence of keel bone fractures and deviations (Wilkins *et al.*, 2004; Casey-Trott *et al.*, 2015). Keel bone fractures were scored using a 3-point scale; 0=no break, 1=slight break and 2=severe break (Wilkins *et al.*, 2004). Deviations were scored as either present or absent. The presence of deviations were any bends or S-shaped deformations of the keel bone (Casey-Trott *et al.*, 2015), and scored separately from keel bone fractures. Body mass was recorded using scales that gave accuracy to 3 decimal places, hens were placed in a box to assure accuracy of the measurement. A feather scoring system was used from Tauson *et al.* (2005) including a score from 1-4; 4 being full feather coverage and 1 being large patches of feather loss. Foot health was recorded from Tauson et al. (2005) was bumblefoot and foot pad dermatitis was used from Butterworth (2013). For Flock A feather cover and foot pad health were scored according to how they were described in the references. In Flock B, a visual analogue scale was attached to each parameter to make classification more accurate, which showed photos with a further breakdown of the categories. Flock A was assessed at 54, 56 and 61 weeks of age, whereas flock B was assessed before each observation period (21, 23, 24, 25, 26, 27, 28, 31, 33, 35 and 36 weeks of age).

2.2.7. Keel bone palpation intra-observer reliability

To test the reliability of palpation as a tool to determine keel bone fracture prevalence, 13 birds were palpated in flock A at 61 weeks of age and 20 were palpated in flock B at 36 weeks of age. Each of the hens were palpated twice so that intra-observer reliability could be tested, therefore, to see the percentage agreement of scores of the same hens when repeated.

2.2.8. Keel bone palpation accuracy

There were 3 different time points were palpation accuracy was compared to an experienced palpation assessor, whose palpation accuracy had been validated against dissected keel bones. In April 2015 80 hens were palpated by an experienced assessor then again by the assessor in the current study. In May 2015 accuracy was compared again using the same 80 hens. Palpation accuracy was compared again in March 2018 on 30 hens as part of a palpation training course. In May 2018 palpations were carried out on the same 600 hens as a trained assessor one week apart. Therefore, in this incidence the percentage of breaks detected was recorded because after one week those hens that developed a fracture could not be scored.

2.2.9. Statistical Analysis

2.2.9.1. Model fit and selection

Data was analysed using R statistical software (R Core Team, 2017) with R Studio (RStudio Team, 2016) as the interface. The lme4 package (Bates *et al.*, 2015) was used to run the models and the LmerTest package (Kuznetsova *et al.*, 2017b) provided p-values for the output. The lsmeans package (Lenth, 2016) was used to obtain least square means for the model outputs. Histograms of the data showed a left-skewed distribution, thus either a lognormal or gamma model were chosen based on the histograms, Q-Q plots of residuals and AIC of the models because they determine best fit of the models.

Fixed effects included in the first model of all analyses were the 3-way interaction between fall (Y/N) (binary) x collision status (binary) x movement height (factor), 2-way interaction between collision status x direction of movement (binary), location at the start and end of the movement (factor), presence of keel bone score (binary), presence of foot pad dermatitis (binary), presence of bumble foot (binary), feather conditions (factor), body mass (continuous) and flock. In flock A the data collected closest to the time point hens were monitored were used. For flock B if hens had any keel bone or foot pad problems during either assessment, this was included. Body mass was averaged in flock B. A stepwise generalised mixed effect model was used with bird nested within pen as a random factor, meaning that individual hen was the experimental unit. Interactions and fixed effects were considered significant at $P<0.05$ and if significance was not reached then the interaction or fixed effect was generally removed from the model. Exceptions were the body AV model, where the removal of the collision (Y/N) resulted in a large increase in AIC from the model, and because of this collision status was left in the model but was not significant and flock was controlled for in each model.

Flock remained in the model even when non-significant to control for the difference between flocks. Age was not included in the analyses because it was confounded with flock. The response variables were maximum acceleration vector (AV) at the keel (g), readout duration (s), average acceleration vector (AV) at the keel (g), average AV at the keel x readout duration. Flock A ranged from 50-61 weeks of age and flock B ranged from 21-36 weeks of age.

2.2.9.2. Multiple comparisons within movement height

When significance was detected multiple comparisons using Bonferroni tests in R were used with the lsmeans package in R to determine significance levels and lsmeans. Multiple comparisons tested in the movement height interaction were; 1. Differences between fall status within individual heights, 2. Difference between heights in yes fall group and 3. Difference between heights in the no fall group.

2.2.9.3. Multiple comparisons between locations at the start and the end of the movement

Multiple comparisons were again carried out using Bonferroni comparisons. Difference between heights in no fall group. All comparisons in the location at the start and end of the movement were compared to each other. The direction of movement between the location at the start and the location at the end of the movement are written are interchangeable i.e. litter to tier could also mean tier to litter.

2.2.9.4. Sub-setting – Maximum summed AV during a collision

The data was split into a subset for part of the analysis: The effect of the object the bird collided with was only analysed in the group of outcomes where individuals had a collision and the maximum summed AV at the keel associated with the collision. Fixed effects included in the model were the 3-way interaction between fall (Y/N) (binary) x movement height (factor) x Object the hen collided with, location at the start and end of the movement (factor), direction of movement (binary), presence of keel bone fracture (binary), presence of foot pad dermatitis (binary), presence of bumble foot (binary), feather condition (factor), body mass (continuous) and flock (factor). Any non-significant interactions and effects were removed from the analysis using a stepwise approach until only significant effects, P<0.05, were left in the model.

2.2.9.5. Sub-setting – Maximum summed AV and the reason for a fall

Another subset was the reason for fall and the maximum summed AV at the keel associated with that. Fixed effects included in the first model of all analyses were the 2-way interaction between collision status (binary) x movement height (factor), 2-way interaction between collision status x direction of movement (binary), the reason for a fall (factor), location at the start and end of the movement (factor), presence of keel bone score (binary), presence of foot pad dermatitis (binary), presence of bumble foot (binary), feather condition (factor), body mass (continuous) and flock. Any non-significant interactions and effects were removed from the analysis using a stepwise approach until only significant effects, P<0.05, were left in the model.

Means, standard errors and confidence intervals provided in the text are least square means produced from the final models. Interaction plots were plotted using the “lsmeans” and “ggplot2” (Wickham, 2009) packages in R version 3.4.3. When needed data were optimised using the optimx package in R (Nash, 2014) to correct for any convergence issues in the data.

2.2.9.6. Keel bone palpation intra-observer reliability

Intra-observer reliability of keel bone fracture palpation was tested using a weighted Cohen’s kappa test in R using the irr package (Gamer *et al.*, 2012). Results were interpreted as: ≤ 0 no agreement, 0.01–0.20 as none to slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement (McHugh, 2012).

2.2.9.7. Keel bone palpation accuracy

All evidence shown is descriptive and is given for the severity score and presence and absence of keel bone fracture.

2.2.10. Ethical Statement

All procedures carried out were approved by the Canton of Bern (Switzerland), the experimental number was BE-58/15.

2.3. Results

All together there was 5,000 accelerometer outputs that were recorded that had the potentially to register a behaviour. The full useable dataset represented 1,766 useable accelerometer outputs. Therefore, 35.32% of all the potential accelerometer outputs could not be used, either because the logger became loose in the vest, the bird could not be seen, or the output did not meet the requirements of the threshold e.g. the body and the keel sensor both reaching a maximum summed acceleration vector of 15g. Data showing the maximum summed AV relating to a collision contained and 486 data points 27.52% of the useable dataset. Data showing the maximum summed AV and the reason for a fall contained 579 data points, representing 32.79% of the useable dataset.

The pens sampled each week and all the data relating to each pen is provided in Table 2.3 and 2.4. As can be seen from the table, week of age of the hens, the pen sampled, the number of focal hens used from each pen, the number of accelerometer outputs relating to each pen as well as a breakdown of health parameters per pen are provided. It is worthwhile to note that as the hens age (particularly in Flock B), health parameters tend to deteriorate. For example; more hens tend to have foot pad problems and keel bone fractures and are less likely to have excellent feather condition

***Table 2. 3:** Descriptive data relating to hens from flock A*

Flock A

Week of Age	Pen Number	Number of focal hens	Number of useable accelerometer outputs	Number of hens with keel bone fractures	Number of hens with keel bone deviations	Number of hens with bumble foot	Number of hens with foot pad dermatitis	Number of hens with excellent feather cover
50	11	7	47	5	3	3	0	7
51	12	7	34	4	0	1	0	1
52	3	7	36	2	2	3	0	6
52	6	8	29	5	5	5	0	2
54	3	7	33	2	2	3	0	6
54	6	8	24	5	5	5	0	2
55	3	7	28	5	2	5	0	7
55	6	8	42	6	5	5	0	1
56	4	7	50	2	5	1	0	2
56	7	8	71	8	2	2	0	2
58	11	7	29	7	4	4	1	1
58	18	8	34	8	4	6	5	2
59	12	7	75	6	2	2	4	0
59	17	8	75	8	5	2	6	1
60	4	7	38	6	4	2	1	2
60	17	8	25	8	5	6	6	1

Table 2. 4: *Descriptive data relating to hens from Flock B*

Flock B

Week of Age	Pen Number	Number of focal hens	Number of useable accelerometer outputs	Number of hens with keel bone fractures	Number of hens with keel bone deviations	Number of hens with bumble foot	Number of hens with foot pad dermatitis	Number of hens with excellent feather cover
21	12	8	49	5	1	0	2	8
21	16	7	30	0	0	0	1	7
23	13	6	42	4	0	2	1	6
23	17	7	32	3	3	0	0	6
24	14	6	27	2	1	0	0	6
24	18	6	28	2	1	0	0	6
25	15	6	106	3	2	3	0	6
25	19	6	55	3	0	0	0	6
26	12	6	65	4	2	1	0	6
26	16	6	86	1	0	0	0	6
27	13	7	40	4	1	0	0	7
27	17	7	34	5	2	0	0	7
28	14	7	66	2	1	1	1	7
28	18	7	32	4	1	0	0	7
31	15	7	61	5	1	1	2	7
31	19	7	43	4	1	1	0	7
33	12	8	81	8	0	0	3	8
33	16	7	81	4	1	0	0	7
35	13	8	53	6	2	0	2	8
35	17	7	30	6	1	1	0	7
36	14	8	28	6	3	5	2	5
36	18	7	27	1	2	0	2	6

Table 2.5 provides a breakdown of behavioural characteristics of all the movements shown by the hens. This data is provided to show an overview of the whole dataset and is not broken down into week of age or pen number. The total height of each movement and whether the behaviour consisted of a fall and collisions or both is shown.

Table 2.5: *Total movement height and presence of falls and collision (sample size)*

Total height of movement	***Fall and collision presence in movement***			
	No fall/ no collision	*No fall/ yes collision*	*Yes fall/ no collision*	*Yes fall/ yes collision*
0m	*11*	*2*	*1*	*0*
0-0.5m	*250*	*33*	*61*	*39*
0.5-1.0m	*519*	*19*	*93*	*81*
1.0-1.5m	*27*	*9*	*11*	*75*
1.5-2.0m	*110*	*47*	*33*	*85*
2.0-2.5m	*9*	*1*	*22*	*41*
>2.5m	*63*	*17*	*2*	*14*

2.3.1. Maximum summed acceleration vector (AV) at the keel sensor

There was an interaction effect of fall status and movement height (F= 4.912, P<0.0001) on the maximum AV at the keel. There was a difference in the maximum summed acceleration vector (AV) and whether there was a collision or not (F= 47.866, P<0.0001), between different start and end locations (F= 2.207, P=0.0399) and flock (F= 6.639, P=0.0207). However, flock was added to control for the design of the experiment and will not be discussed. There were no significant differences between different body masses, keel bone fracture presence, foot health or feather condition and the maximum summed AV at the keel.

Non-fall movements that occurred at lower heights (0m-1.0m) had higher maximum summed acceleration vectors (AV) at the keel compared to those occurring at 1.5m-2.0m (Z-ratio = 3.841; P value = 0.0060; Z-ratio = 3.292, P-value 0.0487, respectively; Figure 2.6). This was contrary to the prediction movements from higher heights would result in higher maximum summed acceleration vectors compared to heights lower in the system. Although visual inspection indicated that falls from a total height >0.5 m had greater maximum summed AVs than controlled movements, such differences only reached significance for movements occurring at 1-2m (Z-ratio = -3.501, P=0.0227 and Z-ratio= -7.157, P<0.0001) and 2.5m (Z-ratio = -4.008, P=0.0030) (Figure 2.6). Visual inspection of the graph indicates as total height increases the maximum summed AV increase for falls, but this was not statistically significant.

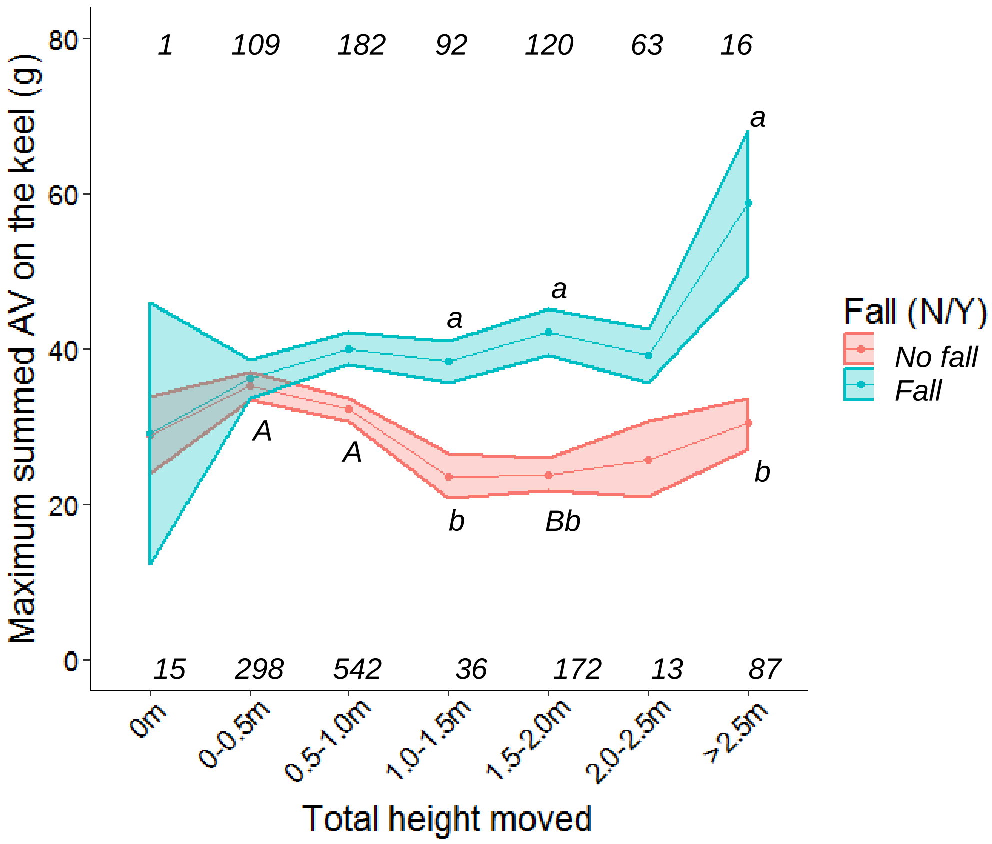

Figure 2.6: *Maximum summed acceleration vector at the keel depending on fall status and actual height of movement (LS Mean ± SE).*

Numbers at the top of the graph are the sample sizes for falls and those at the bottom of the graph are sample sizes for non-falls. Differences between falls and non-falls are only compared within single heights. Significant differences are shown as small letters. Differences across heights are compared independently for falls and non-falls. Significant differences are shown as capital letters

There was no interaction between collision presence and the height of the movement on the maximum summed AV. Movements that contained a collision (38.49g ± 2.37g) had higher maximum summed AVs at the keel compared with movements that did not contain a collision (29.17g ± 1.69g; Z-ratio = -6.936; P<0.0001).

There was an overall effect of the location of movement and the maximum summed AV on the keel (F= 2.207, P=0.0399), but when comparing individual locations, no differences were seen. Numerically, the structure to structure movement resulted in lower maximum summed AV readings on the keel compared to other movement locations, but it was not significant. Movements between a perch and a grid had the highest maximum summed AV readings on the keel, but this was not statistically significant (Figure 2.7).

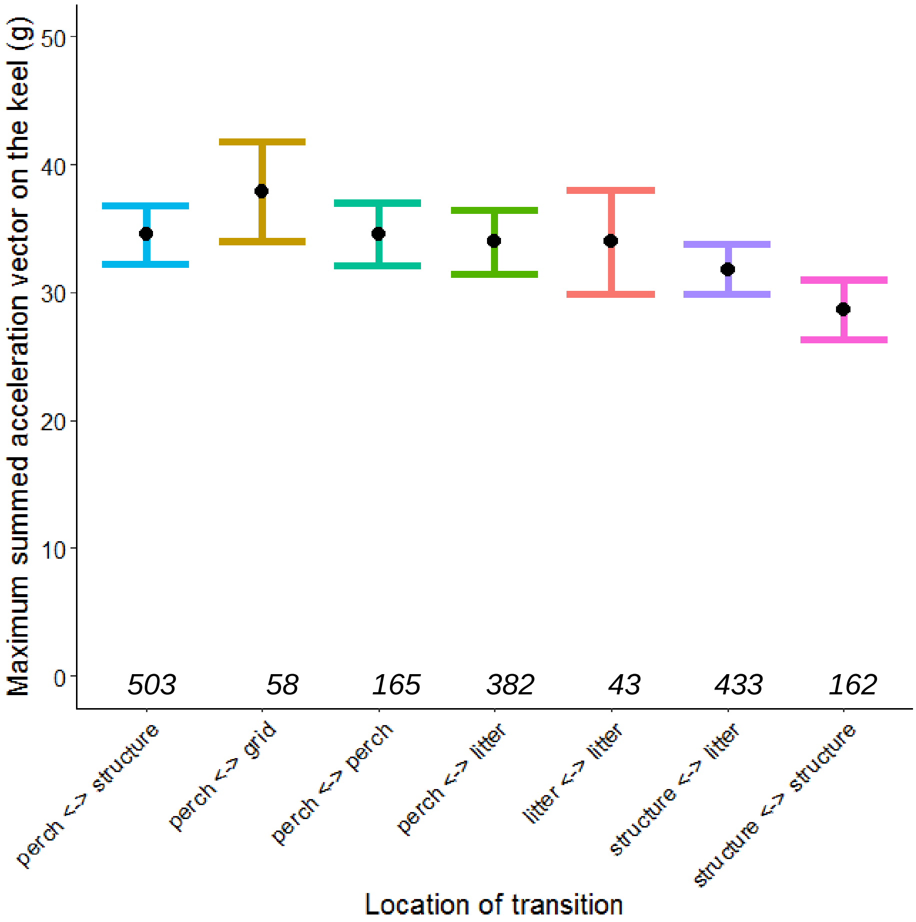

***Figure 2.7:** Maximum summed acceleration vector readings in relation to the location of the transition (start and end of the movement) (LS Mean ± SE).*

Numbers along the bottom refer to the sample sizes.

2.3.2. Readout duration

There was a 3-way interaction effect of fall (N/Y) x collision (N/Y) x total movement height (W= 11.667, P=0.0397) on the readout duration. There was a 2-way interaction between collision (N/Y) and movement direction (up/down) (W= 17.250, P<0.0001). There was an effect of the location of the start and the end of the movement and the readout duration (W= 57.399, P<0.0001). There were no significant differences detected between weight, keel bone fracture presence, foot health or feather condition and the maximum summed AV at the keel. Table 2.5 shows the sample sizes for all the fall (N/Y), collision (N/Y) and actual height moved interactions.

For the readout duration, non-fall/non-collision movements (red, Figure 2.8) at total height 0-0.5m had longer durations compared with total movement heights 0.5-1.0m (Z-ratio = 3.748, P=0.015). Non-fall movements/collisions (green; Figure 2.8) had a longer readout at 0-0.5m compared to 0.5-1.0m (Z-ratio= 3.959, P=0.0063). Non-fall movements/collisions (green, Figure 2.8) at 0.5-1.0m had shorter readout durations compared to 1.5-2.0m (Z-ratio=-4.450, P=0.0007) and >2.5m (Z-ratio=-3.542, P=0.0334). Falls/collisions (purple; Figure 2.8) tended to have shorter readout durations at 0-0.5m compared with 1.0-1.5m (Z-ratio=-3.298, P=0.0819) and had longer readout duration compared with 1.5-2.0m (Z=-3.571, P=0.0299). Fall/collision movements (purple. Figure 2.8) at heights 2.0-2.5m had longer readout durations compared to fall/non-collision movements (blue, Figure 2.8; Z=-3.993, P=0.005). Non-fall/non-collision movements (red, Figure 2.8) had shorter readout durations at total movement heights 0-2.0m compared fall/non-collision movements (blue, Figure 2.8; [0-0.5m; Z=-5.556, P<0.0001; 0.5-1.0m; Z-ratio=-8.743, P<0.0001; 1.0-1.5m; Z-ratio=-4.871, P=0.0001; 1.5-2.0m; Z-ratio=-5.488, P<0.0001]). Non-fall/collision movements (green; Figure 2.8) had lower readout durations at 0.5-1.0m and 1.5-2.0m compared to fall/collision movements (purple; Figure 2.8; Z-ratio=-8.005, P<0.0001 and Z-ratio=-6.343, P<0.0001) and tended to be lower than 1.0-1.5m (Z-ratio=-3.388, P=0.0592).

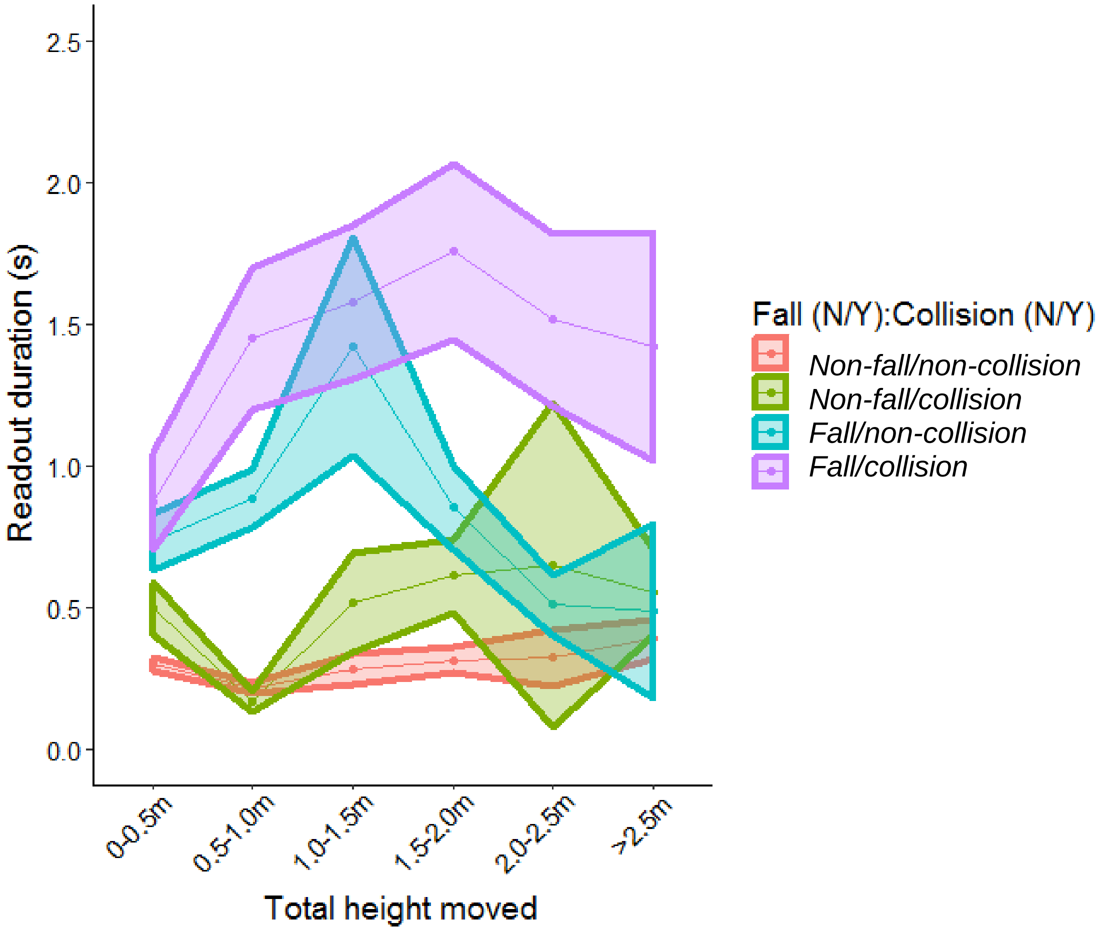

Figure 2.8: *Three-way interaction between fall status (Y/N), collision status (Y/N) and actual height of movement (LS Mean ± SE).*

For collisions, downward movements had shorter readout durations compared to upward movements (Z-ratio = -4.095, P=0.0002; Figure 2.9). For non-collisions, there was no significant difference in duration with respect to direction of the movement (P>0.05). Upward movements that contained a collision had longer durations compared with those that did not contain a collision (Z-ratio = -3.86, P=0.0005). There was no significant difference between downward movements irrespective of whether a collision occurred (P>0.05) (Figure 2.9).

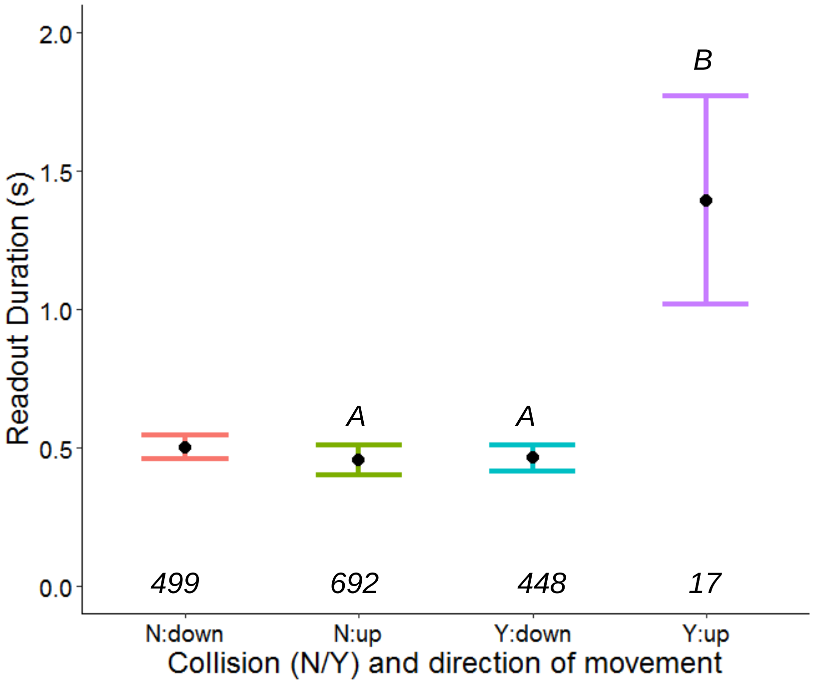

Figure 2.9: Interaction between the collision status (N/Y) and the direction of the movement (up/down) for impact duration (s) (LS Mean ± SE).

Different letters show a statistical difference.

There was an overall difference in the readout duration between different locations of movements (W=57.40, P<0.0001; Figure 2.10). Movements between a structure and the litter had longer readout durations compared with movements between a perch and a structure (Z-ratio=-7.344, P<0.0001), a perch and the grid (Z-ratio=-4.2, P=0.0005), a structure to another structure (Z-ratio=2.848, P=0.0925) and from a perch to another perch (Z-ratio=-2.894, P=0.08; Figure 2.10). Movements between a perch and another structure had lower readout durations compared with movements between a perch and another perch (Z-ratio=3.517, P=0.0092; Figure 2.10).

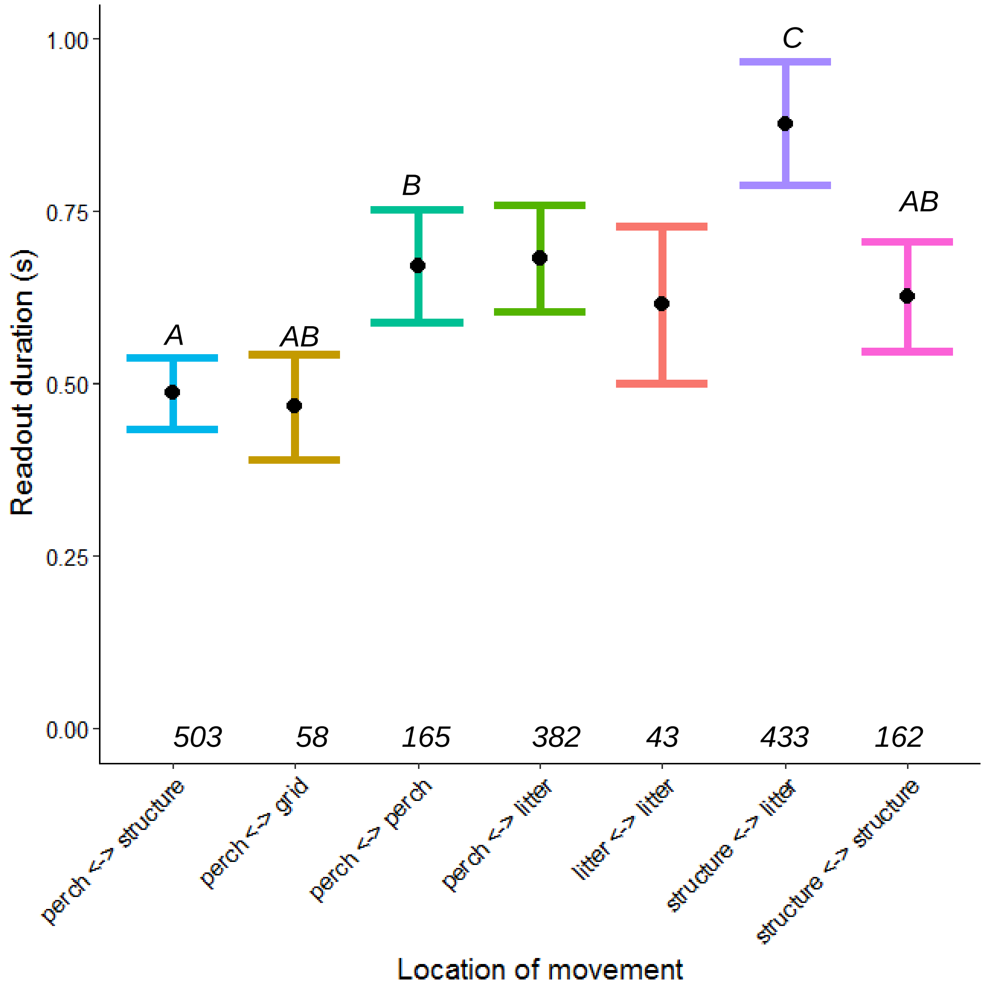

Figure 2.10: *Duration of impact in relation to the location of the movement (LS Mean ± SE).*

Different letters show different significance. Sample sizes are displayed at the bottom of the graph.

2.3.3. Average summed AV at the keel

There was a significant 3-way interaction between fall/non-fall and collision/non-collision and total movement height (F=4.067, P=0.0011). There was a 2-way interaction between collision/non-collision and direction of the movement (F-statistic= 5.679, P=0.0173). There was also an effect of the location of the movement (start and destination area) (F=8.460, P<0.0001). There were no significant differences detected between weight, keel bone fracture presence, foot health or feather condition and the maximum summed AV at the keel.

Movements that were non-falls/collisions (green; Figure 2.11) had higher average summed AVs compared to non-falls/non-collisions (red; Figure 2.11; Z-ratio=-4.252, P=0.0019). Movements that were non-falls/collisions (green; Figure 2.11) at total height moved of 0-0.5m had lower average summed AVs compared to non-falls/collisions at total heights of 0.5-1m (Z-ratio=-5.572, P<0.0001). Movements that were non-falls/non-collisions (red; Figure 2.11) at 0-1.0m, had higher average summed AVs compared with falls/non-collisions (blue; Figure 2.11) at the same height (z-ratio=3.282, P=0.0886 and Z-ratio=3.967, P=0.0064, respectively). Movements that were non-falls/collisions (green; Figure 2.11) at 0.5-1.0m had higher average summed AVs compared to falls/collisions (purple; Figure 2.11) at the same height; Z-ratio=6.353, P<0.0001.

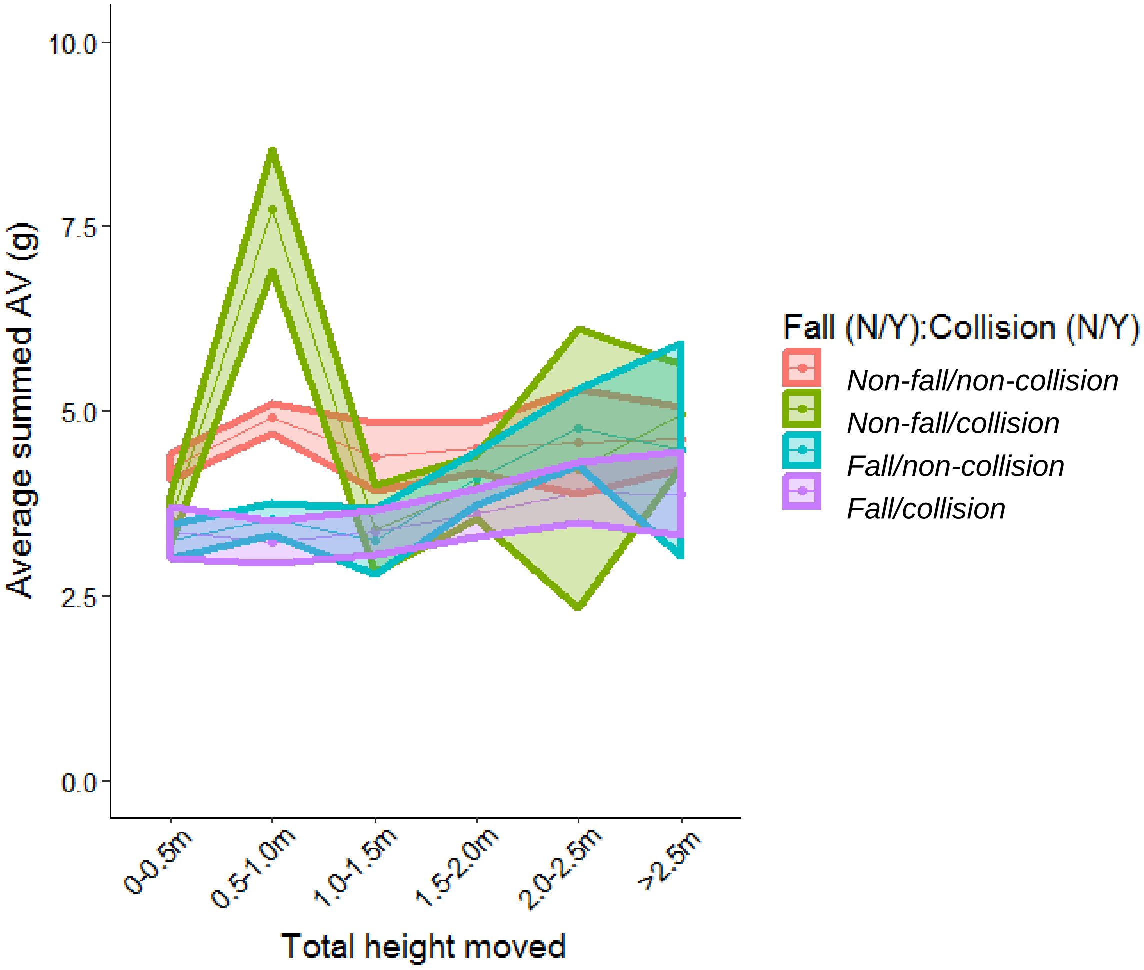

Figure 2.11: *Three-way interaction between fall status, collision status and actual height moved (LS Mean ± SE) for the average summed acceleration.*

Upward movements had lower average summed AV at the keel than downward movements, and this difference was significant both when movements included a collision (Z-ratio= 4.089, P=0.0002) and when there was not a collision (Z-ratio= 3.95, P=0.0003) (Figure 2.12). There was no statistical difference within movement direction and whether there was a collision or not.

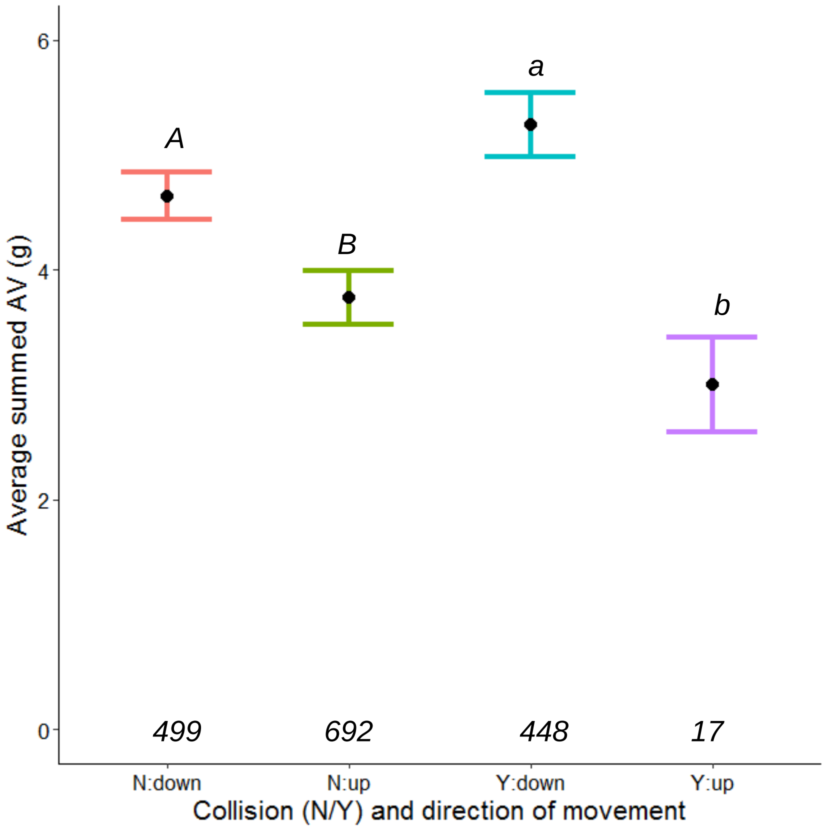

Figure 2.12: *Interaction between the collision status (N/Y) and the direction of the movement (up/down) for the average acceleration (LS Mean ± SE).*

Capital letters denote differences between non-collisions and small letters denote difference between collisions.

Movements from a perch to a structure resulted in higher average summed AV at the keel compared with movements from a perch to the litter (Z-ratio=-3.428, P=0.0128) or between a structure and the litter (Z-ratio=6.772, P<0.0001) (Figure 2.13). Movements between a perch and a grid had higher average summed AV readings compared with movements between a structure and the litter (Z-ratio=4.035, P=0.0011). Average summed AVs were higher when moving from a perch to another perch compared with between a structure and the litter (Z-ratio=3.134, P=0.0362) (Figure 2.13).

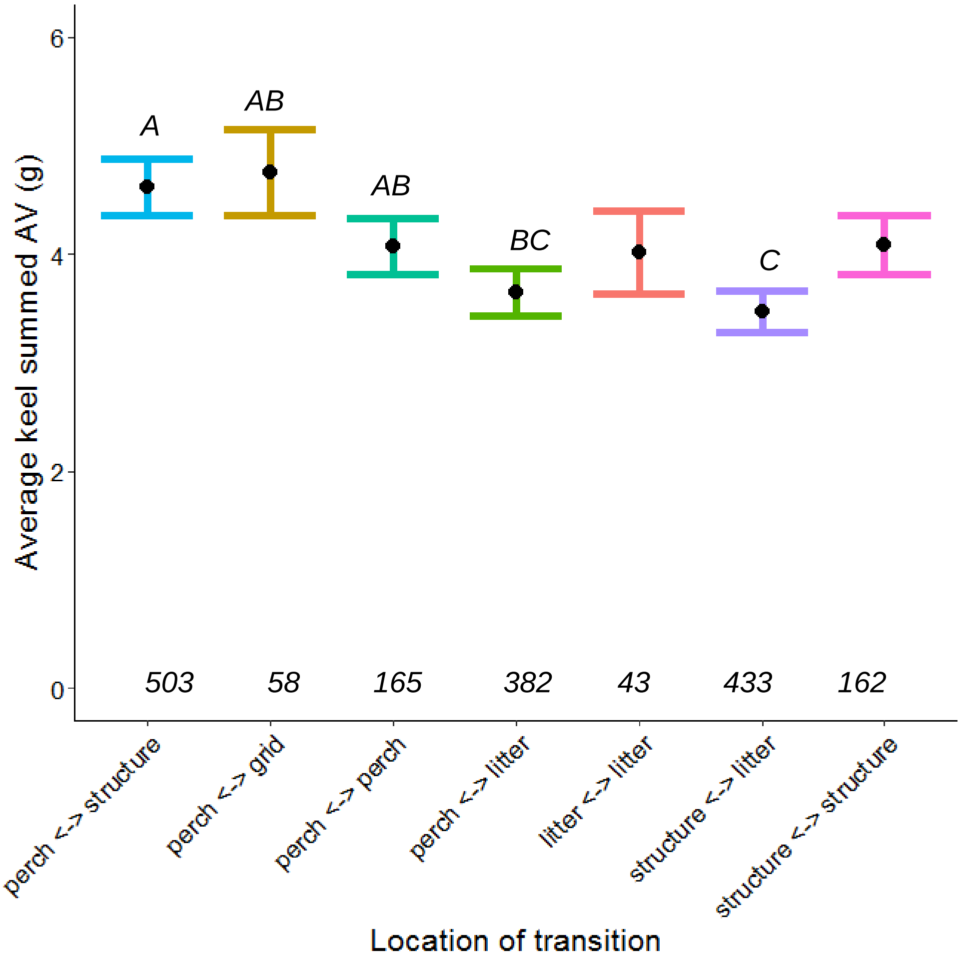

***Figure 2.13**: Start and end location of movement in relation to the average AV at the keel (LS Mean ± SE).*

Sample sizes are displaced at the bottom of the graph. Different letters show different significance.

2.3.4. Average summed AV x readout duration

Overall, there was a 2-way interaction between fall status and total movement height (F=7.496, P<0.0001), a 2-way interaction between collision status and movement height (F=2.792, P=0.0162), a 2-way interaction between collision status and direction of movement (F=6.797, P=0.0092), with the location of the movement (start and destination point) (F=8.161, P<0.0001) and flock

(F=5.250, P=0.0368). There were no statistical differences between keel bone fracture presence, foot pad health and feather condition and the value of the average summed AV x readout duration.

Non-falls had lower average summed AV x readout duration at total movements heights 0.5m-1.0m compared to those 0-0.5m (Z-ratio=3.989, P=0.0024) and those 1.5-2.5m. There was an increase in the average summed AV x readout duration during falls (blue) from 0-0.5m to 1.0-1.5m, then a decrease to 2.0-2.5m, and then an increase again at 2.5m. However, there was only one significant difference from 0-0.5m compared to those 1.0-1.5m (Z-ratio=3.816, P=0.0049), all other differences can only be seen numerically (Figure 2.14). In every total height category except 2.0-2.5m, the average summed AV x readout duration of fall movements were statistically greater than those of non-falls (Figure 2.14).

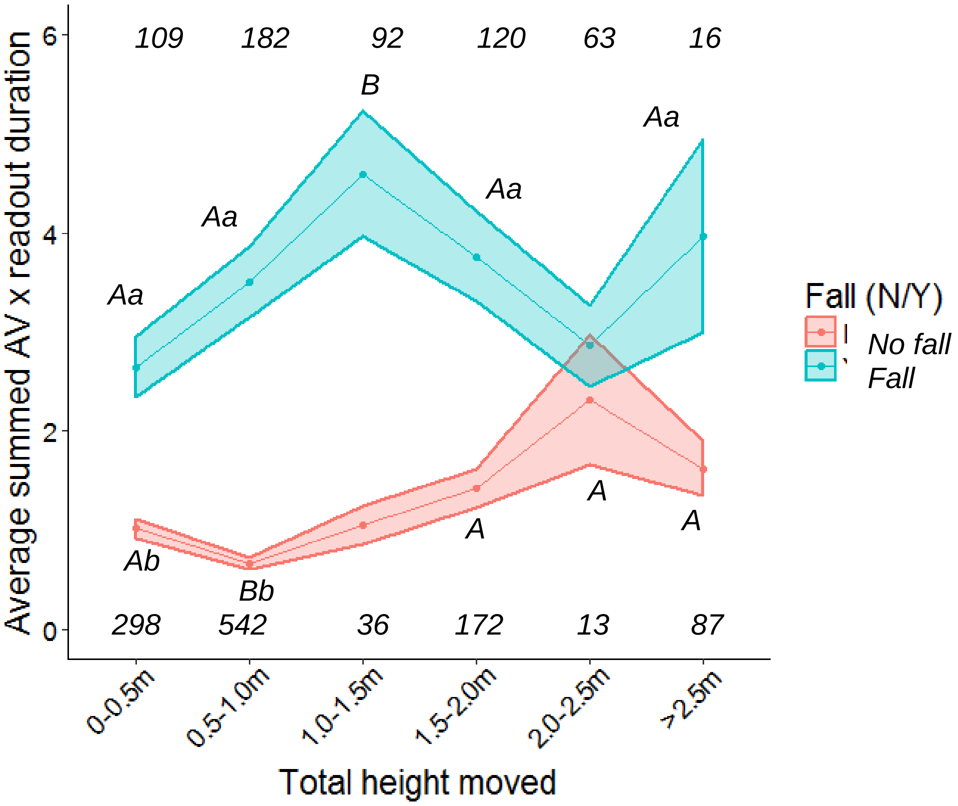

Figure 2.14: *Two-way interaction between fall (N/Y) and actual height moved in relation to the average summed AV x readout duration (LS Mean ± SE).*

Numbers at the top of the graph are the sample sizes for falls and those at the bottom of the graph are sample sizes for non-fall movements. Differences between falls and non-falls are only compared within single heights. Significant differences denoted by small letters. Differences across heights are compared independently for falls and non-falls. Significant differences denoted by capital letters.

There was no significant difference in the average summed AV x readout duration of movements that did not contain a collision between different total heights (Figure 2.15). However, when a movement did involve a collision, those at total heights 0.5-1.0m had lower readings compared with total heights 1.5-2.5m. There were differences between average summed AV x readout duration and whether a collision was present or absent at total heights; 0-0.5m (Z=3.079, P=0.0749) and 1.5-2.5m. In each instance, the readings were higher when a collision was present compared to those where a collision was absent (Figure 2.15).

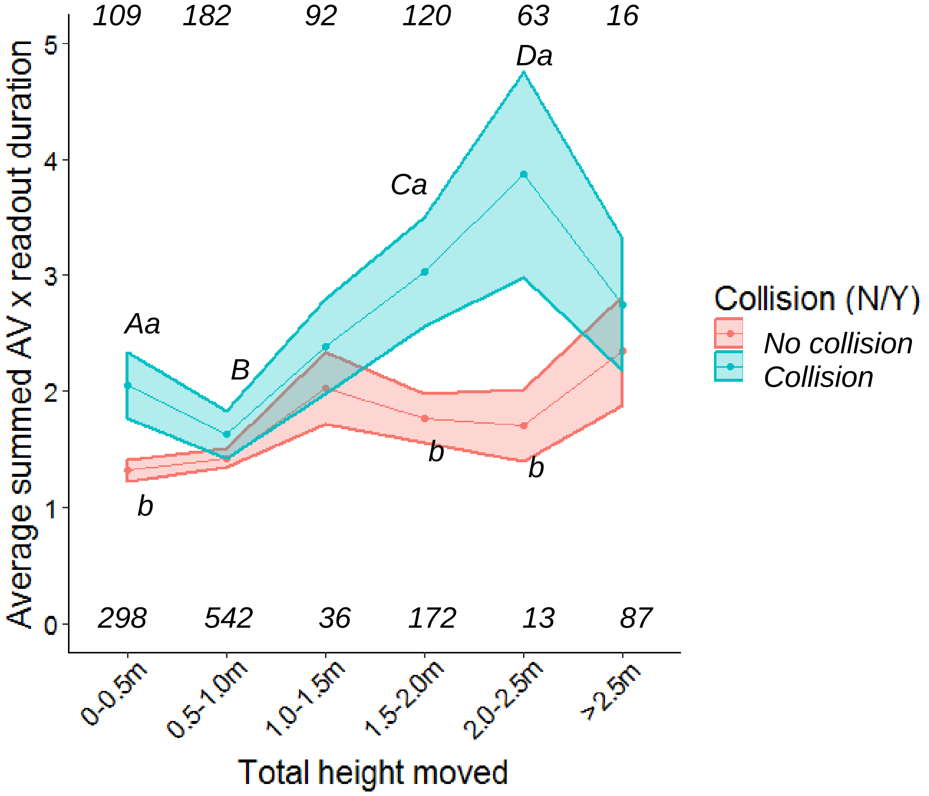

Figure 2.15: *Two-way interaction between collision status (Y/N) and actual height moved in relation to the Average summed AV (LS Mean ± SE).*

Numbers at the top of the graph are the sample sizes for falls and those at the bottom of the graph are sample sizes for non-fall movements. Differences between falls and non-falls are only compared within single heights. Differences denoted with small letters. Differences across heights are compared independently for falls and non-falls. Differences denoted with capital letters.

Upward movements that included a collision tended to have higher average summed AV x readout duration readings compared to those that did not include a collision (Z-ratio=3.127, P=0.0071) (Figure 2.16). Downward movements that included a collision had lower average summed AV readings compared with upward movements that included a collision (Z-ratio=-2.989, P=0.0112).

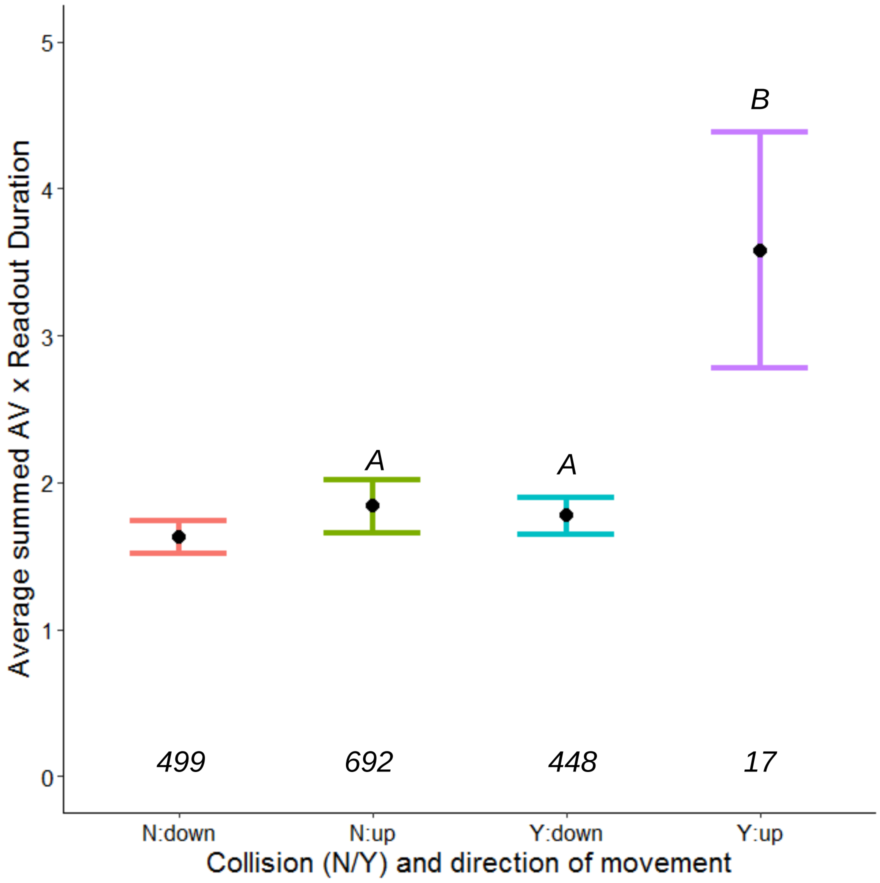

Figure 2.16: *2-way interaction between collision status and the direction of movement relating to average summed AV x readout duration (LS Mean ± SE).*

Sample sizes are displaced at the bottom of the graph. Different letters show different significance.

Movements between a structure and the litter had higher summed AV x readout duration compared movements between a perch and a structure (Z-ratio=-6.849, P<0.0001), a structure and another structure (Z-ratio=3.336, P=0.0178), a perch and the grid (Z-ratio=-3.698, P=0.0046) and a perch and another perch (Z-ratio=-3.175, P=0.0315). Movements between a perch and the litter had higher average summed AV x readout duration compared with movements between a perch and a structure (Z-ratio=3.603, P=0.0066) (Figure 2.17).

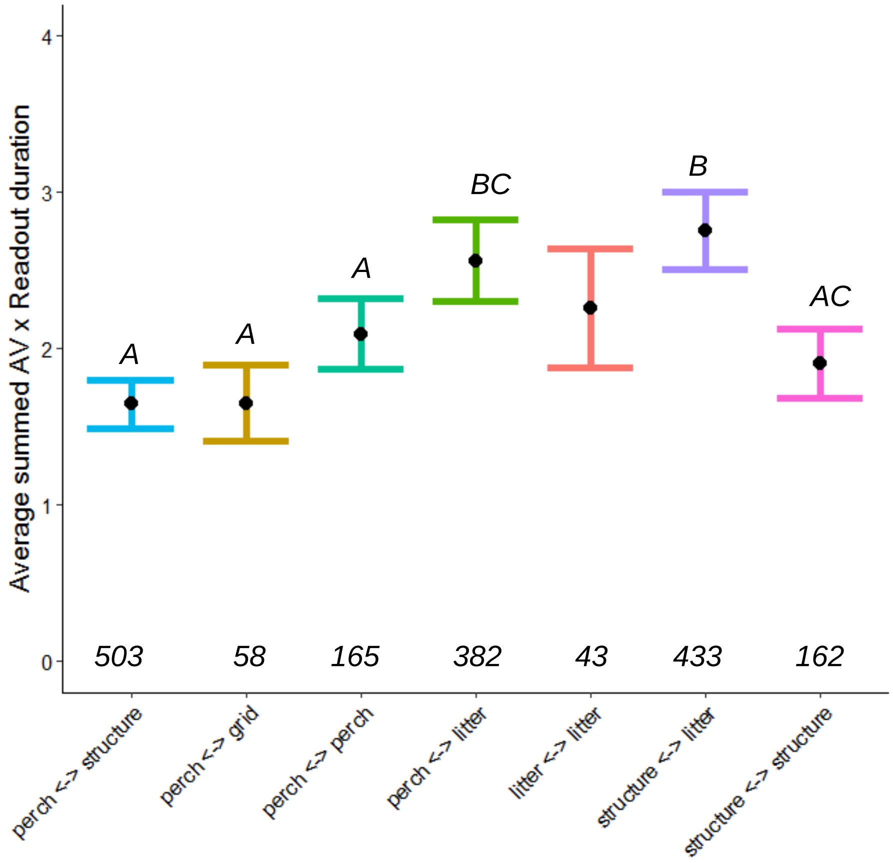

Figure 2.17: *The start or end location of movement in relation to the average summed AV x readout duration (LS Mean ± SE).*

Numbers at the bottom show sample sizes. Letters above the bars represent statistical significance.

2.3.5. Maximum summed AV during a collision

There was an overall effect of the object that a hen collided with and the maximum summed AV (F=5.06, P=0.0019). Quantitively, collisions with a conspecific had the lowest maximum summed AV, followed by the litter, a perch and lastly a structure (Figure 2.18). Statistically, collisions with conspecifics were lower than those with perches (Z-ratio=-3.470, P=0.0031) and structures (Z-ratio=-3.473, P=0.0031).

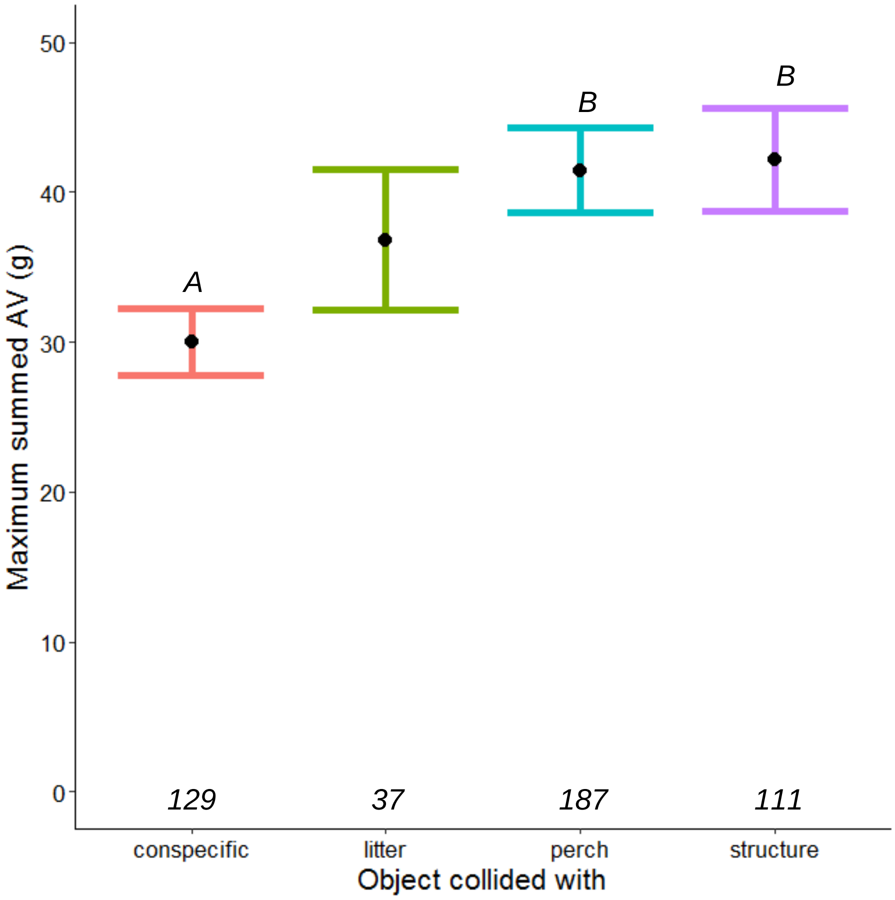

Figure 2.18: *Maximum acceleration vector at the keel relating to the object that is collided with LS mean (±SE).*

Sample sizes for each category are displayed along the bottom. Different letters denote significant differences.

2.3.6. Maximum summed AV and the reason for fall

There was a significant effect of the cause of fall on the maximum summed AV at the keel (F=2.85, P=0.0370). Falls that were caused by missed landings had higher maximum summed AVs compared with than those caused by pushes from conspecifics (Z-ratio=2.675, P=0.0449), (Figure 2.19). There was no statistical difference between a fall caused by an interaction, slip and any of the other categories (all terms are described in Table 2.2).

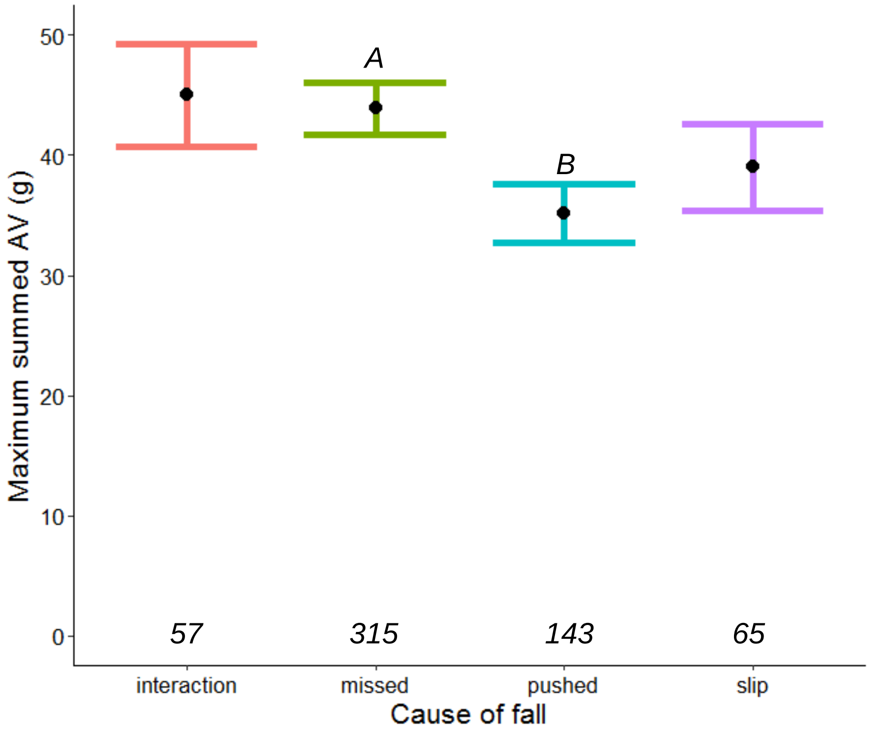

Figure 2.19: *Cause of fall relating to Maximum summed AV at the keel LS means (± SE) shown.*

The numbers at the bottom represent the sample size. Different letters denote significant differences.

2.3.7. Maximum summed acceleration vector (AV) on the body sensor

All other sections of the results to this point, have focussed on data from the sensor placed on the keel bone; this section will focus on data collected from the sensor placed on the body. Whether there was a fall or not (F=67.38, P<0.0001), the height of the movement (F=3.93, P=0.0007; Figure 19), location of movements (F=8.59, P<0.0001), feather condition of the hen (F=7.7, P=0.0006), body mass (F=18.91, P<0.0001) and flock (F=30.82, P<0.0001) were all significantly different for the maximum summed AV on the body sensor. As body mass of hens increased, the maximum AV on the body sensor increased. There was no difference between keel bone fracture presence and foot pad health when analysing the maximum summed AV on the body.

Falls had higher maximum summed AVs (29.21 ±1.09g) compared with non-falls (23.26 ± 0.95g). Movements from total heights 0-0.5m had lower maximum summed AV on the body compared with all total heights between 0.5-2.0m (Z-ratio=-2.879, P=0.0848; Z-ratio=-3.011, P=0.0556; Z-ratio=-3.428, P=0.0131) and those >2.5m (Z-ratio=-3.914, P=0.0020). Total movements height 2.0-2.5m had lower maximum summed AVs on the body compared with total heights >2.5m (Z-ratio=-3.012, P=0.0553) (Figure 2.20).

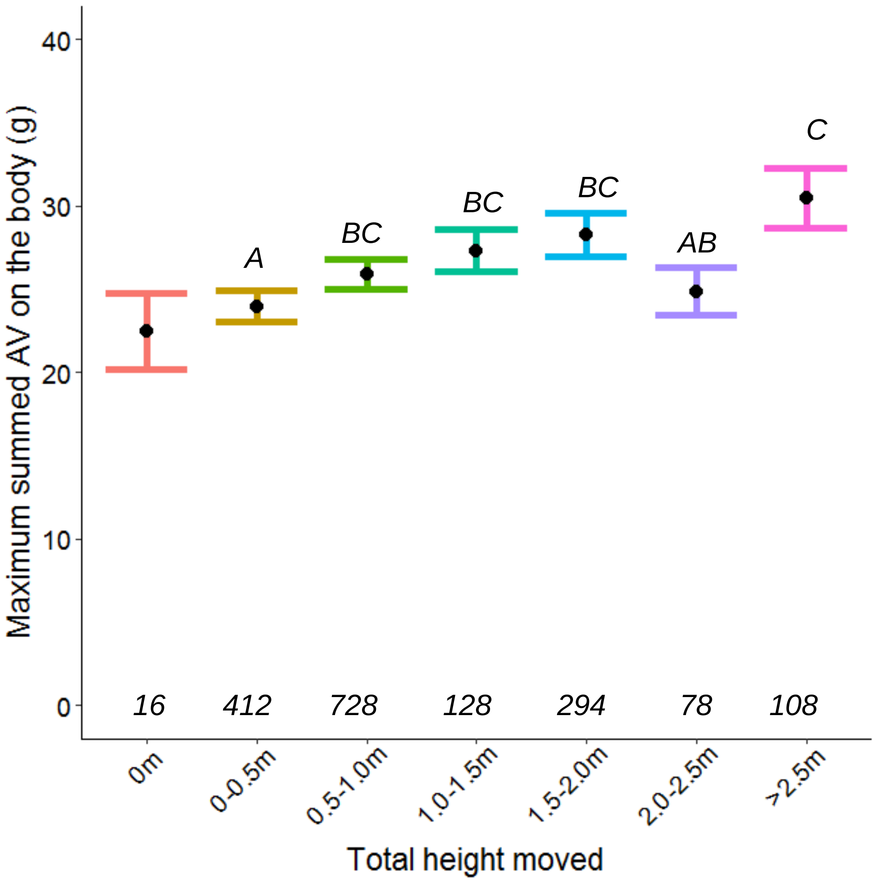

Figure 2.20: *Total height of movement relating to maximum summed AV on the body present as LS means (± SE).*

Sample size is shown at the bottom. Different letters indicate significant differences.

Movements between a structure and the litter has higher summed AV on the body sensor compared with movements between a perch and a structure (Z-ratio= -6.577, P<0.0001), a structure and another structure (Z-ratio=4.130, P=0.0008), a perch and the litter (Z-ratio=-3.334, P=0.0184) and between a perch and another perch (Z-ratio=-3.076, P=0.0447) (Figure 2.21).

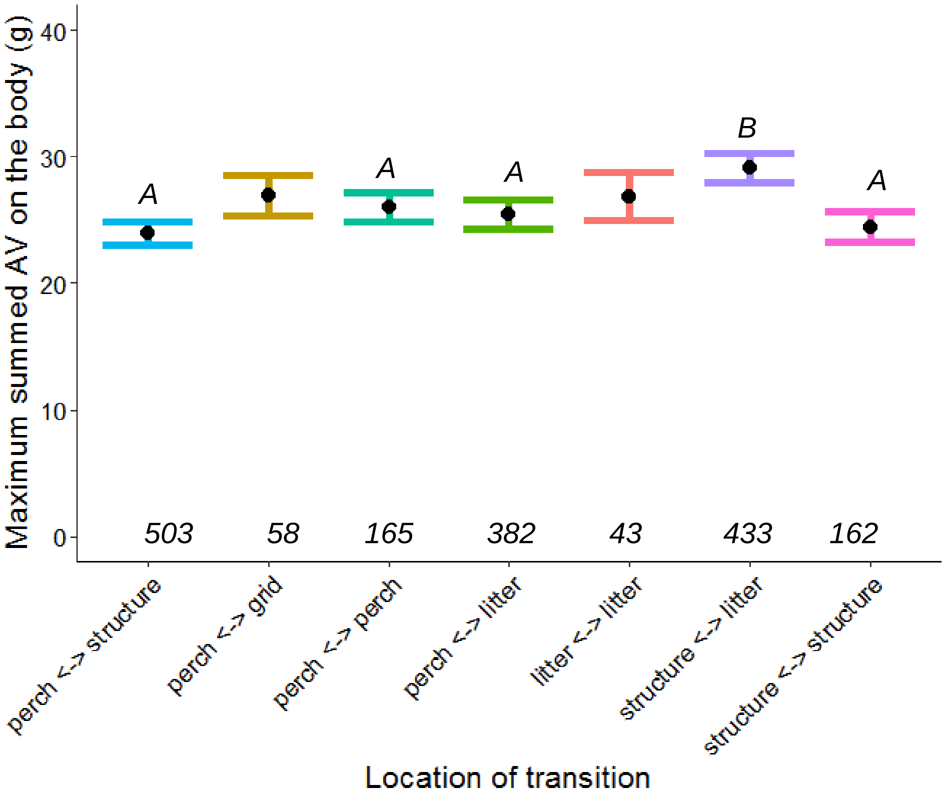

Figure 2.21: *Location of transition (start and end) relating to the maximum AV on the body present as lsmeans (±SE).*

Sample sizes shown on the bottom of the graphs. Different letters show significant differences.

Hens with poor feather condition had lower maximum summed AVs on the body sensor compared with those with excellent feather condition (Z-ratio=4.011, P=0.0002) or good feather condition (Z-ratio = 3.776, P=0.0005). There was no difference between those with excellent feather condition and good feather condition (P>0.01) (Figure 2.22).

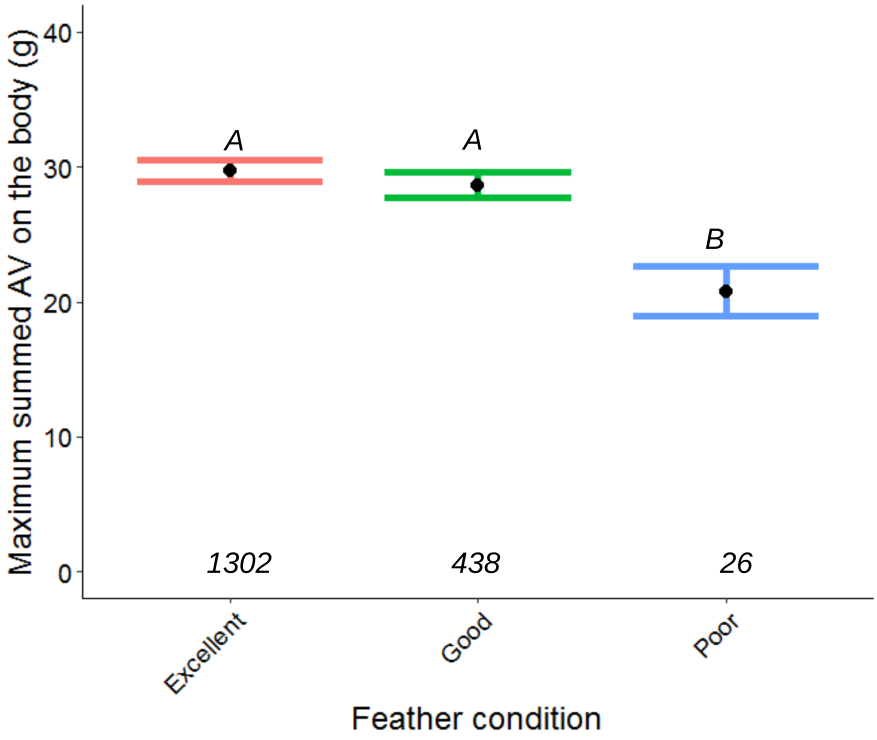

Figure 2.22: *Feather condition in relation to hens with excellent, good and poor feather condition, relating to the maximum AV on the body presented as lsmeans (±SE).*

Numbers at the bottom show sample size and different letters show significance.

2.3.8 Keel bone palpation reliability

The intra-observer reliability of palpation was graded as a perfect score (z = 4.73, Cohen's kappa = 0.834) (McHugh, 2012).

2.3.9. Keel bone palpation accuracy

In April 2015, when comparing fracture severity with a trained assessor, the accuracy was 69.2%, when comparing no breaks and breaks the accuracy was 75.6%. In May 2015 accuracy was compared again using the same 80 hens.

The percentage of fracture severity scores that were the same was 77% and the percentage of no breaks and breaks that were the same was 85.1%. Palpation accuracy was compared again in March 2018 on 30 hens, the severity score matches to the trained assessor were 83.3% and the percentage of no breaks and breaks detected was 93.3%. In May 2018 palpations were carried out on the same 600 hens as a trained assessor one week apart. Out of the 600 hens 158 had fractures in week one (detected by the trained assessor) then in week 2 all the same 158 fractures were detected by the assessor of the current study, with a fracture detecting rate of 100%.

2.4. Discussion

This is the first study that analysed the accelerometry patterns resulting from transitions within a multi-tier system. The current study has shown that different behaviours (falls and collisions), total heights, start and destination areas and, in the case of the summed AV on the body, the plumage condition of hens, can have an impact on the forces experienced by the hen around the keel bone and on the body. The results show that falls, collisions and increasing heights have the potential to increase the energy of interactions experienced by laying hens in multi-tier systems.

2.4.1 Maximum summed AV at the keel

Falls and collisions have been suggested to be the cause of high levels of keel bone fractures in laying hens housed in non-cage systems (Gregory and Wilkins, 1996; Wilkins *et al.*, 2011; Stratmann *et al.*, 2015a). Experimental work using an impact tester on dead hens has shown that greater forces lead to a higher proportion and more severe levels of keel bone fractures (Toscano *et al.*, 2018). Force was calculated using acceleration x body mass (Toscano *et al.*, 2018). Thus, movements that result in greater forces on the hen (i.e., those that result in a higher summed AV), likely pose a greater risk to the hen's skeletal integrity. In the current study falls and collisions had higher summed AVs on the keel compared with non-fall and non-collision movements. This would suggest that falls and collisions increase the likelihood of fracture, compared to non-fall and non-collision movements, and supports prediction **1.** that falls, and collisions would result in higher average AVs and greater average AVs x readout duration than controlled movements.

Furthermore, in the current study, controlled movements (non-falls) that were under 1m, had higher average maximum AVs at the keel compared with heights over 1m. This contradicts findings from previous researchers who found that landing forces were higher at 61cm compared with 41cm in laying hens (Banerjee *et al.*, 2014). However, the previous study did not look at the differences between falls less than 1m and those more than 1m, which makes comparisons difficult. In the current study, there was an increase in AVs again as the height increased to over 2.5m. This did not match prediction **2.** that movements from higher areas in the system would result in higher AV than lower heights. One potential reason is that transitions under 1m may not provide the hen with enough time to ready herself for movement, whereas transitions from greater heights allow the hen to adjust their movement to minimise energy. It may also be that difficult transitions within the housing system i.e. moving upward between perches and between perches and tiers, were under 1m. In the current study, upward movements tended not to exceed 1m. This would mean that heights under 1m include upward and downward movements whereas those over 1m contain solely downward movements (the interaction between total height moved and direction of movement was not controlled for in the analysis). However, previous work contradicts what was shown in the current study. Previous work concluded that hens find downward transitions more difficult to navigate in comparison to upward transition (Scott *et al.*, 1997; Moinard *et al.*, 2004b). Both previous studies only included heights under 1m and had no comparison for movement over 1m that were downward, making it difficult to relate directly to the current study.

Using qualitative observation from videos, when hens moved upwards, they would wing flap vigorously to allow their claws to grip the area they were wanting to reach. Controlled downward movements, at greater heights result in birds using their wings to glide down, rather than vigorously wing flapping; potentially reduce the summed AV at the keel. Previous work on acceleration forces has shown that finches and doves perform twice as many wing beats during landing as during take-off (Provini *et al.*, 2014). The wing flapping that occurred more in upward movements compared with downward movements in the current study may be the reason why maximum summed AVs are high at lower heights because lower heights could be skewed towards upward movements.

It is known that as distance increases between take-off and landing region, the landing becomes less accurate (Moinard *et al.*, 2004a). The previous study attributed this to hens having too high wing loading for their body size (Moinard *et al.*, 2004a). However, for non-fall movements, this did not match what was found in the current study, in that the higher the movement height, the great the maximum summed AV at the keel. Conversely, in the current study, as the total height of a fall increased, the maximum summed AV on the keel increased.

For transitions that included a fall, the maximum summed AV at the keel gradually increased until >2.5m. This result was expected because the higher the height of the fall the more turning during free fall the hen may be doing. This turning motion would result in substantial changes in acceleration across different axes, which would then result in a higher summed maximum AV. Falling from higher heights would also increase the force upon landing, making the height more likely to create a greater impact on the keel, which would correspond to the higher maximum summed AV on the keel in this study (Bertocci *et al.*, 2004).

2.4.2 Readout duration

The readout duration was calculated as this gives a measure of how immediate an impact event occurred. In general, there was a split between falls and controlled movements. If the movement was controlled, hens moved relatively fast and when the movement was a fall the readout duration diverged depending on whether there was a collision or not. If there was a collision, then the readout duration increased as the total height of the movement increased. When there was no collision, the readout duration peaked at total heights of >1.0-1.5m, then steeply declined. Movements that were upward and had collisions had longer readout durations compared with other types of movements. However, this does not match results found previously (Moinard *et al.*, 2004b). Downward movements in the previous study took longer to gain balance compared to upward movements. One reason for this difference may be that the current study was in a semi-commercial environment and hens jumping/flying was influenced by other hens in the system and variable structures within the system. Whereas the study by Moinard et al., (2004b) was carried out in a controlled environment with one hen being studied at a time.

It was expected that if a hen had a fall and a collision then the readout duration would be lower than a hen that had a fall but did not have a collision. The theory behind this assumption was that if a collision occurred this would stop the hen’s movement, however, it appeared to be that the collision prolonged the readout duration. Interestingly, birds that fell from a height above 2 m but did not have a collision had similar readout durations to birds that did not fall. Due to the height of the transitions these birds were potentially able to correct their posture and gain a controlled landing. In line with prediction **2**, movements that were falls/collisions resulted in higher readout durations at higher total heights compared with lower heights. However, for falls/non-collision movements the readout duration increased until 1.5m and then began to steeply decrease in duration as the total height moved increased to >2.5m. This may possible be a result of a fall with a collision ending sooner than a fall without a collision at higher heights because the hen will be in free fall and there is no collision interrupting and prolonging the fall.

2.4.3 Average summed AV at the keel

Falls had lower average summed AVs compared to controlled movements, contrary to prediction **1**. There was a peak of average acceleration at a height of 0.5m-1m for non-fall/collision movements. This peak may be because these movements had short impact durations and the collision may have caused a peak in the acceleration. Therefore, a sharp momentary increase in acceleration accompanied by a short duration may have led to an increased average AV for non-fall/collision movements at heights of 0.5m-1.0m. This result suggest that these movements may be potentially hazardous.

2.4.4 Average summed AV x readout duration

Falls and collisions tended to have higher average summed AV x readout durations compared to controlled movements and the trend stayed the same as the height of movement increased. The result, in line with prediction **1**. was expected because average summed AV x readout duration increases as height increases. This would suggest that falls and collisions are potentially more hazardous than controlled movements and that the hazardous effect becomes stronger as the height increases.

2.4.5 Maximum summed AV related to colliding object

When looking at what hens collided with, and the effect this would have on the maximum summed AV, results revealed that collisions with conspecifics had the lowest maximum acceleration. This makes sense as other hens are more compliant than furnishings in the system and a conspecific can move on their own, which may reduce energy of the impact.

2.4.6 Maximum summed AV and the reason for a fall

A hen being pushed resulted in the lowest maximum acceleration at the keel. This could be because pushes were often seen to be from behind the hens, away from the keel sensor and may not result in the immediate peak in acceleration that may be associated with a collision. This result was in line with prediction **8**, that missed landings would have higher maximum summed AVs at the keel than pushes.

2.4.7 Maximum summed AV on the body

There was a relationship between the maximum summed AV on the body and feather condition. Hens with poor feather condition had a lower maximum summed AV on the body compared to those with good or excellent feather condition. This was contrary to prediction **4**; that hens with poor feather cover would have higher maximum AVs compared to those with excellent feather cover. Hens with poor feather cover have been shown in previous studies to use the free-range environment less than those with good feather cover (Mahboub *et al.*, 2004). This suggests that potentially hens with poor feather cover are less adventurous and would be less likely to take part in hazardous behaviour, meaning there were less likely to take part in a movement that could result in a higher maximum summed AV on the body.

The maximum summed AV on the body provided different results compared with the maximum summed AV on the keel. There was no interaction between total height of the movement and whether there was a fall when looking at results from the body sensor, whereas this interaction was present on the keel sensor. There was an increase in the maximum summed AV on the body as height increased and the maximum summed AV on the body was greater for falls compared with non-falls.

This highlights the importance of attached body-mounted sensors to animals in locations that are closely linked to the behaviour you are wanting to investigate, in this case, the keel. A similar result was seen when accelerometers were used on wild birds, if behaviours of interest involved the head, the accelerometer outputs were more accurate when the device was placed in a neck collar compared to a back pack (Kölzsch *et al.*, 2016). Therefore, for general movements and specific behaviours, such as dustbathing, body mounted accelerometers may be an appropriate option. In the current study, the keel sensor is likely to be more relevant because the aim of the study was to detect falls and collisions.

2.4.8 Limitations of the study

It is important to note that all the data comes from a small number of hens within each pen. It is possible that due to behavioural differences, or indeed similarities, that the range of behaviours shown is limited. Also, even though individual birds were used as a nested factor within pen, it is also possible that the same hens may find certain navigation paths difficult. This would mean that high accelerations may have always originated from the same individuals. Therefore, future studies would benefit from looking at individual differences between hens. Indeed, the data generated from this study could also be used to look at this. This represents a future beneficial use of the data presented here.

A limiting factor of the study was that hens had to wear vests to hold the accelerometers in order to record the acceleration outputs during the different behaviours. Attaching extra equipment may weigh the hen down and cause her to act differently with the equipment than without. A previous study looked at the behaviour of laying hens immediately after equipped with a backpack and up to 3 days after backpack placement (Buijs *et al.*, 2018). What they found was that the backpack tended to affect the hens immediately after placement but effects became more subtle and had limited significance as the time after placement progressed (Buijs *et al.*, 2018). Another study by Daigle *et al.* (2012) suggests that behaviour may be affected up to 48 hours after backpacks were placed on hens. Therefore, for future studies it would be beneficial to have a longer period of acclimatisation to the accelerometers or vests. However, this would only be possible with the improvement in battery life of these devices. If future studies could use more devices this would allow this to be achievable, in the current study there were only 15 accelerometers available meaning that they were needed each week, making the acclimatisation period limited. A method of remotely downloading the data while the hen is wearing an accelerometer would be the ideal situation. This would mean that checks on whether the equipment is working correctly could be made, otherwise, equipping hens with accelerometers, just to realise that they were not recording for weeks would set back experimental time.

2.5. Conclusion

This study shows that it is possible to use accelerometers to understand more about how animals interact with their environment and the technology can aid in improving housing design for laying hens. This is the first study that has shown that both falls, collisions and increasing heights result in higher maximum AV at the keel bone of laying hens. Previously, research has shown this in experimental conditions (Toscano *et al.*, 2018) and has theorised that falls, collisions and increasing the heights of systems in practice would result in an increased energy at the keel. The current study is the first step in understanding the forces that may be exposed to laying hens in commercial conditions.

Future work should look at the change of keel bone fractures and foot pad health over time to determine whether an effect can be seen in those hens. It may be more relevant to look at how individual behaviour varies over time as they accumulate health issues instead of comparing differences in behaviour between different individuals. In particular, different individuals may have different coping styles (Koolhaas *et al.*, 1999).

A more targeted study at the causal nature of falls and collisions on fracture occurrence would be worthwhile. This could be achieved by directly monitoring hens that have a fall or a collision (when wearing an accelerometer) to determine whether high summed AVs do indeed cause keel bone fractures.

Chapter 3

Navigation paths in a multi-tier system: Controlled movements and falls

3.1. Introduction

Multi-tier systems give hens the choice to roost high in the system as well as the opportunity to jump and fly and carry out more species-specific behaviours such as foraging and dustbathing in comparison to cages (Tanaka and Hurnik, 1992; Colson *et al.*, 2007). However, due to the increased total height in the environment birds can fall from heights above 2m. As hens fall, they can collide with perches and other structures. The higher the hens are when they fall, the more likely they are to collide with an object through the multi-tier system as there are more structures to encounter (compared with falls from shorter heights). The higher the fall the more forceful the impact is likely to be. These collisions are thought to be the cause of keel bone fractures (Gregory and Wilkins, 1996), which may explain their increased prevalence in multi-tier systems (Wilkins *et al.*, 2011).

Falls occur when hens attempt to move from one structure in a system to another. However, falls can occur at any area within a system with more structures. In one aviary study, falls occurred more when hens were moving towards perches compared to when they were moving to the litter (Campbell *et al.*, 2016c). This may be because perches have smaller landing areas compared with structures or the litter, so are more likely to result in falls. However, the previous study was only done in one commercial aviary and video recordings were made throughout the lights-on period, observing the whole flock. No one has ever looked at the locations of falls during lights off and followied individual hens for consecutive days. Movements between the litter and lower tier and movements between a nest box rail and perches have been described as the most common movement (Carmichael *et al.*, 1999). They also found that approximately 50% of the flock were on the structure at all times (Carmichael *et* *al.*, 1999). This suggests that laying hens prefer to be elevated and it is important that the system is designed in such a way to facilitate movement to prevent keel bone breaks during movement to elevated structures. However, these authors only observed the hens during the daytime. The dusk phase, when lighting is gradually dimmed, may be a particularly relevant phase to include as many hens will move upward into the system to roost, resulting in a high risk of falls.

Furthermore, low light levels (anything under 10lux) (DEFRA, 2015) during the dimming (dusk), night and dawn phase may make it more difficult for hens to move through the system due to the inability to see structures. It has been shown that low light intensity increases an individual hens latency to jump (Taylor *et al.*, 2003), suggesting that navigation is more challenging during low light intensities. Stratmann *et al.* (2015a) looked at movements by hens during the dusk phase and whether the use of different structures within the system reduced the number of falls and collisions during dusk. It was found that by adding in ramps and platforms falls were reduced compared to the same system without the addition of ramps or platforms.

The morning can also be expected to be a period with an increased risk of falls, as perches on the top level and (pathways leading to) nest boxes may become overcrowded due to behavioural synchrony as hens lay eggs in the morning (Odén *et al.*, 2002; Schrader and Müller, 2009; Collins *et al.*, 2011; Brendler *et al.*, 2014; Brendler and Schrader, 2016). This overcrowding can be expected to lead to obstructed take-offs and landings, which can lead to falls (Moinard *et al.*, 2005; LeBlanc *et al.*, 2016). However, it is presently unknown if falls occur more near nest boxes in the morning.

Due to 50% of hens being on the top tier of a multi-tier system at any one time (Carmichael *et al.*, 1999) and hens roosting high in the system at night (Brendler and Schrader, 2016), these areas can become overcrowded. The percentage of falls (the number of falls/total number of movements x 100) from the top tier has the potential to be higher compared to the middle or lower tiers in a system with elevated perches because of the large volume of hens that are often present in the top tier.

Acute angles have the potential to cause a greater percentage of falls compared to wide angles. This is based on previous evidence that steep angles are difficult for hens to negotiate compared to other areas within the system (Scott *et al.*, 1997). In addition to the angle, the direction of movement towards the angle may influence the percentage of falls. Downward movements would result in more falls compared with upward movements, based on the previous evidence that downward movements are more difficult for hens to navigate than upward movements (Moinard *et al.*, 2004b).

As well as the system, the health of the hen may affect the number of times a fall occurs. Hens with keel bone fractures may have a higher incidence of falls compared to those without keel bone fractures, due to the potential pain and increased latency to move from perches in hens with keel bone fractures (Nasr *et al.*, 2012b; Nasr *et al.*, 2013a; Nasr *et al.*, 2015). Previous work has shown that hens with footpad lesions and poor feather cover move from perches earlier than healthy birds (LeBlanc *et al.*, 2016), this could lead to more falls as movements are less planned. Hens with foot pad lesions, such as bumblefoot of foot pad dermatitis are likely to be in pain and this may influence how the hen moves (Weitzenbürger *et al.*, 2006). However, it is possible that all these health parameters have the opposite effects, in that hens with keel bone fractures, footpad lesions and poor feather condition are less likely to move from/to perches or tiers and therefore, are less likely to have a fall or a collision.

3.1.1. Aims and predictions

The main aim of this study was to identify hazardous areas within a commonly used multi-tier system (Bolegg Terrace). Specific predictions were formulated to facilitate the identification of potential improvements to such systems that would reduce falls. To identify hazardous pathways, the prevalence of falls compared to controlled movements within each pathway and frequency of use of each pathway are needed.

The main predictions were:

1. Navigation paths in the top tier of the system would have a higher prevalence of falls compared to those in the lower tier of the system.
2. Navigation paths with steeper inclines mainly between the 2nd perch and the 3rd perch would result in a higher percentage of falls compared with more acute inclined navigation paths.
3. Downward movements would result in more falls compared with upward movements.
4. Landings and take-offs from perches would result in a greater percentage of falls compared with landings and take-offs from other structures in the system.

5. A greater percentage of falls compared with total movements would occur at dawn and dusk compared to other times of day.
6. Keel bone fractures, footpad lesions and poor feather cover may either result in a higher percentage of falls compared with total movements, or a lower percentage of falls compared with total movements.

3.2. Materials and Methods

The results in this Chapter follow on from those discussed in Chapter 2. Ethical approval, animal husbandry and health recordings of all individuals were the same as in Chapter 2. The hens used were the same as those in Chapter 2, with the same protocol being used for the attachment of accelerometers because the data was collected from the same accelerometers that were used in Chapter 2.

3.2.1. Breakdown of data

The data in this chapter are taken from two different methods of analysis. One of these is the data that was generated from focal hens that were wearing accelerometers and is described in the headings as: “related to accelerometry output – focal hens only”. The second type of data is pen level, meaning that video observations of movement between tiers was taken from the pen as a whole and not only generated from hens that were wearing accelerometers. This data is always refered to as: “non-accelerometry data – observations on a pen level”.

The dataset used in related to accelerometry output from focal hens only represents 1517 data points and corresponds to certain pathways that are shown by the blue arrows in Figure 3.1 and represents 85.9% of the total dataset used in Chapter 2 of this thesis.

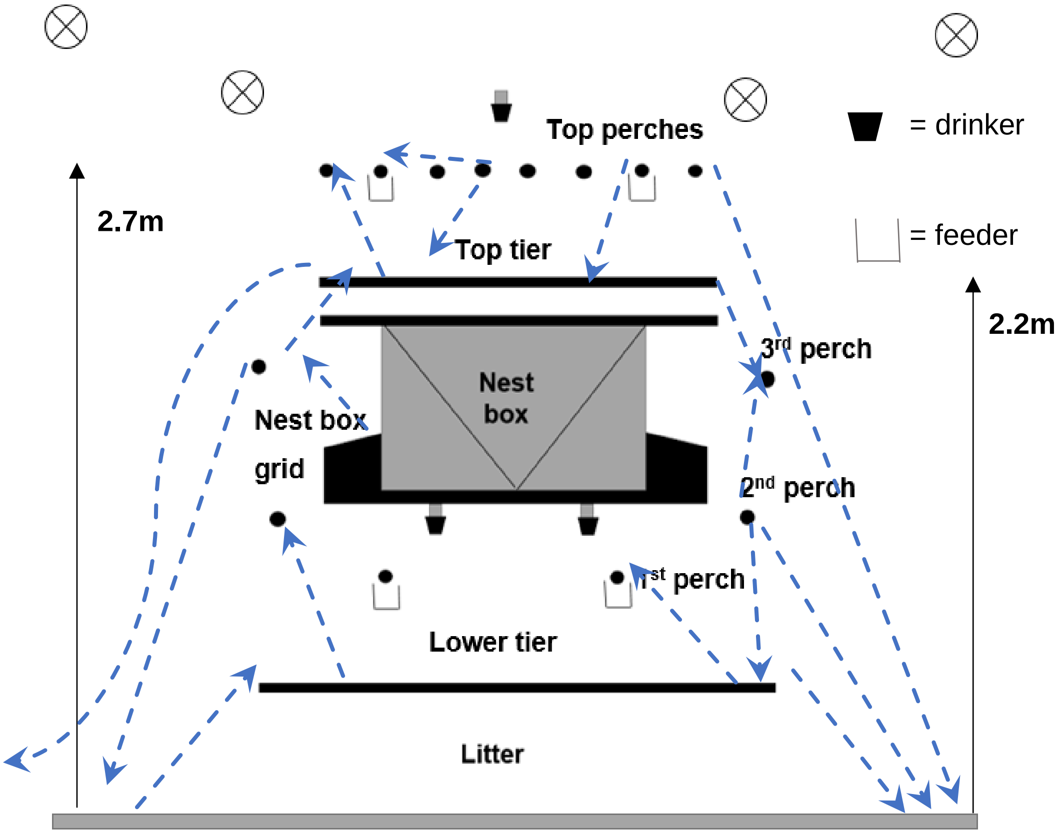

Figure 3.1: *Schematic of the side view of a Bolegg Terrace multi-tier system with the navigation paths used in all the analysis.*

All labels refer to areas of the system named later in the text. Feeders and drinkers have been added and camera placement is noted using circles with crosses. Top perch and tier heights have been labelled within the system. Perch names labelled on the right-hand side of the figure are also present on the left-hand side of the figure.

3.2.2. Navigation path (related to accelerometry output – focal hens only)

Once an accelerometry output was recorded, the videos were analysed to determine what behaviours and/or movements the focal hen was doing at that time. This subset of data was then analysed to determine the number of falls compared to controlled movements in each navigation path. This resulted in a percentage of falls (fall/all movements x 100) that could be used to compare the percentage of falls between navigation paths.

Movements between different areas of the system were classed as “navigation paths”. The starting area was defined as where the hen was at the beginning of the movement and the end was where the hen landed first. If the hen then moved further, this was not included in the navigation pathway. To be defined as a navigation path for all the analysis, the movement had to occur more than ten times on the accelerometer. Figures 3.1 shows all the navigation pathways included in the analysis. In contrast to the observer analysis of movements described in the next paragraph, navigation paths depended on direction: e.g. movements from the 3rd perch to the litter were distinguished from movements from the litter to the 3rd perch. This was to understand in more detail the navigation paths hens were using and the percentage of falls in each navigation path compared to total movements.

3.2.3. Navigation paths – Multiple comparison groupings (<u>related to accelerometry output – focal hens only</u>)

From the video data, because the navigation path was known it was possible to determine: 1. The starting area of the movement (and subsequent height), 2. The first landing area of the movement (and subsequent height), 3. Whether the first part of the movement was upward or downward. Due to each accelerometer creating a time stamp, it was possible to match the accelerometry output with the time of day.

3.2.4. Reason for fall (related to accelerometry output – focal hens only)

When viewing the video data (to pair behaviour with an accelerometry event), if a bird had a fall it was split into different categories. They are discussed in Chapter 2 and include:

1. Missed – The focal hen shows intentional behaviour, indication movement to a specific location but lands in another location
2. Slipped - Focal hen does not intend to change position but does change position
3. Pushed - Focal hen knocked from the area they are currently occupying by a conspecific and are displaced to another location
4. Interaction- Contact with a conspecific that can constitute; scratching, grabbing, climbing onto and chasing. Also refers to a panic reaction in the system

3.2.5. Extrapolation of accelerometer data to represent 200 hens (related to accelerometry output – focal hens only)

The total number of hours of video recorded for each time point was divided by the number of hens that had generated usable data from the accelerometers, this was 271 hens (because some hens were used more than once). The total number of useable hours collected from the accelerometers was 1740 hours and 45 minutes (13 hours and 40 minutes of dawn, 21 hours and 5 minutes of dusk, 747 hours and 5 minutes of night and 958 hours and 55 minutes of the day). The reason that the number was calculated from focal hen movements and not pen level movements was is that pen level data consisted of hens during a 4-hour period and included all movements in the navigation path. Whereas, the focal hen data takes movements into account if they exceed the predetermined summed acceleration vector (AV) threshold (as discussed in Chapter 2). This was also done so that the number of times events were recorded on the accelerometers per path could be compared to the number of movements hens made in "real time" within the same navigation paths. The number of movements represents the number of movements by 200 hens. Two-hundred hens were chosen instead of per hen because the number per hen was extremely low making it difficult to interpret and there were 200 hens in each pen, so 200 hens represent one pen.

3.2.6. Movements within a 4-hour period (non-accelerometry data – observations on a pen level)

To determine the use of individual navigation pathways the movement of all 200 hens (without accelerometers) in one pen were monitored in Observer

10XT (Noldus, the Netherlands). Any upward or downward transition was recorded, where the hen started and finished on a different structure in the system. Navigation pathways were not split into upward and downward movements, they were analysed in either direction. This means that movements from the lower tier to the litter were classified into one category, litter to lower tier, and were given a score representing how many hens used that one pathway. This is slightly different in how navigation path (for birds with accelerometers) in section 3.2.2. was analysed.

At 33 weeks of age all hens in one pen were observed at four-time points between 0130-0230hr, 0600-0800hr, 1200-1400hr, 1630- 1730hr, respectively. The dawn period (lights gradually turning on) was from 02:00-02:10hr, Lights on (day) ran from 02:10-16:40hr, the dimming period (dusk) ran from 1640-1700hr and the light-off period (night) ran from 1700-0200hr. In the 06.00-08.00 and 12.00-14.00 sessions, movements were recorded for five minutes and then the recording was skipped for five minutes. This was to try and get an even spread of day-time hours. In the 01.30-02.30 and 16.30-17.30 sessions movements were monitored continuously to capture the entire dawn and dusk period, as well as the time immediately before and after. This was considered important because more movements were predicted during the dawn and dusk periods. The reason this analysis was done was to get an idea of the frequency of use of each navigation path, that was used in analysis section 3.2.2,

3.2.7. Statistical and descriptive analysis

3.2.7.1. Navigation path (<u>related to accelerometry output – focal hens only</u>)

All statistical analysis was carried out using R (R Core Team, 2017) with R Studio as the interface (RStudio Team, 2016). The model contained fall (yes/no) as the response variable. Fixed effects included navigation path (factor), time of day (factor), flock (binary). Bird nested in pen was in the model as a random factor, with individual hen being the experimental unit. A stepwise regression was used to determine the significance of each factor in the model to determine the best fitting model. AIC was also used as an indication into the model fit. The model was then checked using the blmeco package (Korner-Nievergelt *et al.*, 2015) for overdispersion and was found to meet the parameters of the model. The optimx package (Nash, 2014) was used to optimise the model in order to deal with any convergence issues. Other parameters such as; keel bone fracture presence, foot pad dermatitis presence, bumblefoot presence, body mass and feather condition were used in the original model but removed from the final model due to non-significance.

3.2.7.2. Multiple comparison testing

The analysis was first done comparing all navigation paths to each other using Bonferroni corrections. Then comparisons between different groups of data were made. The comparisons were: 1. Starting area of the movement (and subsequent height), 2. The first landing area of the movement (and subsequent height), 3. Whether the first part of the movement was upward or downward. The movements were grouped within R, using the lsmeans package (Lenth, 2016), and analysed using Bonferroni corrections.

3.2.7.3. Reason for fall (related to accelerometry output – focal hens only)

Data regarding the reason for falls was looked at but only descriptively. The data are presented here and shows the reasons hens had falls data collected from the accelerometers but has been corrected to represent per hour per 200 birds. The falls included come from the accelerometry data and relate to the navigation paths discussed in this chapter.

3.2.7.4 Movements within a 4-hour period (non-accelerometry data – observations on a pen level)

Movements within a 4-hour period have been are shown as descriptive statistics only and have been corrected to show the number of movements per hour per 200 hens.

3.3. Results

There was a statistically significant difference between the percentage of falls relating to navigation path (P<0.0001), time of day (P<0.0001) and flock (P=0.0055). All other factors (keel bone fracture (Y/N), feather condition (excellent, good, poor), bumblefoot (Y/N), foot pad dermatitis (Y/N) and weight) were not significant (P>0.05) and were removed from the final model.

3.3.1. Navigation paths (<u>related to accelerometry output – focal hens only</u>)

There was a difference in the percentage of falls between navigation paths (W=7.71, P=0.005; Figure 3.2). All relevant Bonferroni comparisons are shown in Table 3.2. Only those that reach significance are shown, due to many comparisons, trends are not shown.

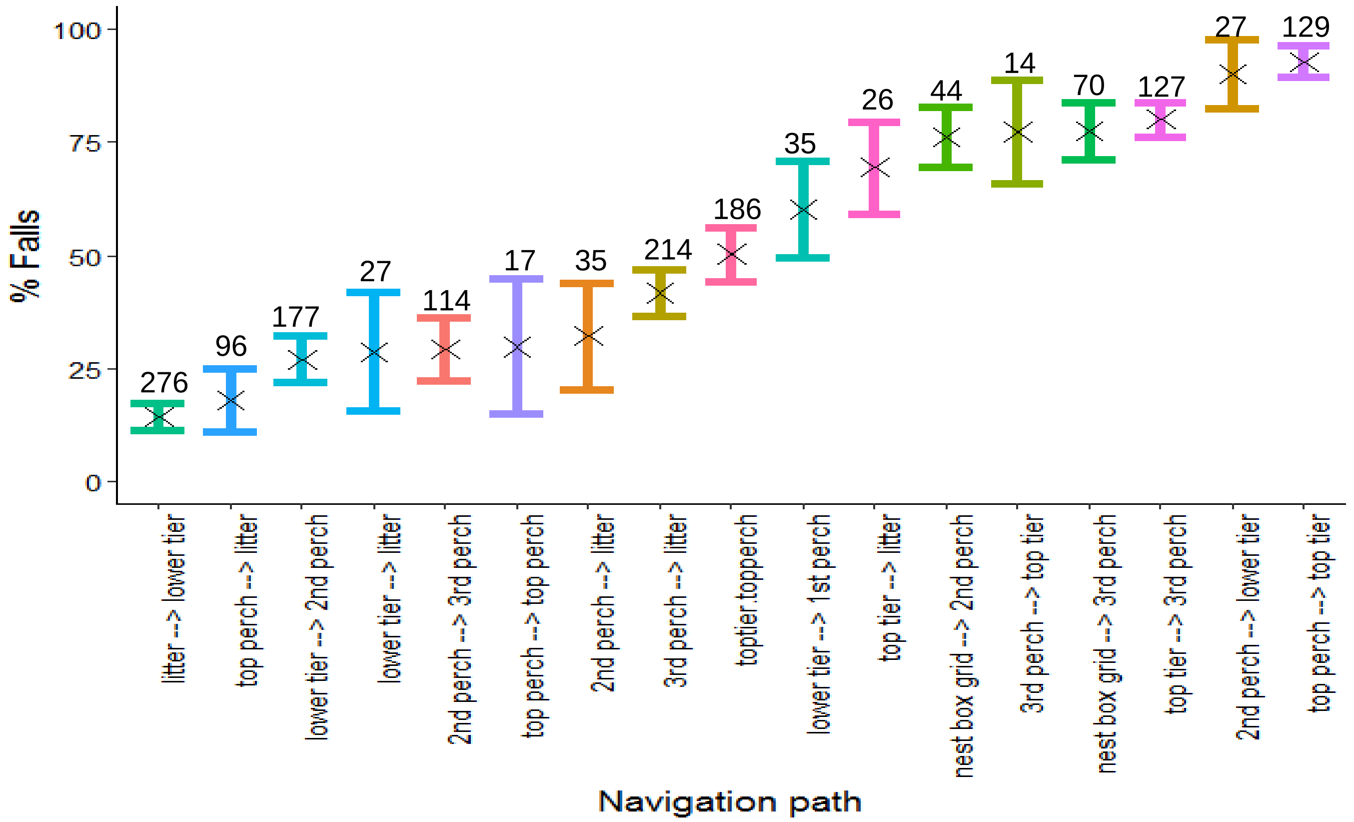

***Figure 3.2** The percentage of falls relating to each navigation pathway (lsmeans ± SE). Each navigation pathway is presented in ranked order from least falls to most falls.*

Data presented here represent movements recorded from accelerometers only. The number of times each navigation path was recorded on the accelerometers are shown above each navigation pathway.

Table 3.1: *Results from Bonferroni multiple comparisons relating to the navigation path (from birds recording accelerometry readings).*

Comparison of navigation paths	**Z-ratio (test statistic)**	***P* value**
2nd perch to 3rd perch vs nest box grid to 2nd perch	-4.382	0.0016
2nd perch to 3rd perch vs nest box grid to 3rd perch	-4.507	0.0009
2nd perch to 3rd perch vs top perch to top tier	-5.711	<.0001
2nd perch to 3rd perch vs top tier to 3rd perch	-6.048	<.0001
2nd perch to litter vs top perch to top tier	-4.445	0.0012
2nd perch to litter vs top tier to 3rd perch	-3.722	0.0268
2nd perch to lower tier vs litter to lower tier	4.584	0.0006
2nd perch to lower tier vs lower tier to 2nd perch	3.656	0.0349
2nd perch to lower tier vs top perch to litter	3.852	0.0160
3rd perch to litter vs nest box grid to 2nd perch	-3.806	0.0192
3rd perch to litter vs nest box grid to 3rd perch	-3.964	0.0100
3rd perch to litter vs litter to lower tier	4.831	0.0002
3rd perch to litter vs top perch to top tier	-5.273	<.0001
3rd perch to litter vs top tier to 3rd perch	-6.235	<.0001
3rd perch to top tier vs litter to lower tier	4.405	0.0014
Nest box grid to 2nd perch vs litter to lower tier	7.085	<.0001
Nest box grid to 2nd perch vs lower tier to 2nd perch	5.173	<.0001
Nest box grid to 2nd perch vs top perch to litter	4.638	0.0005
Nest box grid to 3rd perch vs litter to lower tier	7.459	<.0001
Nest box grid to 3rd perch vs lower tier to 2nd perch	5.372	<.0001
Nest box grid to 3rd perch vs top perch to litter	4.737	0.0003
Litter to lower tier vs lower tier to 1st perch	-4.552	0.0007
Litter to lower tier vs top perch to top tier	-7.584	<.0001
Litter to lower tier vs top tier to 3rd perch	-9.964	<.0001
Litter to lower tier vs top tier to litter	-5.042	0.0001
Litter to lower tier vs top tier to top perch	-5.727	<.0001
Lower tier to 2nd perch vs top perch to top tier	-6.194	<.0001
Lower tier to 2nd perch vs top tier to 3rd perch	-7.450	<.0001

Lower tier to litter vs top perch to top tier	-4.275	0.0026
Top perch to litter vs top perch to top tier	-5.897	<.0001
Top perch to litter vs top tier to 3rd perch	-5.780	<.0001
Top perch to litter vs top tier to litter	-3.584	0.0460
Top perch to top perch vs top perch to top tier	-3.905	0.0128
Top perch to top tier vs top tier to top perch	4.608	0.0006
Top tier to 3rd perch vs top tier to top perch	4.904	0.0001

Each location analysed can be seen in Figure 3.1. The first navigation path is always compared to the 2nd navigation path indicated by "vs". A positive Z-value indicated that the first navigation path has a higher percentage of falls than the 2nd navigation path. A negative Z-value indicates that the first navigation path has a lower percentage of falls compared to the 2nd navigation path. Only statistically significant results are shown.

Movements from the litter to the lower tier had the lowest percentage of falls and those from the top perch to the top tier had the highest percentage of falls (Figure 3.2). Movements from the 2nd to the 3rd perch represent the steepest angle in the system (75°) and was predicted to be the most hazardous pathway in the system. However, movements from the 2nd perch to the 3rd perch resulted in a lower percentage of falls compared to movements around the nest box and the top tier region (Figure 3.2). There is a large variation in the number of times navigation paths were recorded on the accelerometers, ranging from 14 times for movements from the 3rd perch to the top tier to 276 times for movements from the litter to the lower tier (Figure 3.2). Navigation paths that had a low number of accelerometer readouts associated with them, such as 3rd perch to the top tier, top perch to top perch and top tier to the litter appeared to show differences between other navigation paths graphically (Figure 3.2). However, because of the small sample size for these navigation paths and many multiple comparisons, statistical significance was not usually reached.

3.3.2. Navigation paths (comparing accelerometry output from focal hens and non-accelerometry movement at the pen level)

The number of accelerometer readouts per hour per 200 birds was always lower than that found when hens were not equipped with accelerometers (Table 3.2), because the latter represents all movements, whereas the former is a subset of movements. However, some of the navigation paths are consistently high in frequency. For instance, the litter to the lower tier occurred 0.117 times per hour per 200 birds when just considering movements with an accelerometry reading compared with 441.25 times per hour for all movements along this pathway. It should be noted that when hens were not wearing accelerometers navigation paths were recorded in both directions (litter to lower tier and lower tier to litter), whereas those with accelerometers were only recorded in one direction (litter to lower tier). This was because the accelerometry data was looked at in detail and was the main aim of the current study. Once accelerometry data was collected it was considered important to know the frequency of movements within each navigation path. It was considered more important to know how often a navigation path is used in total rather than whether the movements are upward or downward in that navigation path.

Table 3.2: *Navigation paths and the number of times movements that were recorded both on and off accelerometers*

Path	**Number of times hens moved in this path (one way) /200 birds/ hour (accelerometer data)**	**Total number of times hens moved in this path (both ways) /200 birds/ hour (non-accelerometer data)**
litter – lower tier	0.1170	441.25
top perch – litter	0.0407	4.25
lower tier – 2nd perch	0.0750	47.5
lower tier – litter	0.0114	441.25
2nd perch – 3rd perch	0.0483	10.75
top perch – top perch	0.0072	385.25
2nd perch – litter	0.0148	34.5
3rd perch – litter	0.0907	48.75
top tier – top perch	0.0789	174.5
lower tier – 1st perch	0.0148	91
top tier – litter	0.0110	0.5
grid NB – 2nd perch	0.0187	135
3rd perch – top tier	0.0059	129.25
grid NB – 3rd perch	0.0297	81.5
top tier – 3rd perch	0.0538	129.25
2nd perch – lower tier	0.0114	47.5
top perch – top tier	0.0122	174.5

In each of the columns, the figure shown is the number of movements per 200 hens per hour. Some duplicated are present in column 2 because the number of movements in each navigation path recorded without accelerometers was taken in either direction. Those from column 1 were in one direction.

However, there are also times when the proportions do not match. Movements from 3rd perch to litter occur seldomly on the accelerometers (0.0059 times per hour per 200 birds) but a high number of times when hens are not wearing accelerometers (129.25 times per hour per 200 hens) (Table 3.2). This discrepancy may be explained when looking at movements from the top tier to the 3rd perch, which resulted in 0.0538 recordings per hour per 200 birds, this was the 6th highest reading for hens wearing accelerometers (Table 3.2). Hence, the relative proportions between the data sets are dependent on whether that pathway is associated with high or low accelerometer readings.

The top perch to the top perch is an example of when the number of times recorded on the accelerometer is much lower (0.0072 – 2nd lowest) compared to the number without accelerometers (385.25 – 2nd highest) (Table 3.2). This may represent a navigation path of little concern (in terms of hazard) due to the absence of movement vigorous enough to cause an accelerometer output. Top perch to litter had a relatively frequent number of recordings on the accelerometers (0.0407 – 7th highest) but a relatively low frequency recorded without accelerometers (4.25 – 2nd lowest) (Table 3.2). Navigation paths like these may not pose much of a risk because they are either seldom used or are less likely to result in a high energy behaviour. The data indicate that movements like the top perch to the litter may often result in high energies but are seldom used. The pathways of greatest concern are those that are frequently used and demonstrate high accelerometry readings.

3.3.3. Navigation paths – Multiple comparison groupings (<u>related to accelerometry output – focal hens only</u>)

3.3.3.1. Relationship with the starting area of the movement

The percentage of falls were different depending on the starting location of the movements. Movements starting on the nest box grid had a higher percentage of falls than those starting on a tier (Z-ratio=3.735, P=0.0011), a perch (Z-ratio=-3.178, P=0.0089) or the litter (Z-ratio=9.065, P<0.0001) (Figure 3.3). Compared to movements starting from the litter; those from a tier (Z-ratio=-6.883, P<0.0001) or a perch (Z-ratio=6.548, P<0.0001) had a higher percentage of falls.

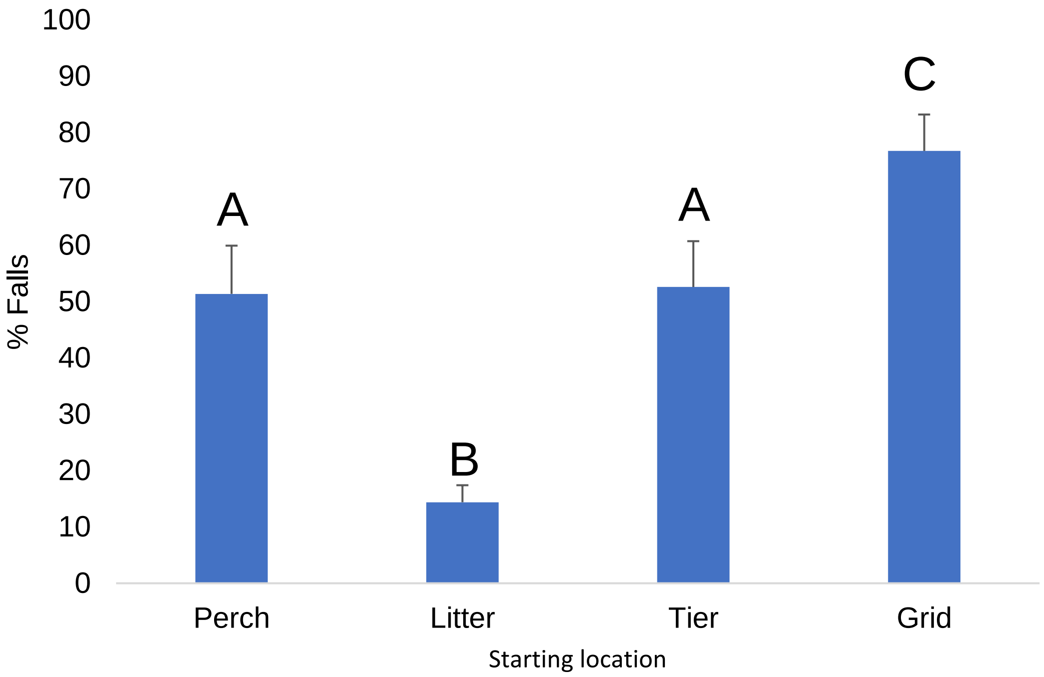

Figure 3.3: Starting location and the percentage of falls (compared to all movements). Different letters signify statistical difference (lsmeans ± SE).

3.3.3.2. Relationship with starting height of the movement

Falls were more likely to occur when the starting location of the transitions was over the nest box compared to below the nest box (Z-ratio=3.901, P=0.0001; Figure 3.4).

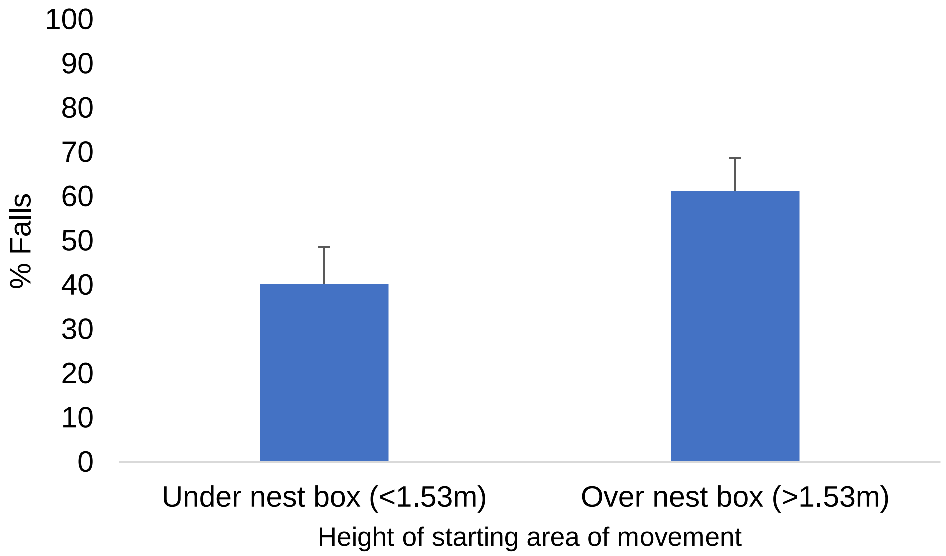

Figure 3.4: *Percentage of falls and whether the starting area of the movement was above or below the nest box (lsmeans ± SE).*

3.3.3.3. Relationship with the landing area of the movement

When looking at differences between the first landing destination or the intended landing destination, there was a significant difference between regions (Figure 3.5). This data incorporates when a hen wanted to land on a structure or the litter and did land on a structure or the litter. It also includes if a hen intended to land on an area but did not land on that area. Therefore, successful movements and unsuccessful movements are grouped together. The first landing area could either be a result of an intentional movement or the first area the bird contacts after a push or a slip.

Landings (or attempted landings) on a tier had a higher percentage of falls compared to those on perches (Z-ratio=2.643, P=0.0246) and the litter (Z-ratio=4.332, P<0.001). Landings on perches had a higher percentage of falls compared to those on the litter (Z-ratio=-2.845, P=0.0133).

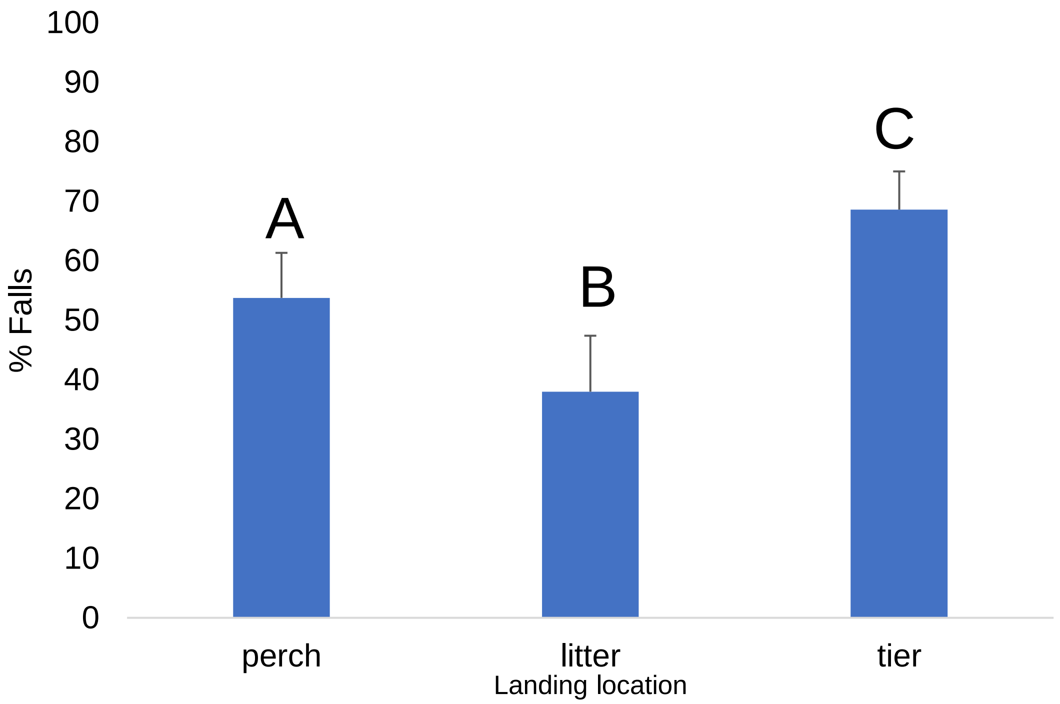

Figure 3.5: *The first (or intended) landing area and the percentage of falls compared to controlled movements that correspond to that landing area (lsmeans ± SE).*

Different letters signify the statistical difference. The grid is not included because the grid never appeared as a landing area in the accelerometry analysis.

3.3.3.4. Relationship with end height of the movement

The end height of the movement (the height of the first landing position of the hen) showed a significant difference in the percentage of falls depending on whether the landing was above or below the nest box (Z-ratio=3.568, P=0.0004; Figure 3.6).

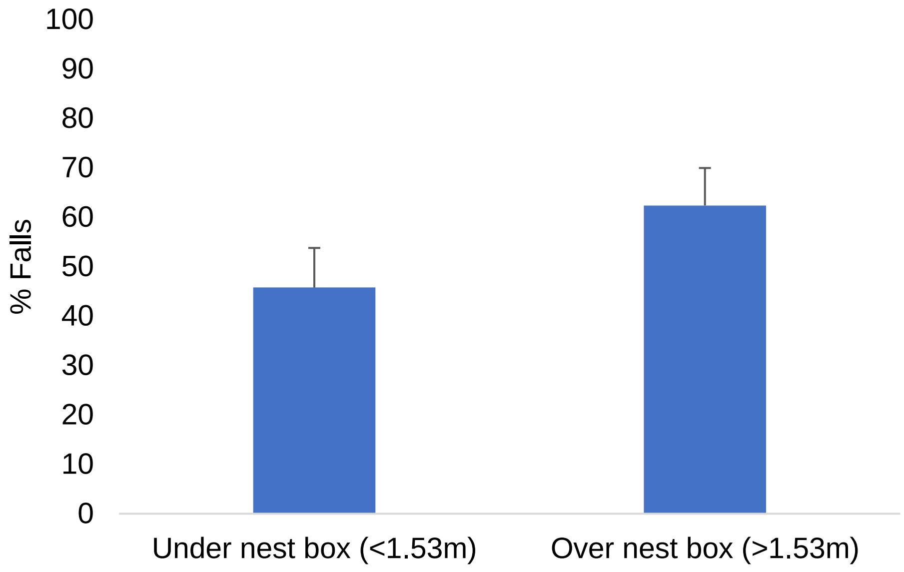

Figure 3.6: Percentage of falls and whether the first contact point or the intended destination was above or below the nest box grid (lsmeans ± SE).

3.3.3.5. Relationship with an upward or downward direction of the movement

When hens were moving downwards (including intentional and non-intentional movements) there was a significantly higher percentage of falls compared to upward movements (Z-ratio=-2.827, P=0.0047; Figure 3.7). However, upward movements only contained intentional movements because a fall or a push could not result in an upward movement, which may bias the data.

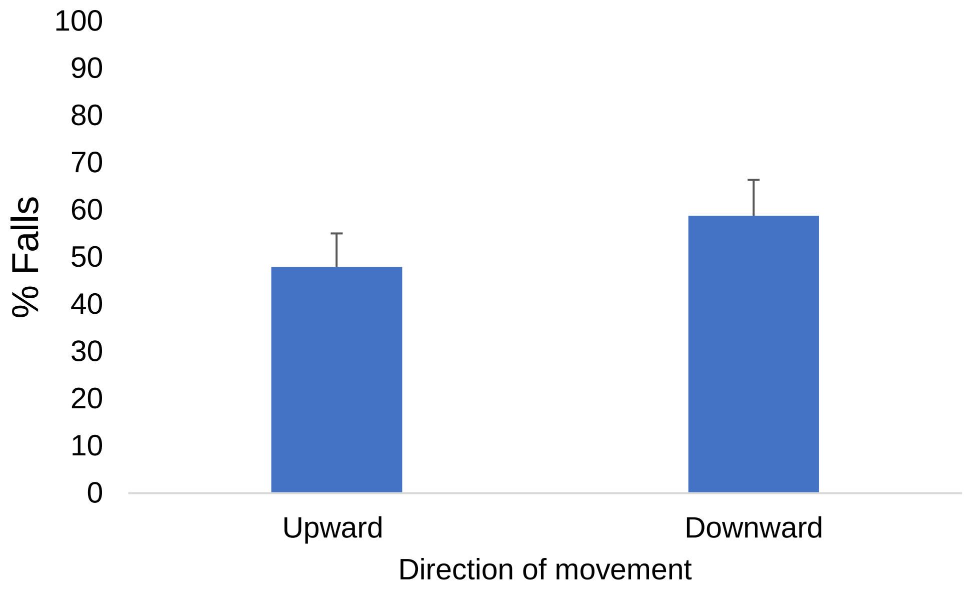

Figure 3.7: The percentage of falls that occur when the first part of the movement was either upward or downward (lsmeans ± SE).

3.3.3.6. Relationship with time of day

There was a significant difference between the time of day and the percentage of falls for a given navigation path (W=145.43, P<0.0001; Figure 3.8). There was a higher percentage of falls during the night compared with the day (Z=-10.589, P<0.0001), dawn (Z=-4.716, P<0.0001) and dusk (Z=-2.704, P=0.0411; Figure 3.8). There was a higher percentage of falls during dusk compared with the day (Z=-8.551, P<0.0001) and dawn (Z==3.022, P=0.0150). No significant differences were seen in the percentage of falls between day and dawn (P>0.05; Figure 3.8).

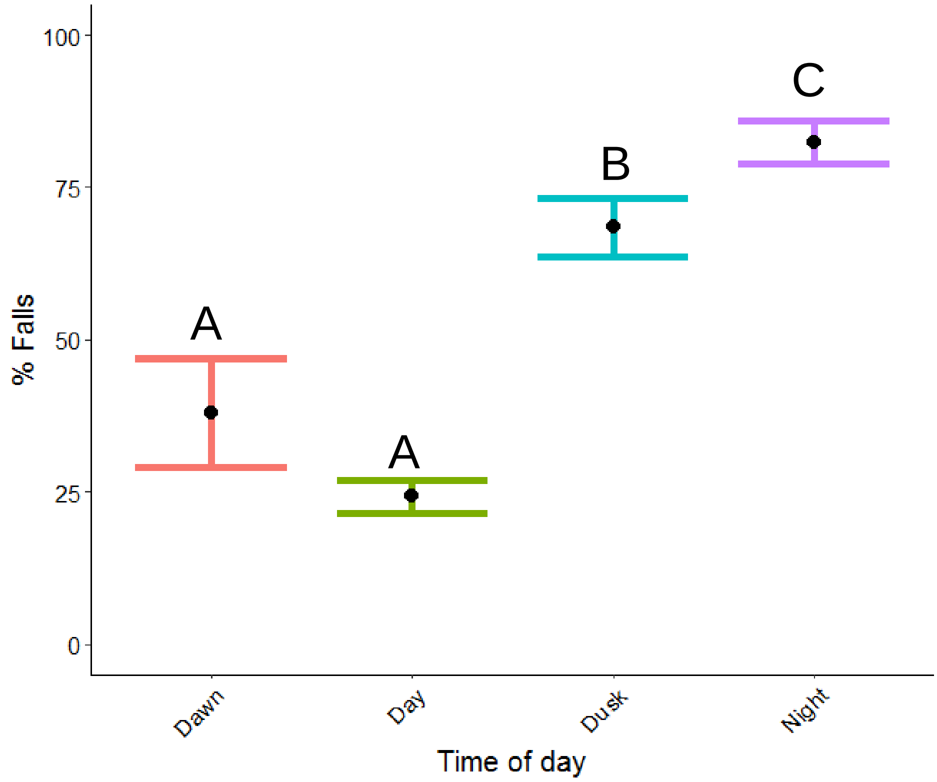

Figure 3.8: Percentage of falls in relation to the time of day (lsmeans ± SE). *Dawn = 10 minutes after lights on, Day = lights on, Dusk= 20 minutes before lights off and Night = lights off. Different letters represent statistical differences.*

Although Figure 3.8 considers the percentage of falls compared to total movements, it is important to look at the number of movements and falls at each time of day, regardless of the percentage, as this may relate to the *actual* hazard of a navigation pathway. Figure 3.9 shows the number of movements per 200 hens (associated with the accelerometry dataset) per time period. When considering the percentage of falls the results show the same general pattern except in the case of night-time movement (Figure 3.9). When looking at the number of movements per hour per 200 birds and the number of falls per time point, there are far fewer movements and falls during the night, then day compared with dawn and dusk (Figure 3.9). Most falls during the night happened in the hour after dusk, when 63.2% of the falls occurred. This indicates that dawn and dusk are the most active and the most hazardous periods. However, any movement at night has a high likelihood of becoming a fall but because the frequency of movement is low, the actual frequency of falling is also low.

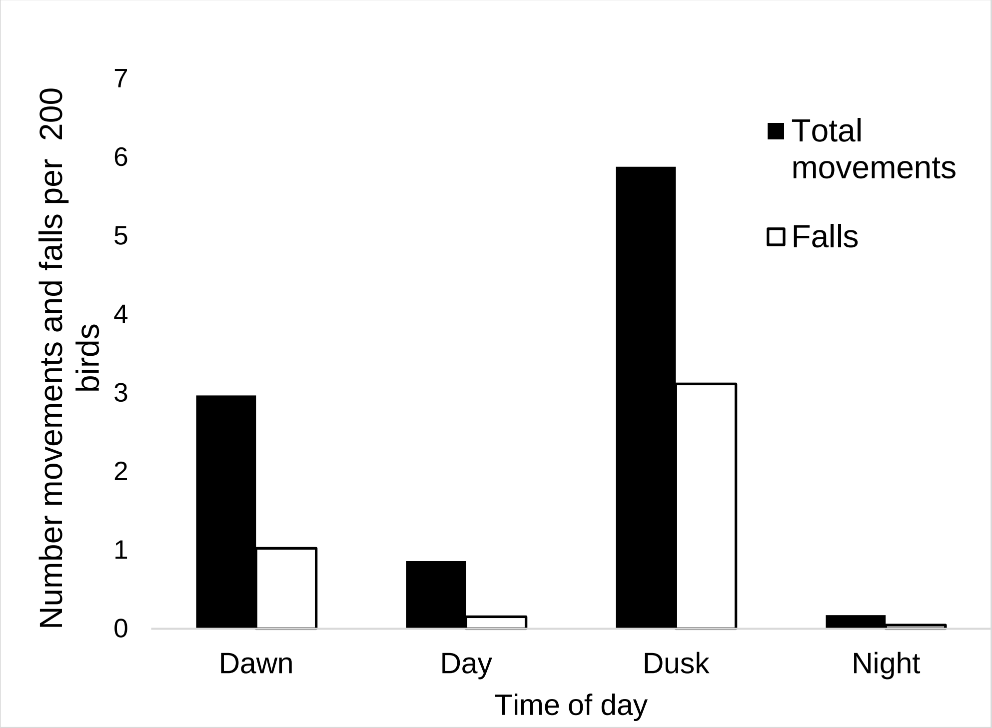

Figure 3.9: Movements and falls per time period per 200 birds (extrapolated from hens equipped with accelerometers).

To allow a better understanding of how hens move around the multi-tier system the navigation paths were split into different times of day. This was done using descriptive statistics only because there was only one number per time point so statistical analysis was not possible. Movements from 3rd perch to litter and top tier to 3rd perch have the highest number of total movements and falls during dawn (Table 3.3). The litter to lower tier and the top tier to top perch pathway had the highest number of total movements during the day but top tier to top perch and top tier to 3rd perch have the highest number of falls during the day. Litter to lower tier and nest box grid to 3rd perch have the highest number of movements during dusk but nest box grid to 3rd perch and 3rd perch to litter had the highest number of falls during dusk. Litter to lower tier and lower tier to 2nd perch have the highest number of movements during the night but nest box grid to 3rd perch and lower tier to 2nd perch have the highest number of movements during the dusk (Table 3.3).

Table 3.3: Navigation paths relating to the total number of movements and the total number of falls during dawn, day, dusk and night per hour per 200 hens *(related to accelerometry output – focal hens only)*

	Total movements				**Falls**			
Navigation pathway	**Dawn**	**Day**	**Dusk**	**Night**	**Dawn**	**Day**	**Dusk**	**Night**
2nd perch → 3rd perch	0	0.077	0.46	0.00099	0	0.0069	0.14	0.00099
2nd perch → Litter	0.11	0.021	0	0.0059	0	0.00077	0	0.0059
2nd perch → Lower tier	0	0.0023	0.18	0.019	0	0.00077	0.18	0.018
3rd perch → Litter	1.35	0.12	0.77	0.017	0.22	0.0092	0.67	0.017
3rd perch → Top tier	0	0.0077	0.035	0.0030	0	0.0038	0	0.0030
Nest box grid → 2nd perch	0	0.029	0.070	0.0040	0	0.014	0.070	0.00099
Nest box grid → 3rd perch	0.054	0.012	0.81	0.030	0.054	0.0038	0.77	0.027
Litter → Lower tier	0.11	0.15	1.40	0.039	0	0.015	0.21	0.011
Lower tier → 1st perch	0	0.012	0.14	0.016	0	0.0062	0.070	0.0099
Lower tier → 2nd perch	0	0.10	0.77	0.025	0	0.0092	0.14	0.021
Lower tier →Litter	0	0.018	0.035	0.0020	0	0.0015	0.035	0.00099
Top perch → Litter	0.47	0.065	0.070	0.0020	0	0.0038	0.070	0.00099
Top perch → Top perch	0.054	0.010	0.11	0	0.054	0.0015	0	0
Top perch → Top tier	0.16	0.013	0.25	0.00099	0.16	0.0077	0.25	0.00099
Top tier → 3rd perch	0.54	0.084	0.14	0.0030	0.49	0.037	0.11	0.0030
Top tier → Litter	0.054	0.018	0.035	0.0020	0.054	0.0069	0	0.0020
Top tier → Top perch	0.054	0.12	0.63	0.0049	0	0.025	0.42	0.0020

Some movement pathways are never picked up by the accelerometers at certain times of day; for example, there are no movements from 2nd perch to litter during dusk. When movements end on the litter during dusk, they are usually falls (Table 3.3).

During dusk (the dimming phase) there are some navigation paths that only occurred as falls and these were always downward movements; such as movements from the nest box grid to the 2nd perch and the lower tier to the litter (Table 3.3). This indicates these navigation paths were either overcrowded or difficult to navigation during the dusk.

3.3.4. Reason for fall (<u>related to accelerometry output – focal hens only</u>)

The greatest number of falls were caused by missed landings, followed by pushes, interactions with conspecifics and then slips (Table 3.4). Certain navigation pathways are always associated with a specific type of fall. All falls from 2nd perch to 3rd perch, lower tier to 1st perch and lower tier to 2nd perch were caused by missed landings. Most of the falls from nest box grid to 2nd perch, nest box grid to 3rd perch, top tier to 3rd perch, top tier to top perch and litter to lower tier were caused by missed landings (Table 3.4). Navigation pathways that ended on the litter never had a missed landing, as it was not possible to miss the litter. Most falls from 2nd perch to lower tier and 3rd perch to litter were caused by pushes (Table 3.4). All the other pathways either had no single type of fall that predominated, or they had very few falls.

Table 3.4: *Navigation paths and the type of fall from accelerometry output (total number of accelerometry outputs)*

	Type of fall			
Navigation pathway	**Missed**	**Slipped**	**Pushed**	**Interaction**
2nd perch → 3rd perch	14	0	0	0
2nd perch → Litter	0	1	3	2
2nd perch → Lower tier	2	2	20	0
3rd perch → Litter	0	1	41	10
3rd perch → Top tier	4	1	0	3
Nest box grid → 2nd perch	17	1	1	2
Nest box grid → 3rd perch	48	1	2	4
Litter → Lower tier	36	0	0	1
Lower tier → 1st perch	20	0	0	0
Lower tier → 2nd perch	37	0	0	0
Lower tier →Litter	0	1	3	0
Top perch → Litter	0	1	4	0
Top perch → Top perch	2	0	0	1
Top perch → Top tier	2	9	6	3
Top tier → 3rd perch	44	7	4	9
Top tier → Litter	0	5	5	1
Top tier → Top perch	44	0	2	1
Total	**270**	**30**	**91**	**37**

3.4. Discussion

To our knowledge, this is the first study that looked at the navigation paths within a multi-tier housing system and their effect on the frequency of use and frequency of falls. Other studies have looked into the way that birds move around a multi-tier system (Stratmann *et al.*, 2015a; Campbell *et al.*, 2016a; Campbell *et al.*, 2016c) but our study is unique in that individual laying hens were followed throughout lay using accelerometers to evaluate where in a multi-tier system birds were travelling and falling.

The aim of this chapter was to understand where most of the falls occur in a multi-tier system. It was also important to understand at what time of day there is a higher percentage of falls. This is because if system changes are to be made then it is vital that it is known whether a navigation path is difficult for hens and whether this is dependent on the time of day.

A summary of the results shows that the highest percentage of falls occur around the nest box area and on the top area of the system. The dusk time point is the most hazardous time point because, although a higher percentage of falls occurred during the night period (82.5% of movements), few movements occurred during the night. This suggests that the night-time is less hazardous than dusk. Similarly, dawn has a high total number of movements with around 35.7% of these being falls.

3.4.1 Navigation paths

There was a significant effect of navigation path on the percentage of falls within a multi-tier system. This is the first time accelerometers have been used to identify areas where falls occur in a housing system for laying hens. These findings can be the basis for future modifications for multi-tier systems for laying hens.

One of the original predictions **(1)** was: navigation paths high in the system would have a higher percentage of falls compared to those low in the system. Navigation paths from litter to lower tier (14.4 ± 3.0%) and lower tier to litter (28.7

± 13.1%) had the lowest percentage of falls compared to all other navigation paths. When looking at the number of movements in the system during a 4-hour period (using pen level, not focal bird data), litter to lower tier was high at 0.117 times per hour per 200 hens. Therefore, these movements from the litter to the lower tier are highly used, but rarely result in such vigorous movement that a readout is generated on the accelerometer, and of these, they have the lowest percentage of falls compared to other navigation paths. This means that movements between the litter and the lower tier should not be classed as hazardous because hens use them a lot and they do not appear to be difficult for the hens to transition.

Movements from litter to lower tier were recorded less frequently on the accelerometers, at 0.0114 times per hour per 200 hens. This could have two explanations. One could be that litter to lower tier is a pathway that is used more and would be more likely to have movements registered on the accelerometer. Another reason could be that movements from litter to lower tier are more vigorous compared to those from the lower tier to the litter, and therefore again being more likely to register on the accelerometer. It is likely to be the first reason in this case because moving from litter to lower tier is the main way hens enter the multi-tier system. A previous study has shown that re-entries into a multi-tier system from the litter happen throughout the day, whereas exits from the system are mainly focussed in the morning (Campbell *et al.*, 2016c). Hens can also leave the multi-tier system, in the current study, through a variety of navigation paths, meaning that movements from the lower tier to the litter may be lower in number in general compared to movements up from the litter.

If we take movements from top perch to top tier (92.8 ±3.5%) and top tier to top perch (50.3 ± 6.0%) as an example, they have a higher percentage of falls compared to other navigation paths in the system. Although these movements were frequently seen (both ways) when monitoring movements of all hens at a pen level (174.5 times per hour per hen), top perch to top tier had relatively low frequencies on accelerometers (0.0072 times per hour per hen) and top tier to top perch had a higher frequency (0.0789 times per hour per hen). The higher frequency of movements from top tier to top perch compared to top perch to top tier could be because of the need for hens to roost on high perches and structures (Olsson and Keeling, 2000; Schrader and Müller, 2009). This potentially means there are a greater number of movements up towards the higher perches from the top tier compared to down from the perches to the top tier. This may be because there is a variety of alternative navigation paths that can be used to move down from the top perches; such as to the 3rd perch or directly to the litter. It was also shown in this study that movements higher than the nest box resulted in more falls than those lower than the nest box. This finding is supported by previous work that hens fall more from higher regions in multi-tier systems compared to lower regions in the system (Stratmann *et al.*, 2015a).

However, it is important to note that there may be many more movements occurring within each navigation pathways that are not being picked up by the accelerometer. The accelerometer was set to record only when the predefined threshold was reached on both the body and keel sensor. The threshold was set low enough that controlled movements were recorded and this provides some confidence that all falls that occurred were recorded. Movements between top tier and top perches (0.5m) were shorter than that between the litter to the lower tier (0.73m), therefore it is possible that more controlled movements were missed in the top tier region than were missed between the litter and the lower tier due to the lower acceleration generated when moving. Moreover, movements along navigation paths that are less likely to result in falls are likely to be under-represented in the subset of events linked to accelerometry outputs. However, this does not apply to the direct observations not linked to accelerometer outputs.

Another prediction **(2)** was whether navigation paths with steep angles, mainly between 2nd perch and 3rd perch, would result in a higher percentage of falls compared with wider angled navigation pathways. This was not supported by the data. Movements between 2nd perch to 3rd perch resulted in a low percentage of falls, 29.3 ± 7.1%. These movements were recorded on the accelerometer 0.0483 times per hour per hen. On a pen level (without accelerometers), the frequency of pathway use was 10.75 times per hour per hen. This suggests that controlled movements between 2nd perch to 3rd perch may produce more overall acceleration but that falls are rare. In a previous study, upward movements of 60° were easier to negotiate than 30° angles and there was no difference between downward movements of 30° and 60° when the vertical distance between perches was 50cm (Scott *et al.*, 1997). In the current study, the vertical distance between 2nd and 3rd perch was 65cm and the horizontal distance was 18cm. The short horizontal distance may explain why there was a lower failure rate than expected in transitions between the two perches (Scott *et al.*, 1997).

However, there could be many reasons why movements from 2nd perch to 3rd perch did not result in a high proportion of falls. One could be that there were other navigation paths that could be used, not solely the transition from 2nd perch to 3rd perch. In between the 2nd perch and the 3rd perch was the nest box area. To access the top-level of the system, individuals could move from 2nd perch to grid in front of the nest box and then to the 3rd perch, thereby bypassing the steep angle. This was shown in the frequency of use data with movements from the nest box grid to the 2nd perch and the nest box grid to the 3rd perch being used more compared with movements from the 2nd perch to the 3rd perch. It could be theorised that only confident birds will attempt the steep angle, and this accounts for the high proportion of controlled movements (and a low proportion of falls) in this path. It may also be possible that a higher number of falls do occur between 2nd perch and 3rd perch but they do not result in high acceleration outputs and are thus not recorded by the accelerometers. When hens fall, they may land on the grid in front of the nest box (a height of 0.37m from the 3rd perch). This shorter distance may not generate enough force to trigger the accelerometer to generate an output. Another reason may be that hens use 2nd perch to 3rd perch movement more during the day compared with movements from nest box grid to 3rd perch, which was used more during dusk and night. Therefore, movements from nest box grid to 3rd perch may be more of an issue due to poor visibility. Individuals may also only use movements between 2nd perch and 3rd perch when they feel confident about making the transition i.e. during the day. However, this does not change the conclusion that the movements between 2nd perch and 3rd perch represented a low hazard in this study.

It is important that access to and from the nest box is made easily accessible to prevent falls because movements from and to the nest box lead to a high number of falls and are frequently used. A recent study has shown that hens access the nest box to the same extent regardless of litter substrate used, whereas other resources in the system were used to varying degrees across litter substrate (Campbell *et al.*, 2016b). This would suggest that a nesting area is a resource that all birds need regardless of other factors present in the system. Previous studies have shown that hens are highly motivated and willing to overcome obstacles to gain access to a nest box and will actively seek out nest boxes (Cooper and Appleby, 1995; 1996). The occurrence of a high proportion of falls compared to controlled movements near the nest box may be heightened in commercial systems due to laying hens tending to show a preference for certain nest box positions (Riber, 2010). However, it is crucial to understand whether the nest box is a hazardous area in commercial systems. The current study was carried out in a system containing 200 birds and 4 group nest boxes. Whether the same behaviours would be seen on a farm with 4,000 birds and 80 group nest boxes is not known, however safe access to nest boxes is important in all systems.

It may also be that areas with perches (e.g. the 3rd perch and 2nd perch) show up frequently because they are difficult to grip, or it is difficult for hens to judge the distance between them. The vision of laying hens may be poor in dim light conditions due to the flattened shape of the hen eye (Prescott *et al.*, 2004). Metal perches were present in the system and were dark in colour. Black perches have been shown to increase latency to jump in laying hens compared to wooden or white perches and it is possible metal perches may have a similar effect, particularly in dark conditions (Taylor *et al.*, 2003). One study showed that hens changed perches more often at night when white perches were present, suggesting that white perches are more visible to the hens in the dark (Chen and Bao, 2012).

Some falls around the nest box were from pushes and interactions with other conspecifics. A previous study had shown that lower rank hens received more aggressive pecks in the hour before egg laying compared to the hour that followed egg laying (Freire *et al.*, 1998). They were also uprooted from their chosen nest more frequently in the 30-minutes before oviposition compared with other hens (Freire *et al.*, 1998). Future studies should aim to make access to the nest boxes easier and facilitate the smoother transition between other areas of the system and the nest box.

Prediction **3** was: downward movements would result in more falls compared to upward movements. This was confirmed in the study and corroborated results found previously (Moinard *et al.*, 2004a; Moinard *et al.*, 2004b).

3.4.2. Reason for fall

Different navigation pathways resulted in different types of falls; either misses, slips, pushes or interactions. This difference in fall behaviour suggests that some destinations are more difficult to reach (misses) and others result in a greater amount of slips or pushes. There could be several reasons why these areas are difficult to reach. It could be that they are associated with high numbers of movements and the high bird volume could lead to a higher likelihood of falls. It could also relate to the structure that hens are trying to reach. It is known that falls from perches are more frequent than falls from any other structures (Campbell *et al.*, 2016a).

Landings on perches were expected to have a greater number of falls compared with landings on tiers and the litter. Although prediction **4** stated that landings and take-offs from perches would result in a greater percentage of falls was true in the instance of perch vs litter, it was not true of perch vs tier, with landings on tiers having a higher percentage of falls compared with landings on perches. One reason for this could be that landing on perches may be more controlled as hens ready themselves more to jump to perches compared to when they jump to tiers. Also, hens may have less grip on tiers than they do on perches. Tiers, particularly the edges, can become slippery due to faecal build up which means that it is more difficult for hens to grip and are therefore are more likely to have falls. However, this did not match previous research where the addition of extra tiers (platforms) reduces the number of falls compared to a system without platforms (Stratmann *et al.*, 2015a). Interestingly, when movements end on the litter during dusk, they usually are falls. This suggests that during dusk hens do not usually want to move towards the litter, and when they do, it tends to be unintentional. This is supported by previous studies were hens move up during dimming to roost at night (Stratmann *et al.*, 2015a; Brendler and Schrader, 2016).

3.4.3 Time of day

The dusk period had the highest percentage of falls (focal hen observations) and the highest number of falls per hour per 200 birds (pen level observations) as predicted (prediction **5** – that a greater percentage of falls compared with total movements would occur at dawn and dusk). Dawn had the second lowest percentage of falls (focal hen observations) but the second highest number of falls per 200 birds (pen level observations). This suggests that dusk and dawn are the most hazardous time points of the day, due to greater general activity, and that night-time is not as hazardous, simply because of the very low number of movements that occur during the night.

This shows that birds struggle with movements at these times. Movements during the dimming period have been found in previous studies to be difficult for birds to navigate (Tanaka and Hurnik, 1991; Taylor *et al.*, 2003; Stratmann *et al.*, 2015a). The high proportion of falls could be due to the inability of the birds to see accurately during these times as well as the high bird traffic during dusk when all individuals are trying to reach the optimal roosting position on the top perches (Taylor *et al.*, 2003; Brendler and Schrader, 2016). The high proportion of falls when lights are dimmed and during lights off would suggest that birds are unable adequately to see structures due to limited lighting.

Although the data suggest that a higher proportion of falls occur during night and dusk compared to during the day it is important to understand that much more movements occurred during the daylight period compared to night and dusk. The night is a prolonged period and there are not many controlled movements at night as hens are all on the system roosting. Dusk is a short 20-minute period and within this time there are controlled movements and falls. During dusk, there is a substantial number of falls within a short period. The dimming period that is needed for hens to successfully transition to perches at night has been discussed in the literature, with one study by Stratmann *et al.* (2015a) looking at the number of falls birds have during this period, within multi-tier systems.

3.4.4 Health parameters

It was thought that hens with poor health, such as increased keel bone fractures, poor foot pad health and poor feather condition would have a higher percentage of falls compared to healthy hens - prediction **6**. The results contradicted this, as there were no differences found between any of the health parameters and the percentage of falls between hens. With regard to feather cover, one reason could be that overall cover was used in the current study and not wing feather cover as in the previous study (LeBlanc *et al.*, 2016). Keel bone fracture scoring was performed using palpation and therefore, hens were classed as either having a keel bone fracture or not. Each hen may have been at different stages of fracture healing and this may affect the level of pain in each bird, and therefore influence mobility to a variable extent (Nasr *et al.*, 2013a; Nasr *et al.*, 2015). Acute pain in humans is associated with nociceptive and neuropathic pain whereas chronic pain is usually neuropathic (McCormick and Law, 2016). Furthermore, calves that have been castrated show different behaviours in the moments after castration compared with 48-hours after castration, indicating that acute and chronic pain can have different behavioural effects (Molony *et al.*, 1995). This is like the outcome for footpad lesions, as hens were classed as either having foot pad dermatitis or bumblefoot or not, the effect of having severe lesions may be masked.

3.4.5. Limitations of the study

One of the main limitations of this work is all movements in a navigation path are not picked up due to the accelerometers only recording data when the pre-set threshold is exceeded. Movements (including falls) that occur generating accelerations below the pre-determined threshold will not be available, and therefore the true percentage of falls in each navigation path is unknown. A practical limitation of this study is the need to watch individual hen behaviour through videos, which is very time-consuming. No algorithms or machine learning techniques were used to make any conclusive findings from this data.

It should also be noted that only a small sample of focal hens was used to collect the data. This may be important if each hen has individual behavioural responses linked to personality traits and respond to pain and stressful situations differently (Cockrem, 2007). As in Chapter 2; individual hens were nested within pen in the statistical model. Therefore, it may be that the same hens are falling within the same navigation pathways. Even though including hen nested within pen in the model should solve this issue, it is still worthwhile and important to note that this can influence the validity of the results.

Another important point is that when analysing the number of movements within the system (non-accelerometry data – observations on a pen level) it was only carried out in one pen at one time point. For the data to be more representative, it would be important to analyse data over different pens and across different time points. It is important to note that this was not done in the current study due to time restraints when watching videos. The data present here provides an indication of the navigation pathways that are used by hens and the number of times these pathways are used at different times of day, but it is important to remember the frequency of use may be different at different ages or in different pens. In the same analysis, upward and downward movements were grouped together. In future, it would be beneficial to separate the direction of the movement to understand whether different pathways are being used more for upward or downward movements, again, this was not done in the current study due to timing restraints but it would be recommended that this be done in future studies.

3.5. Conclusions and future work

The current study has evaluated system specific navigation paths that represent hazards leading to falls. General principles of the nature of the navigation path and the time of the transition have been established. Due to the nature of the study, it was difficult to causally relate patterns of utilisation of the system with health parameters. However, such studies could and should be performed in the future.

Studies such as this, providing data on hazards within systems, is crucial for the development of custom-built multi-tier systems to allow laying hens the optimal safe means to transition between different tiers. Further studies should focus on controlling lighting regimes to determine whether there are fewer falls when the dimming period is extended. Most falls in the night period occurred around one hour after lights off, which would suggest the hens find it difficult to obtain an optimal roosting location during the 20-minute dimming period provided. Modifications should be made to the housing system, particularly around the nest box area and the top tier to reduce falls and potentially keel bone fractures.

System specific navigation pathways have been identified that represent hazards in leading to falls. Also, general principles of the nature of the pathway and the time of the transition have been established. Because of the nature of this study, it was difficult to causally relate patterns of utilisation of the system with health parameters, however, such studies could and should be performed.

Chapter 4

Modifications to a multi-tier housing system: Effects on health and behaviour

4.1. Introduction

Keel bone fractures are a huge welfare problem in the laying hen industry with several studies indicating keel bone fractures are painful (Nasr *et al.*, 2012b; Nasr *et al.*, 2013a; Nasr *et al.*, 2015). One study on a small sample of hens indicates that those with keel bone fractures consume more feed and water (Nasr *et al.*, 2013b), meaning that potentially keel bone fractures can be an economic issue as well as a welfare one. The potential welfare and economic impacts are enormous due to large number of laying hens housed in commercial farms globally.

Multi-tier systems were first introduced as an alternative to the cage and single-tiered systems. Due to the addition of elevated perches and tiers, there is an increased risk of falls in these systems (Gregory and Wilkins, 1996; Moinard *et al.*, 2004a), which has been linked in previous studies to keel bone fractures (Wilkins *et al.*, 2011). Chapter 2 in this thesis has also discussed how higher heights can results in higher levels of summed acceleration vectors (AVs) at the keel, meaning potentially they can cause higher levels, or probability, of keel bone fractures.

To reach essential resources, such as food and drink, hens must navigate the multi-tier system using the perches and tiers provided. The complex movements required may be difficult for some hens, depending on previous experience during rearing (Gunnarsson *et al.*, 2000). Keel bone fracture prevalence of over 90% has been detected in systems with elevated perches (Wilkins *et al.*, 2011). However, elevated perches are provided because laying hens prefer to roost in high areas and preventing access to perches can cause frustration behaviours in hens (Olsson and Keeling, 2000; Brendler and

Schrader, 2016). Elevated perches in multi-tier systems are crowded at night and used frequently during daytime (Brendler and Schrader, 2016). Perches over 90cm are the preferred height for roosting and the number of birds resting on perches with heights of 80cm or below was not different from those resting on the floor (Brendler *et al.*, 2014). It has been shown that hens prefer to fill up the top perches and tiers when roosting at night, leaving lower levels empty (Schrader and Müller, 2009).

The addition of extra perches has the potential to limit overcrowding due to increased space availability for the hens to perch, reducing displacement from perches. It has been shown that the length of time spent flying and the latency to balance when moving from one perch to another were both reduced when a landing perch had a gap of 30cm between two obstructions compared to a gap of 15cm (Moinard *et al.*, 2005). This suggests that increasing perch space may potentially make movement easier for hens because they may have a wider area without any interactions with conspecifics. Although there were no clear differences between the number of falls and the distance between obstructions (Moinard *et al.*, 2005), increasing perch space may still have the potential to reduce falls on-farm compared to a controlled setting. If falls resulting from displacement from perches are limited, this may reduce the prevalence of keel bone fractures because as it is thought the collisions with structures in the system lead to keel bone fractures due to the keel bone being in a prominent and vulnerable location anatomically (Gregory and Wilkins, 1996). However, the addition of two extra elevated perches, as well as four extra non-elevated perches, did not show any difference in keel bone fracture prevalence at the end of lay in a Bolegg Terrace multi-tier system (Stratmann *et al.*, 2015a).

Nevertheless, the addition of a greater number of elevated perches, instead of non-elevated perches may show a significant effect at reducing keel bone fracture prevalence during lay and has yet to be studied.

As well as the addition of perches on the top tier, placement of perches around essential resources, such as nest boxes, may help to reduce overcrowding; reducing falls, collisions and keel bone fractures. Results from Chapter 3 of this thesis indicate that there are a high proportion of falls compared to controlled movements around the nest box in a Bolegg Terrace multi-tier system. Therefore, providing an extra perch around the nest box may facilitate a new movement path, potentially reducing falls and subsequently keel bone fractures. However, a potential problem when adding extra perches into a system is the possibility of an increased prevalence of keel bone deviations because deviations are thought to be caused by pressure on the keel bone (Warren, 1937; Harlander-Matauschek *et al.*, 2015).

As well as the addition of perches, and placing perches around essential resources, perch cross-sectional shape can affect perching ability (Scholz *et al.*, 2014). Round, metal perches are the most common perch design in a multi-tier system, but round perches have been shown to have fewer safe landings compared with mushroom-shaped perches (Scholz *et al.*, 2014). Provision of mushroom perches in a commercial system may reduce the incidence of falls and collisions, potentially reducing keel bone fracture prevalence. Previous studies have looked at mushroom-shaped perches and have found that the contact area of the keel bone is greater compared with round perches (Pickel *et al.*, 2011). However, the mushroom-shaped perches used had a greater width compared with the round perches and round perches of greater widths were not compared. Therefore, it cannot be concluded with certainty whether the increase contact area was due to the perch shape or width. The addition of mushroom-shaped perches may potentially decrease keel bone deviations by having a larger surface area for a more even pressure distribution (Pickel *et al.*, 2011). In the same study, standing hens generally had lower peak forces on their foot pads on mushroom-shaped perches compared with round perches (Pickel *et al.*, 2011).

Other furnishings within multi-tier systems have been studied in previous research such as platforms and ramps (Stratmann *et al.*, 2015a; Heerkens *et al.*, 2016a). Ramps allow hens to walk instead of jump or fly between tiers, this can reduce the likelihood of falls within the system (Stratmann *et al.*, 2015a). Previous studies have looked at movements from the lower tier to the litter in the absence and presence of ramps (Pettersson *et al.*, 2017a), and falls and collisions during dusk with ramps compared to no ramps (Stratmann *et al.*, 2015a). One study examined general movements within a multi-tier system between the system (tier and perches) and litter (Campbell *et al.*, 2016c). Ramps have been shown to reduce keel bone fracture prevalence throughout lay (Stratmann *et al.*, 2015a; Heerkens *et al.*, 2016a) and have been shown to improve foot pad health (Heerkens *et al.*, 2016a). It is hypothesised that the addition of ramps to a commercial farm will improve keel bone fracture prevalence and foot pad disorders. Ramps may also help improve movements within the system by allowing hens to escape negative interactions more readily, potentially reducing falls and allow less confident hens to walk within the system instead of jumping or flying.

Other health parameters; foot pad health, weight and feather condition were also monitored because it is important when making alterations that not only keel bone damage is assessed but the whole health of the hen so that conclusions can be drawn regarding the suitability of these modifications to laying hens. It is particularly important to test footpad lesions because the addition of perches can be detrimental to footpad health and footpad lesions can be painful (Gwatkin, 1940; Wang *et al.*, 1998; Gentle *et al.*, 2001; Shepherd and Fairchild, 2010; Heerkens *et al.*, 2016b). As the addition of extra furnishings and ramps can affect overall activity, it is important to determine whether bone strength increased in the treatments because exercise in hens improves bone strength (Casey-Trott *et al.*, 2017).

4.1.1. Aims and predictions

The main aim of the current study was to modify a standard multi-tier system to examine the affect in reducing keel bone fractures prevalence. Modifications were based on results obtained in Chapter 2 and 3 and using information from previously published studies on structures that allow hens to move more readily or gain more controlled movements. The treatments used are;

1. C - standard multi-tier system (control)
2. EP – all standard perches are replaced by mushroom-shaped perches and there are extra mushroom-shaped perches on the top tier
3. M – all standard perches replaced with mushroom-shaped perches
4. NBP - an extra standard perch around the nest box
5. R - a ramp that runs transverse once in every two multi-tier units in the system.

There were multiple predictions of the study and they are listed below:

1. The main prediction is that all modifications will result in a decrease in keel bone fracture prevalence.

2. It is also predicted that the addition of mushroom perches would reduce footpad lesions.

3. The addition of extra perches on the top tier of the system will increase perching on the top tier of the system, summed acceleration vectors (AVs) on the keel and body would be higher in the non-modified multi-tier system compared to the modified systems.

4. Bone strength will increase in all modified treatments, apart from treatment 3 with standard perches replaced by mushroom-shaped perches.

4.2. Methods

4.2.1. Animals and husbandry

The study was carried out on a commercial Rondeel® laying hen farm. There was a total of 36,000 Lohmann brown Lite laying hens and they were divided into sub-flocks of 3,600 hens. Each sub-flock represented an individual pen for data collection. Figure 4.1 shows the layout of the pens. All animals were housed on the same farm and they experienced the same management conditions. The indoor area comprises a Bolegg Terrace aviary system and the outdoor area has an artificial grass area with raised platforms (winter garden) that leads onto a smaller woodchipped (outside) area. The Bolegg Terrace has a central belt system, whereby the manure belt and nest boxes are in the centre of the system. Stocking density was 7 hens/m^2 and this included the inside area and the winter garden but did not include the outside area. Food and water were provided within the system and water was also provided in the outside area. Standard layer mash was provided, and corn was scattered in all pens once per day. The average light reading on the litter in the centre of the system was 545 lux at the outer-facing side of the system and 6 lux at the inside of the system. The outer-facing side of the system was very bright due to the large pop-holes providing a stream of natural light. Mortality at end-of-lay was 6%. Natural ventilation in the system was provided via curtains that would open and close automatically depending on weather conditions. Hens were reared as pullets in a system with access to perches and raised structures and transported to the Rondeel® at 19 weeks. Rondeel® eggs have been awarded highest star rating in the Netherlands through Beter Leven “better living” but the eggs are sold as barn eggs as the outdoor area does not meet the requirements needed to be called free-range.

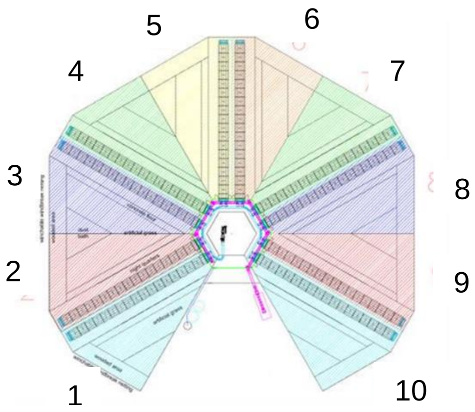

Figure 4.1: *Schematic of Rondeel® split into 10 different compartments operated by Vencomatic, the Netherlands*

4.2.2. Treatment groups

Four treatment groups and a control were used in the study (Figure 4.2).

1. The control (C) (section 5 and 6; Figure 4.2)
2. Extra mushroom-shaped perches on the top level of the system and perches in the system changed from standard round perches to mushroom-shaped perches (EP) (sections 1 and 8). A cross-section of the mushroom-shaped perch design used can be seen in Figure 4.3.
3. Mushroom-shaped perches (M) in place of standard round perches (sections 2 and 7; Figure 4.3)
4. Extra perch in front of the nest box area (NBP) (sections 3 and 9; Figure 4.2)
5. Ramps (R) that run the height of the system, beginning on the lower tier and moving towards the top tier. Ramp angle was transverse to the system (45° and 20cm wide. (Sections 4 and 10; Figure 4.2). A photograph of the ramp used can be seen in Figure 4.3.

All perches and ramps were made of metal and all other furnishings in the system were the same. In the current study, we were limited to an n=2 for each treatment group because the CASE industrial partner had experimental requirements beyond our control and wanted to maximise the number of treatments in our groups, hence the reasons for the small replicate number. All modifications were installed before pullets were placed into the pens. The mushroom-shaped perches were used in the study and the addition of ramps and an extra perch around the nest box were added because of the results from Chapter 2 and 3.

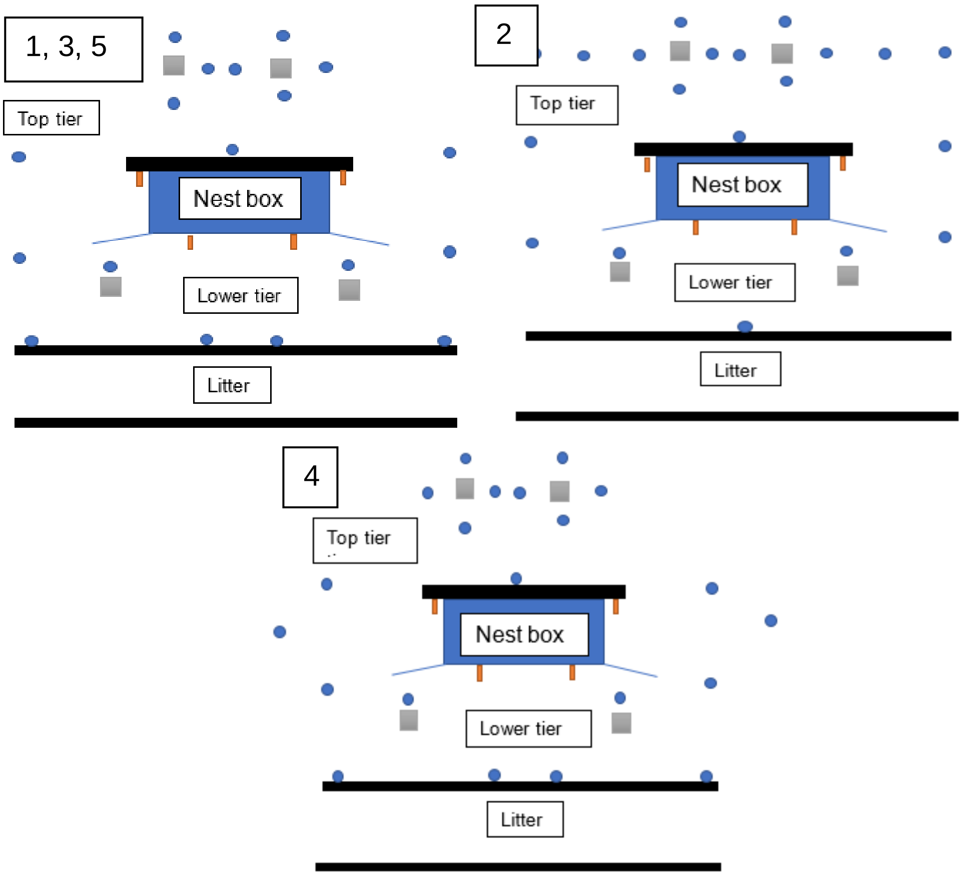

Figure 4.2: Schematics taken from a transverse view of the modification pens.

1, 3, 5 = C, M and R groups. 2 = EP group and 4 = NBP group (see figure 4.1). Blue circles represent perches, Orange rectangles represent drinkers and grey rectangles represent feeders. The ramp treatment group has a ramp running transverse to the system.

C = Control, EP = Extra perches, M = Mushroom perch, NBP = Nest box perch, R = Ramp

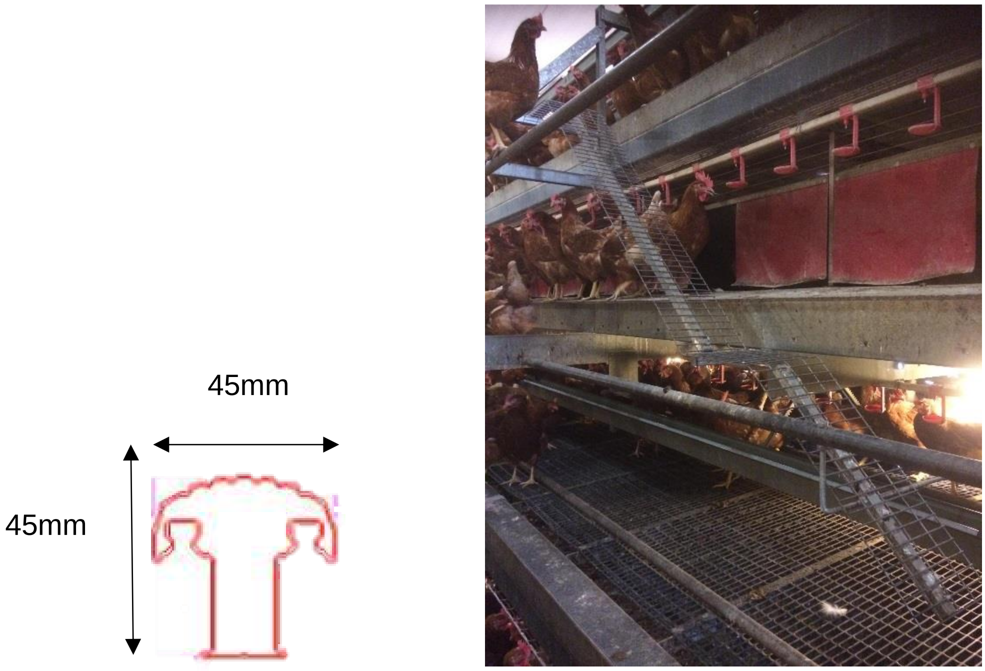

Figure 4.3: *Cross-section and measurements of mushroom-shaped perch (left). Photograph showing the way the ramp was placed in the system*

4.2.3. Keel bone damage assessment

One-hundred and fifty birds were palpated per pen, except at 83 weeks of age when only 50 birds were palpated per pen due to time constraints beyond our control. Different birds were caught and scored at each time-point because it would not have been feasible to re-capture the same hens at each time point. The same individuals were used for foot scoring and weighing because a sample of hens from each treatment were already caught and catching new hens was not needed. Keel bone damage was assessed by palpation, where the thumb and forefinger are moved along the sternum of the hen feeling for any deviations (bends of the bone) and fractures (protrusions, callus formation and sharp edges) (Wilkins *et al.*, 2004; Casey-Trott *et al.*, 2015). There were 5 palpation time points - when placed into pens at 19 weeks of age (150 birds per treatment), 25 weeks (150 birds per treatment), 33 weeks (150 birds per treatment), 48 weeks (150 birds per treatment) and 83 weeks (50 birds per treatment) of age. The lights were dimmed, and birds were caught from all levels of the system in the morning. Once captured, birds were placed into crates and taken out of each pen for palpation. It was only possible at 25 weeks to be blinded to treatment group due to limitations from other data being collected at the same time (video and accelerometry data), it was important not to disturb the hens in these groups in a way that could potentially affect the validity of the results collected. Palpations on birds at 19 weeks of age were not blinded because hens were being placed into the pens and were palpated directly from the transport crates. Fractures were recorded using a severity scale 0= no fracture, 1= small fracture and 2= large fracture (Wilkins *et al.*, 2004), deviations were given scores 0= no deviation, 1= slight deviation (<0.5cm curvature from straight plane) and 2= severe deviation (>0.5cm curvature from straight plane) (Heerkens *et al.*, 2016b). In each instance, the deviation only included a bend in one direction from the straight plane of the keel bone.

4.2.4. Foot scoring

Foot pads were scored for dermatitis, bumblefoot and hyperkeratosis in the same hens selected for palpation and was carried out at 33 (150 per pen; dermatitis and bumblefoot only), 48 (150 per pen) and 83 (50 per pen) weeks of age. Hens were not foot scored at 19 weeks of age as time was limited because hens were being removed from the transport crates and keel bone fractures prevalence determination was the main aim of this study. Due to timing issues, hens were not able to be foot scored at 25 weeks of age. Foot pads were scored for footpad dermatitis (FPD) using a scale ranging from 0 = no FPD to 4 = severe

FPD, a visual scale was used to score FPD (Figure 4.4) (Butterworth, 2013). Bumblefoot was scored at 33, 48 and 83 weeks of age using a scale ranging from 0 = no bumblefoot to 3 = severe bumblefoot, adapted from the visual scale by (Kjaer *et al.*, 2006). and hyperkeratosis (proliferation of the skin on the foot pad) (Weitzenbürger *et al.*, 2006) was only scored at 48 and 83 weeks of age.

Figure 4.4: *Schematic of foot pad dermatitis. Left to right (0-4), no FPD to severe dermatitis.*

4.2.5. Plumage scoring

Feathers were scored at 25, 33 and 48 weeks of age. One-hundred and fifty birds from each pen were individually feather-scored by walking through each pen and comparing with photographs of different body parts from laying hens sourced from Tauson et al., 2005. Birds were not handled during feather scoring. The feather scoring system was: 1 = severe feather damage, 2 = moderate feather damage and 3 = mild feather damage and 4 = almost perfect feather coverage. Only the neck, back, wings and tail were scored as these are the sections of the bird that was easy to score when walking through the pen without disturbing the birds’ normal behaviour. A previous study has found this process to be similar in accuracy to feather scoring individual birds by handling them (Kjaer *et al.*, 2011).

4.2.6. Body mass

The same 150 hens used for keel bone assessment and foot pad scoring were weighed at 33 and 48 weeks of age. Hens were weighed with a weighing scales, where they were suspended upside down. Each hen was in the weighing scales for approximately 5 seconds. The scales were accurate to 3 decimal places (0.001kg).

4.2.6. Litter deterioration

At 33 and 48 weeks of age, litter quality was scored according to the quality of the litter at the time. Litter was scored at 6 different locations within each pen at 33 weeks and 7 locations at 48 weeks of age. Score 0 = dry and sand-like textured litter, score 1 = <10% damaged litter (wet, hard or clumped together), score 2 = 10-50% litter damage and score of 3 = >50% damaged (wet, hard or clumped together). The litter scoring system was one which was developed by the industrial partner, Vencomatic, and is commonly used in practice.

4.2.7. Perching behaviour

Due to only one measurement per time point being created, the data were not statistically analysed. At five different time points throughout the day, with observations starting between 0600-0700hr, 0900-1000hr, 1200-1300hr, 1700-1800hr and 2200-2300hr birds were counted on perches in each of the pens by two observers. Counts were carried out between 30-35 weeks of age and 45-50 weeks of age, twice for each of these time points. The method of counting birds was planned, discussed and practised between both observers beforehand for consistency. Birds were counted along perches in 3 sections in each pen. The 1st, 6th and 12th section in each pen was chosen. The 1st section was the first when entering the pen from the walkway, the 6th section was in the middle of the pen and the 12th section was a section closest to the rear of the pen. Birds were counted first along the outer edge, near the pop-hole and then the observer moved around the pen and counted the birds on perches on the inside of the pen. Top tier perches were any perch above the nest box, the middle perches were any perch at the level of the nest box and the lower perches were any perch below the nest box level. The number of hens on the top tier during the night (2200-2300hr) may be underestimated due to not all perches being visible when counting and due to the large number of birds on the top level during the night.

4.2.8. Accelerometer placement

At time point 30-35 weeks of age (8 hens per pen) and 45-50 weeks of age (7 hens per pen) focal hens were selected to wear accelerometers. The same type of vest was used as those described in Chapter 2 and 3 (brown in colour for this study). Hens were caught from the litter and on each level of the system. Only hens with no or minor (score of 1) keel bone fractures, slight deviations, small or no foot pad lesions, perfect or near perfect feather condition (score 3 or 4) were chosen and with as close to average weights as possible (1.7-2.1kg). This was so that focal hens were as similar as possible and that they would not be uncomfortable wearing the vest. Sixteen (at time point 1) or fourteen (at time point 2) hens were attached with accelerometers at one-time point. If the accelerometer fell out of the vest or the vest shifted under the wing the data were not used, to make up the number more hens were fitted with accelerometers.

Accelerometers were programmed to produce an output when a pre-set threshold of 12g-force (g) on the keel and body sensor were reached. Attached to the body sensor was a location device (Tile®) using electrical tape. A Tile® is advertised as a device to allow you to accurately locate your keys, bag or any other personal item. The Tile® can be activated from a smartphone or tablet and a map appears, using Bluetooth. Once connected to the Tile®, Bluetooth will show the last place the Tile® was and then update to the current location of the Tile®. This reduces the time needed to catch the focal birds. All birds were recorded for approximately one week. For analysis 3.5 days were used to allow comparisons to be made between groups because the accelerometers were on the hens in different pens for slightly different lengths of times. Therefore, it was important that the same length of time for each pen was compared. Due to problems with the vest and the accelerometer, Pen 5 (control) only has data for 7 hens at time point 1.

4.2.9. Tibia radiological and biomechanical analysis

Twenty hens per pen (200 hens in total) were culled at the end-of-lay (83 weeks) and their left and right tibias were removed by dissection, with only the left tibias being used in the analysis. Tibias were used as a surrogate for overall bone strength due to their relatively uniform shape and cross section. The close relationship between keel and tibia properties has been shown previously (Toscano *et al.*, 2013). Hens were weighed, keel bones were checked for fractures through palpation and footpads were checked for abnormalities before dissection. Bones were then frozen at -20˚C until analysis.

Before analysis, the bones were defrosted at room temperature and a HB pencil was used to mark the centre line of the bone. Three tibiae at once were placed in a dual X-ray absorptiometry (DEXA) machine (Lunar PIXImus densitometer, Lunar Corp) and a metal wire was used to indicate the central line of the bone that was previously marked. Data were automatically generated from the machine, with bone mineral density (mg/cm^2), bone mineral content (mg/cm) and the area being recorded (mm).

The same bones used for DEXA measurements underwent biomechanical testing under three-point bending for their breaking strength using an Instron mechanical testing frame (Instron 6022, Instron, UK), fitted with a 10kN load cell. Before analysis, the horizontal and vertical diameters at the centre line of each bone were measured using callipers. The length of the tibia was measured to the closest mm. The midpoint of the tibia was marked previously with a HB pencil to ensure that the same point of the tibia was measured in all tibias. The tibia was placed on the machine with a supporting bridge gap of 4cm and the impactor at the midline perpendicular to the bone. The bone was tested to failure, and a load/deformation curve was generated, with maximum (breaking) load, extension at maximum load, and elastic modulus recorded. Following removal from the apparatus, the inner diameters of the bone were measured using callipers. The inner thickness of the cortical bone was measured at the top and bottom (parallel to the direction of load), with the average calculated, and the left and right (perpendicular to the direction of load) with the average calculated, to get the cortical bone thickness in both the vertical and horizontal direction. This was to determine the thickness of the structural bone bone “cylinder” (the medullary bone is non-structural) so that the values could be entered into the computer and the outputs of material properties, stress, strain and Young's modulus, could be generated automatically.

Data collected was the maximum load (N), which was the force needed to break the bone; the energy at maximum load (KJ), which was the energy needed to break the bone; and the stress (MPa), which had considered the material property of the bone and the force needed to break the bone having accounted for the bones size and dimensions. Stress was measured using the following equation:

Stress = (load*span*external RA/2) / 4(π(external RA^3 *external RB)-(internal RA^3*internal RB))

Where:

- external A is the outer radius of the bone in the direction of load,
- internal A is the inner radius of the bone in the direction of load,
- external B is the outer radius of the bone perpendicular to the direction of load
- internal B is the inner radius of the bone perpendicular to the direction of load.

4.2.10. Statistical analysis

Statistical analysis was carried out in R using R studio as the interface (RStudio Team, 2016; R Core Team, 2017). Health parameters (keel bone fractures and deviations, footpad dermatitis, bumblefoot, hyperkeratosis) scores were pooled to represent either presence or absence of deformity. The data were then analysed using a generalised linear model with family specified as binomial.

Treatment (factor) and age (factor) were included as fixed effects and when significant (P>0.05) the interaction between treatment x age was included in the model. The pen was included as a random factor, with pen as the experimental unit. For keel bone fractures and deviations, the average (of the score between 0 and 1 for 150 hens placed at 19 weeks of age) keel bone fracture and keel bone deviation presence were used in the respective model as a baseline. This was not done for any of the other measurements because of timing issues making it only possible to test keel bone fracture prevalence (the main factor being studied) because hens were being removed from transport containers at the time measurements were taken.

Body mass (kg) was analysed using a linear mixed effect model with treatment and age as fixed effects and pen as a random factor. Pen was the experimental unit.

All parameters from the tibia analysis were measured using generalised linear models with treatment (factor), keel bone fractures status (0,1,2: factor) and keel bone deviation status (0,1,2: factor) as fixed effects. Body mass of hens was kept in the model as a covariate. If keel bone fracture or keel bone deviation prevalence reached a tendency (P<0.1), they were left in the model. The data were checked for normality and when it did not fit normality, the data were log transformation before analysis. Feather cover was analysed as either perfect or not using a generalised model with a binomial distribution. Treatment (factor) and age (factor) were in the model, with pen as a random factor (making pen the experimental unit).

For analysis of the accelerometer data, the average of all the maximum summed acceleration vectors (AVs) on the keel and body for each bird was generated to give one point per bird per age (2 ages). For both sensors; treatment (factor), age (factor), body mass (continuous) and the number of accelerometer outputs recorded (continuous) from each hen were included as fixed effects and pen was included as a random effect (pen was the experimental unit). Data were first log transformed and analysed using a generalised linear model in R.

In all cases, when modelling optimisation was needed, the optimx package was used to deal with any convergence issues in the dataset. Data are presented as lsmeans ± SE unless otherwise stated (Lenth, 2016). In all models an ANOVA, using the car package (Fox and Weisberg, 2011), was used to test the significant effects of all effects in the model. Dunnett's post-hoc test in the multcomp package in R (Hothorn *et al.*, 2008) was used to test for significance between each treatment group (EP, M, NBP, R) and the control (C). When using multiple comparisons to test the age x treatment effect, the lsmeans package specifying multivariate comparison, "mvt" was used as it is specifically designed to be used with multivariate models and be less conservative than the Bonferroni method (Lenth, 2016). For binomial models, overdispersion was checked using the blmeco package (Korner-Nievergelt *et al.*, 2015). In all linear models, model fit, and normality was determined using histograms and QQ-plots of the residuals.

Perching behaviour is shown as descriptive statistics only with means ± standard deviations presented. An average for each pen per time point was created and then the average of both time points was calculated with the degree of variation taken as the difference between the two-time points (called the standard deviation here). To calculate the number of hens per perch; the number of hens that were seen were divided by the number of perches on each level of the system.

4.2.11. Ethical statement

The study was approved by the University of Bristol Animal Welfare Ethics Review Body: UB/16/075.

4.3. Results

All health parameter results can be found in Table 4.1 with results showing the prevalence associated with each age and treatment category, as well as whether any age x treatment interaction was present.

Table 4.1: *Least square means of weeks of age effects and treatment effects for each health parameter, the test statistics and p values are also given.*

Health parameter	**Week of age**				**Treatment**					**Test statistic and P values**		
	25	33	48	83	C	EP	M	NBP	R	Treatment x age	Treatment	Age
Keel bone fracture	14.9 ± 0.9	30.5 ± 1.2	64.0 ± 1.2	87.5 ± 1.5	44.0 ± 2.2	51.3 ± 2.0	53.8 ± 2.2	49.7 ± 2.1	49.8 ± 2.0	NS	W=9.91, P=0.0420	W=10,23.34, P<0.0001
Keel bone deviation	7.0 ± 0.7	11.6 ± 0.8	13.9 ± 0.9	16.8 ± 1.7	11.0 ± 1.0	10.6 ± 1.0	11.3 ± 1.0	15.3 ± 1.3	11.3 ± 1.0	NS	W=10.64, P=0.0310	W=50.66, P<0.0001
Foot pad dermatitis	NA	27.6 ± 1.4	30.7 ± 1.4	23.3 ± 2.0	26.0 ± 2.3	41.4 ± 2.8	33.4 ± 2.6	22.3 ± 2.1	16.4 ± 1.8	NS	W=67.83, P<0.0001	W=10.48, P=0.0053
Bumble foot	NA	3.6 ± 0.5	10.1 ± 1.0	4.2 ± 1.0	4.0 ± 1.0	10.3 ± 1.9	5.9 ± 1.4	4.5 ± 1.2	4.1 ± 1.2	W=19.15, P=0.0141	W=2.80, P=0.5921	W=2.00, P=0.3671
Hyperkeratosis	NA	NA	67.6 ± 1.5	61.4 ± 2.4	59.2 ± 3.6	61.8 ± 3.5	60.5 ± 3.6	68.0 ± 3.3	72.6 ± 3.2	W=13.88, P=0.0077	W=10.40, P=0.0343	W=4.55, P=0.0329
Plumage condition (% with perfect feather score)	98.2 ± 0.4	97.9 ± 0.5	79.5 ± 1.1	NA	96.0	96.3	94.0	93.6	94.4	NS	W=16.09, P=0.0029	W=299.47, P<0.0001

C = control, EP = Extra mushroom-shaped perches, M = Mushroom-shaped perches, NBP = Extra nest box perch and R = Ramp

NA = data were not collected for this parameter

4.3.1. Keel bone fractures

There was an effect of treatment on keel bone fracture prevalence (W=9.91, *P*=0.0420) (Figure 4.5). Overall keel bone fracture prevalence was lower in the standard multi-tier systems (C) compared to the mushroom-shaped perch treatment (M) (Z-value=2.974, *P*=0.0102), extra mushroom-shaped perch (EP) (Z-value=2.489, *P*=0.0425) and there was a tendency with the extra nestbox perch (NBP) (Z-value=2.162, *P*=0.0963). Keel bone fracture prevalence increased with age (Wald=1023.24, P<0.0001). If the baseline level were to be removed from the model (percentage of fractures upon placement into the system at 19 weeks of age), there was no significant difference in keel bone fracture prevalence and treatment. When looking at the raw data for overall fracture prevalence, EP pens (45.0% and 39.3%) the M pens (41.1% and 44.7%), and the NBP pens (41.5% and 43.6%) always had a higher keel bone fracture prevalence compared with the C pens (38.6% and 39.2%). However, the raw results only showed a slight difference.

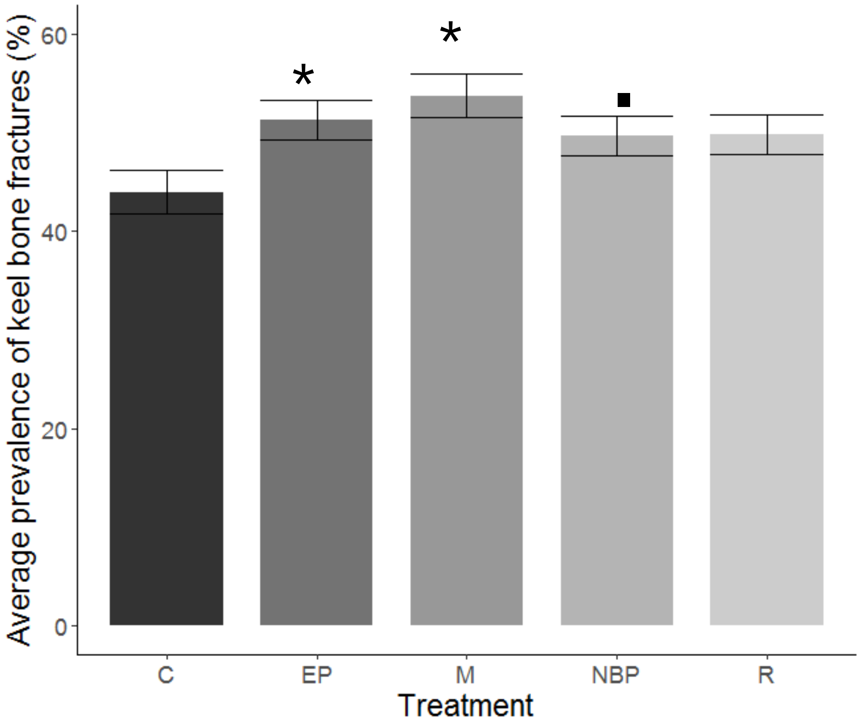

Figure 4.5: *The average prevalence (%) of keel bone fractures across treatment groups. Data are presented as lsmeans (± SE).*

C = control, EP = Extra mushroom-shaped perches, M = Mushroom-shaped perches, NBP = Extra nest box perch and R = Ramp

**** = P<0.0001, **=P<0.01, *=P<0.05, · =P<0.1: different from the control*

4.3.2. Keel bone deviations

There was not an interaction effect between treatment x age on keel bone deviations. There was an overall effect of treatment of the prevalence of deviations (W=10.64, P=0.0310). The NBP treatment had higher levels of keel bone deviations compared with the control group (Z-value=, *P*=0.041) (Figure 4.6). The raw results show that the NBP treatment deviation prevalence per pen was higher (14.6% and 12.8%) compared to the C group (10.6% and 12.2%). If the baseline level (percentage of deviations upon placement into the system at 19 weeks of age), were to be removed from the model, there was no significant difference in keel bone deviation prevalence. and treatment. Keel bone deviations increased with age (W=50.66, P<0.0001).

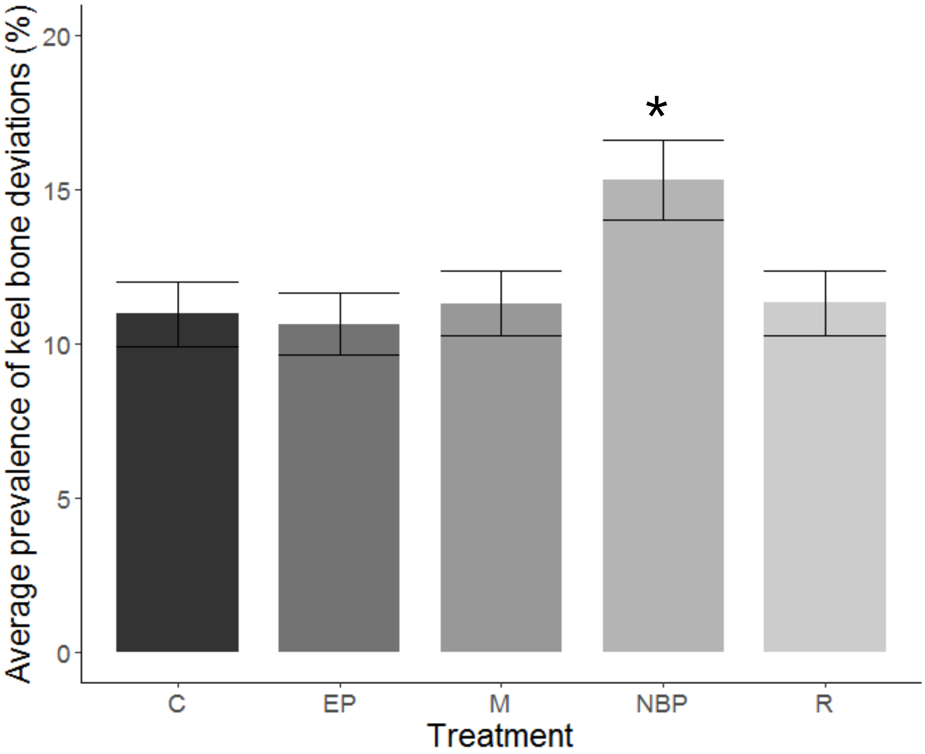

Figure 4.6: *The average prevalence (%) of keel bone deviations across treatment groups. Data are presented as lsmeans (± SE).*

C = control, EP = Extra mushroom-shaped perches, M = Mushroom-shaped perches, NBP = Extra nest box perch and R = Ramp

**** = P<0.001, **=P<0.01, *=P<0.05, · =P<0.1: different from the control*

4.3.3. Footpad health

There was an interaction effect of age x treatment (W=19.15, *P* = 0.041) (Figure 4.7) on bumblefoot prevalence. Bumblefoot prevalence was lower in the C treatment at 48 weeks of age compared to the extra perch treatment (EP) at 48 weeks of age (Z-value=-4.543, *P*=0.0001; Figure 4.7). When looking at the raw results for 48 weeks of age; bumble foot prevalence across pens in the EP treatment (26.7% and 17.9%) was always higher than across the C pens (6.7%

and 4%). Bumblefoot prevalence was lower in the EP treatment at 33 weeks of age compared to 48 weeks of age (Z-value=-6.061, *P* < 0.0001) and 83 weeks of age (Z-value=-3.598, *P* = 0.0075). In the extra nestbox perch (NBP) treatment, bumblefoot prevalence was lower at 33 weeks of age compared to 48 weeks of age (Z-value=-3.826, *P*=0.0031) (Figure 4.7).

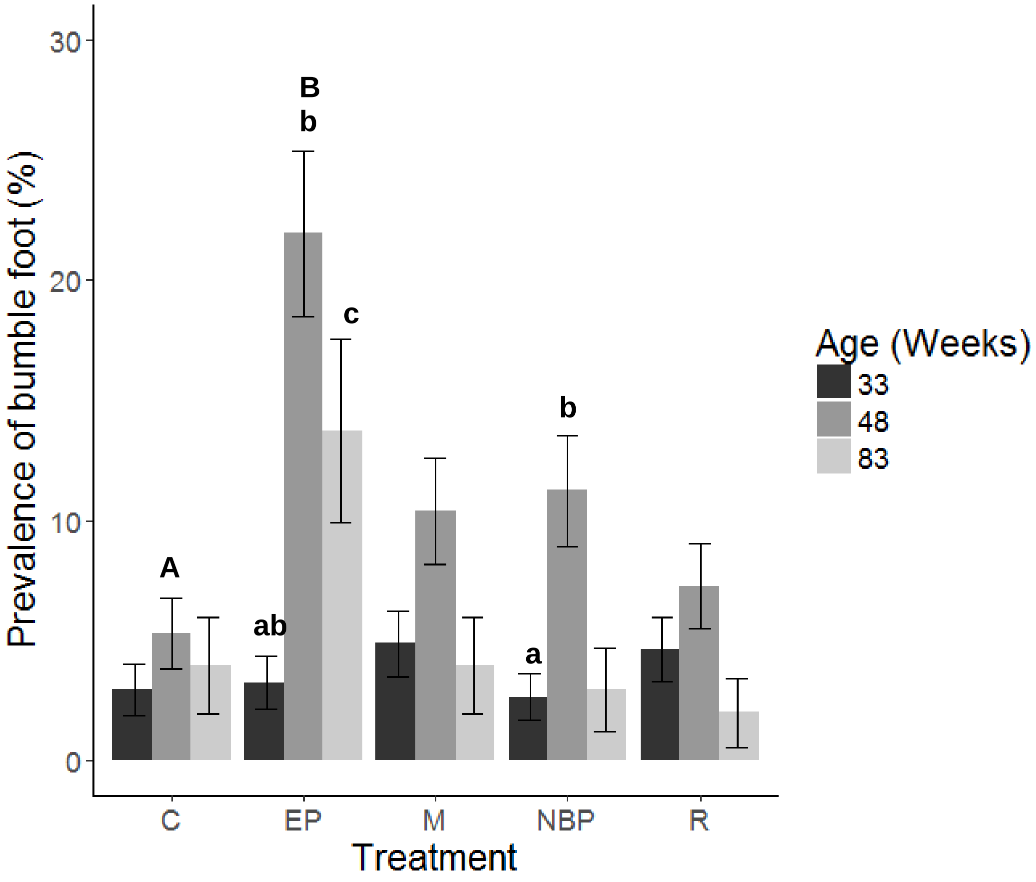

Figure 4.7: *Bumble foot prevalence across treatment groups over different ages.*

Different capital letters denote differences between treatment groups and control within ages. Different lowercase letters denote differences between ages within treatment groups.

C = control, EP = Extra mushroom-shaped perches, M = Mushroom-shaped perches, NBP = Extra nest box perch and R = Ramp

There was an overall effect of treatment on the prevalence of foot pad dermatitis (W=67.83, *P*<0.0001) (Figure 4.8). The EP treatment group had a higher prevalence of foot pad dermatitis compared with the control (Z-value=4.275, P<0.0001). The R treatment group had a lower prevalence of foot pad dermatitis compared with the control (Z-value=-3.313, P=0.0035). When looking at the raw results, the prevalence of overall foot pad dermatitis in the EP pens (38.6% and 47.3%) was higher compared with C pens (28.3% and 26.3%) and was always lower in R pens (13.4% and 21.4%). There was an overall effect of age on the prevalence of foot pad dermatitis (W=10.48, P=0.0053). Prevalence of foot pad dermatitis was higher at 48 weeks of age (30.7 ± 1.4%) compared to 83 weeks of age (23.3 ± 2.0%) (Z-value=3.130, P=0.0047), with no difference at 33 weeks of age (27.6 ± 1.4%).

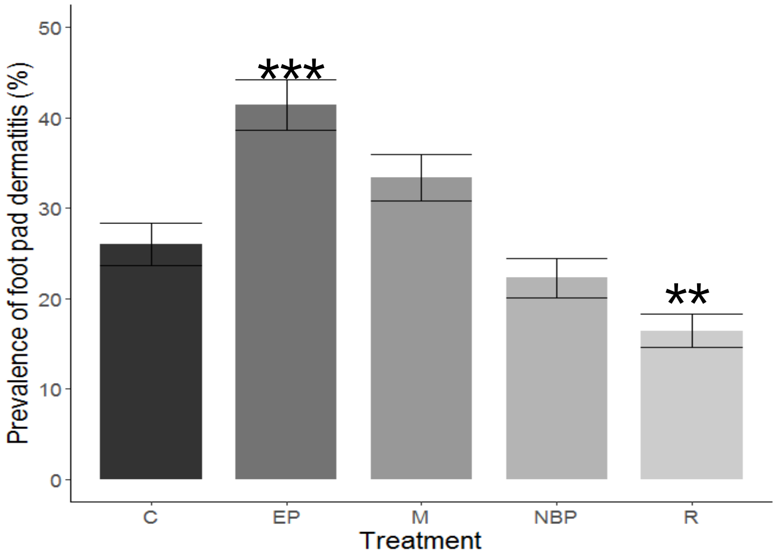

***Figure 4.8:** Prevalence of foot pad dermatitis (%) across treatment groups. Data are presented as lsmeans (± SE).*

C = control, EP = Extra mushroom-shaped perches, M = Mushroom-shaped perches, NBP = Extra nest box perch and R = Ramp

**** = P<0.001, **=P<0.01, *=P<0.05, · =P<0.1: different from the control*

There was an overall interaction effect of treatment x age (W=13.88, *P*=0.008) (Figure 4.9) on the prevalence of hyperkeratosis. The control group at 83 weeks of age had a lower prevalence of hyperkeratosis compared with the ramp treatment group at 83 weeks of age (Z-value=-2.959, *P*=0.0355). When looking at the raw results, the R pens always had a higher prevalence of hyperkeratosis at 83 weeks of age (72% and 78%) compared with the C pens (38% and 68%). However, the C pens had a high variation with 30% difference between pens and may have been due to only 50 hens being sampled at 83 weeks of age instead of 150 hens, as at all the other ages. Hyperkeratosis prevalence was higher at 48 weeks of age for the mushroom perch treatment compared to 83 weeks of age in the mushroom perch treatment (Z-value=3.091, *P*=0.0236).

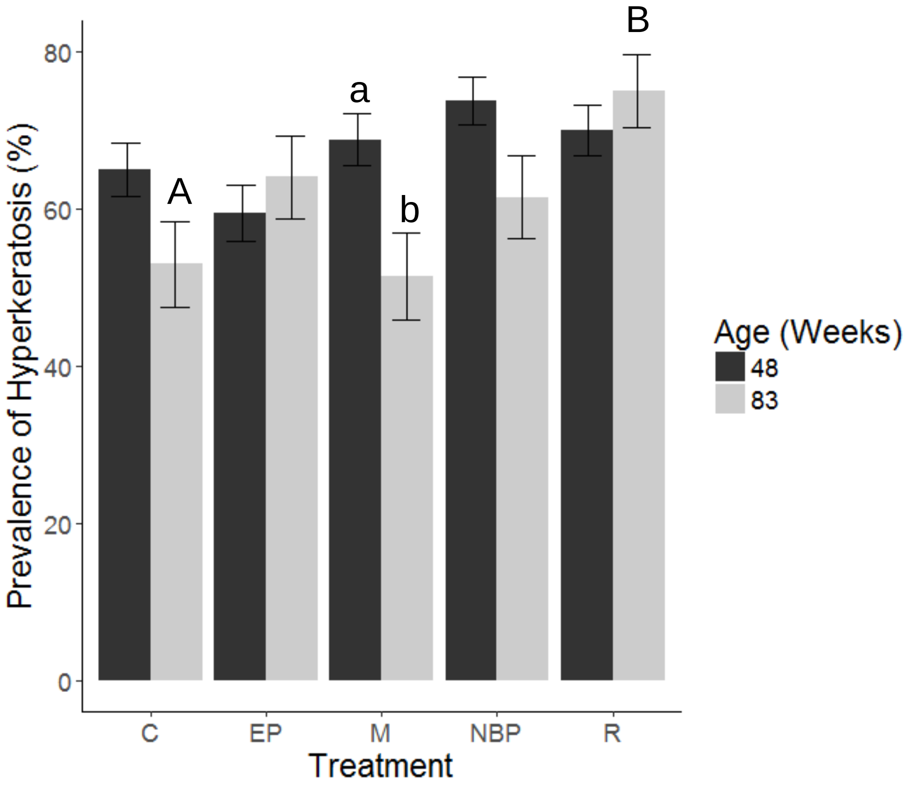

Figure 4.9: *Prevalence of hyperkeratosis across different age and treatment groups. Data are presented as lsmeans (±SE).*

Different capital letters denote differences between treatment groups and control within ages. Different lowercase letters denote differences within treatment groups.

C = control, EP = Extra mushroom-shaped perches, M = Mushroom-shaped perches, NBP = Extra nest box perch and R = Ramp

4.3.4. Feather cover

There was an overall effect of treatment on the percentage of hens with perfect feather cover (W=16.09, *P*=0.003) (Figure 4.10). The NBP had a lower percentage of hens with perfect feather cover compared to the control group (Z-value=-2.723, *P*=0.0223) and there was a tendency for a lower percentage of perfect feather cover in the M treatment (Z-value=-2.325, *P*=0.0653).

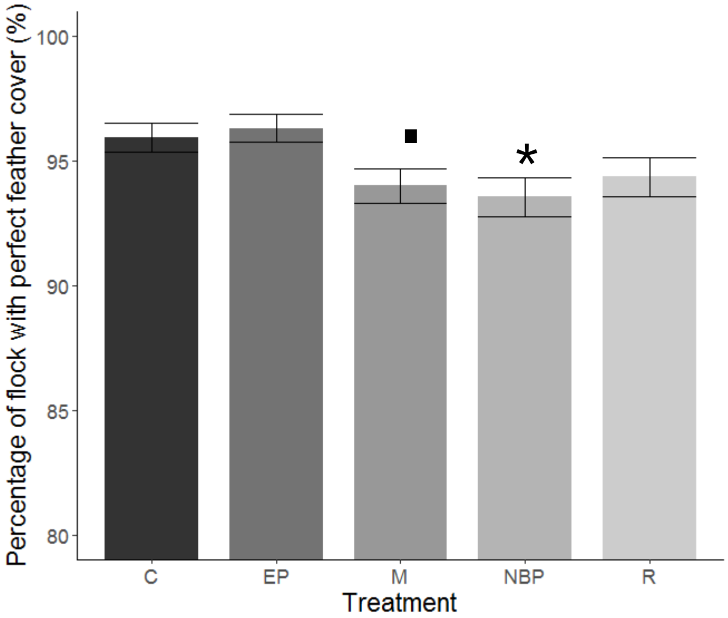

Figure 4.10: *Difference in plumage condition between the control and all treatment groups. Error bars show lsmeans (± SE)*

C = control, EP = Extra mushroom-shaped perches, M = Mushroom-shaped perches, NBP = Extra nest box perch and R = Ramp

*** = P<0.001, **=P<0.01, *=P<0.05, · =P<0.1: different from the control*

4.3.5. Body mass

There was no interaction between treatment x age on the body mass of hens (P>0.05). There was an effect of treatment (F=6.79, P=0.0297) and age (F=9.477, P=0.0021) on the body mass of hens. Hens from the NBP treatment (1.944 ± 0.006kg) weighed less than hens from the C treatment (1.978 ± 0.006kg; Z-value=-3.853; P<0.0001). Hens at 48 weeks of age (1.977 ± 0.004kg) weighed more than those at 33 weeks of age (1.962 ± 0.004kg; Z-ratio=-3.078, P=0.0021).

4.3.6 Litter deterioration

There was an overall effect of treatment on litter quality (W=11.28, *P*=0.0236). There was a difference between the C treatment and the NBP treatment (Z-value=2.889, *P*=0.0138) (Figure 4.11).

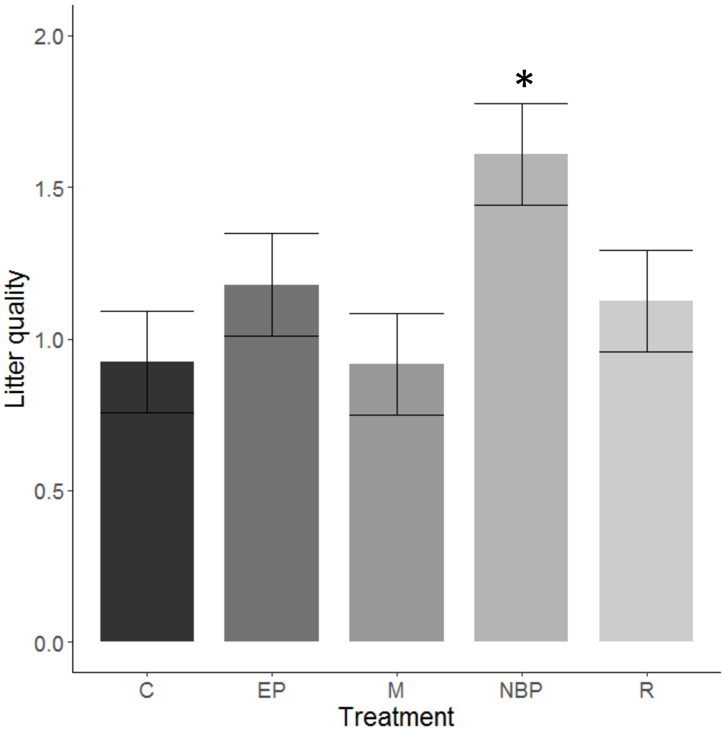

Figure 4.11: Difference in litter quality between the control and all treatment groups. 0=no litter damage, 1=<10% damaged litter, 2=10-50% damaged litter and 3=>50% damaged litter. Data show is lsmeans (±SE)

C = control, EP = Extra mushroom-shaped perches, M = Mushroom-shaped perches, NBP = Extra nest box perch and R = Ramp

**** = P<0.001, **=P<0.01, *=P<0.05, · =P<0.1: different from the control*

4.3.7. Tibia radiological and biomechanical analysis

Table 4.2 shows the bone quality parameters and their significance levels. There was no effect of treatment on the radiological and biomechanical properties of the tibia, except for the vertical diameter of the outer tibia (mm). There was no overall effect of the treatment on the vertical diameter (mm) of the tibia but the NBP treatment tended to have a larger outer vertical diameter compared to the control in the post-hoc analysis (Z-ratio=2.262, P=0.0782). Keel bone deviation score was removed from all models because it never reached a tendency. Keel bone fracture score was removed if it did not reach a tendency. For all parameters body mass was significant and all parameters increased as weight increased (Table 4.2). There was a tendency for the bone mineral density (BMD) (Z-ratio=2.237, P=0.0624) and bone mineral content (BMC) (Z-ratio=2.184, P=0.0709) to be higher in hens without fractures (score 0) compared to those with severe fractures (score 2). The length of the tibia was significantly higher in hens without fractures (score 0) than those with fractures (score 2) (Z-ratio=-2.782, P=0.0142) (Table 4.2). The maximum load (N) was higher in hens without keel bone fracture compared to those with score 1 (Z-ratio=2.213, P=0.0662) and score 2 (Z-ratio=2.929, P=0.0091) respectively (Table 4.2). The energy at maximum load (KJ) was higher in hens with no keel bone fracture compared to those with a keel bone fracture score 1 (Z-ratio=2.202, P=0.0681) and score 2 (Z-ratio=2.594, P=0.0245). There was no difference between score 1 and 2. The flexure stress (Z-ratio=2.55, P=0.0277) was significantly higher in hens without keel bone fractures compared to those with severe keel bone fractures (score 2) (Table 4.2).

Table 4.2: *Bone quality measurements in relation to treatment and keel bone fracture status of hens at 83 weeks of age*

Bone quality parameter	**Treatment**					**Keel bone fracture score**					
	C	EP	M	NBP	R	0 (n=22)	1 (n=90)	2 (n=88)	Treatment	Keel fracture	Body mass
BMD (mg/cm^2)	317.1 ± 11.1	303.6 ± 10.6	304.2 ± 10.5	312.5 ± 10.9	309.3 ± 10.6	321.8 ± 10.2^a	307.4 ± 5.7	299 ± 5.6^b	F=0.29, P=0.883	F=2.73, P=0.065	F=29.94, P<0.0001
BMC (mg/cm)	55.1 ± 1.2	54.8 ± 1.2	53.8 ± 1.2	54.3 ± 1.2	53.8 ± 1.1	56.5 ± 1.6^a	54 ± 0.7	52.7 ± 0.7^b	F=0.26, P=0.89	F=5.05, P=0.08	W=66.57, P<0.0001
Length (mm)	121.8 ± 0.6	121.6 ± 0.6	121.8 ± 0.6	122.1 ± 0.6	120.8 ± 0.6	120.6 ± 0.6^a	121.8 ± 0.3	122.5 ± 0.3^b	F=0.69, P=0.6275	F=4.22, P=0.0162	F=83.5, P<0.0001
Horizontal outer diameter (mm)	7.8 ± 0.1	7.8 ± 0.1	7.8 ± 0.1	7.9 ± 0.1	7.8 ± 0.1	NA	NA	NA	F=0.35, P=0.834	NA	F=30.69, P<0.0001
Vertical outer diameter (mm)	6.7 ± 0.05^a	6.8 ± 0.05	6.8 ± 0.05	6.9 ± 0.05^b	6.8 ± 0.05	NA	NA	NA	F=1.6, P=0.3077	NA	F=26.51, P<0.0001
Cortical bone thickness (mm)	0.795 ± 0.031	0.781 ± 0.031	0.761 ± 0.03	0.760 ± 0.031	0.761 ± 0.031	NA	NA	NA	F=0.027, P=0.8869	NA	F=26.3, P<0.0001
Max load (N)	259.8 ± 13.5	254.5 ± 13.1	250.8 ± 13.0	253.1 ± 13.1	255.0 ± 13.0	275.4 ± 12.3^a	248.7 ± 6.8^b	241.0 ± 6.6^b	F=0.07, P=0.9897	F=4.29, P=0.0151	F=48.08, P<0.0001
Energy at maximum load (KJ)	209.8 ± 8.2	207.8 ± 8.2	196.7 ± 8.2	211.4 ± 8.2	203.3 ± 8.0	224.1 ± 10.3^a	198.8 ± 5.1^b	194.4 ± 5.2^b	F=0.59, P=0.6859	F=3.32, P=0.0382	F=48.83, P<0.0001
Stress (MPa)	62.3 ± 3.2	58.9 ± 3.2	58.2 ± 3.2	61 ± 3.2	60.8 ± 3.2	64.6 ± 2.9^a	59.3 ± 1.7	56.8 ± 1.7^b	F=0.28, P=0.8765	F=3.38, P=0.036	F=4.8, P=0.0297

Different subscripts mean there was a different significant difference between the control and treatment or between severity scores.

4.3.8. Perching behaviour

The data has been presented as both the mean total number of birds perching on elevated perches (Figure 4.12) and the number of birds per elevated perch (Figure 4.13). Although only mean values have been presented to show the general trend of the data, it can be seen in Figure 4.12 that the number of hens perching is numerically higher during the night compared to other time points. The absolute number of hens on elevated perches is generally higher in the EP treatment group compared with the other treatments and the control. At night there are more hens on the middle perches in the NBP treatment. This trend does not continue for the other time points, where the M treatment tends to have the highest number of hens perching in the middle region. In all instances, the number of hens perching on elevated perches on the lower tier is fewer than those on the middle or top tier.

However, when looking at the number of hens per perch, the M treatment appears to have the highest number of hens per perch in the middle and top tier of the system. The lower tier perches are more variable, with the R treatment having one of the highest levels of perching per perch (Figure 4.13).

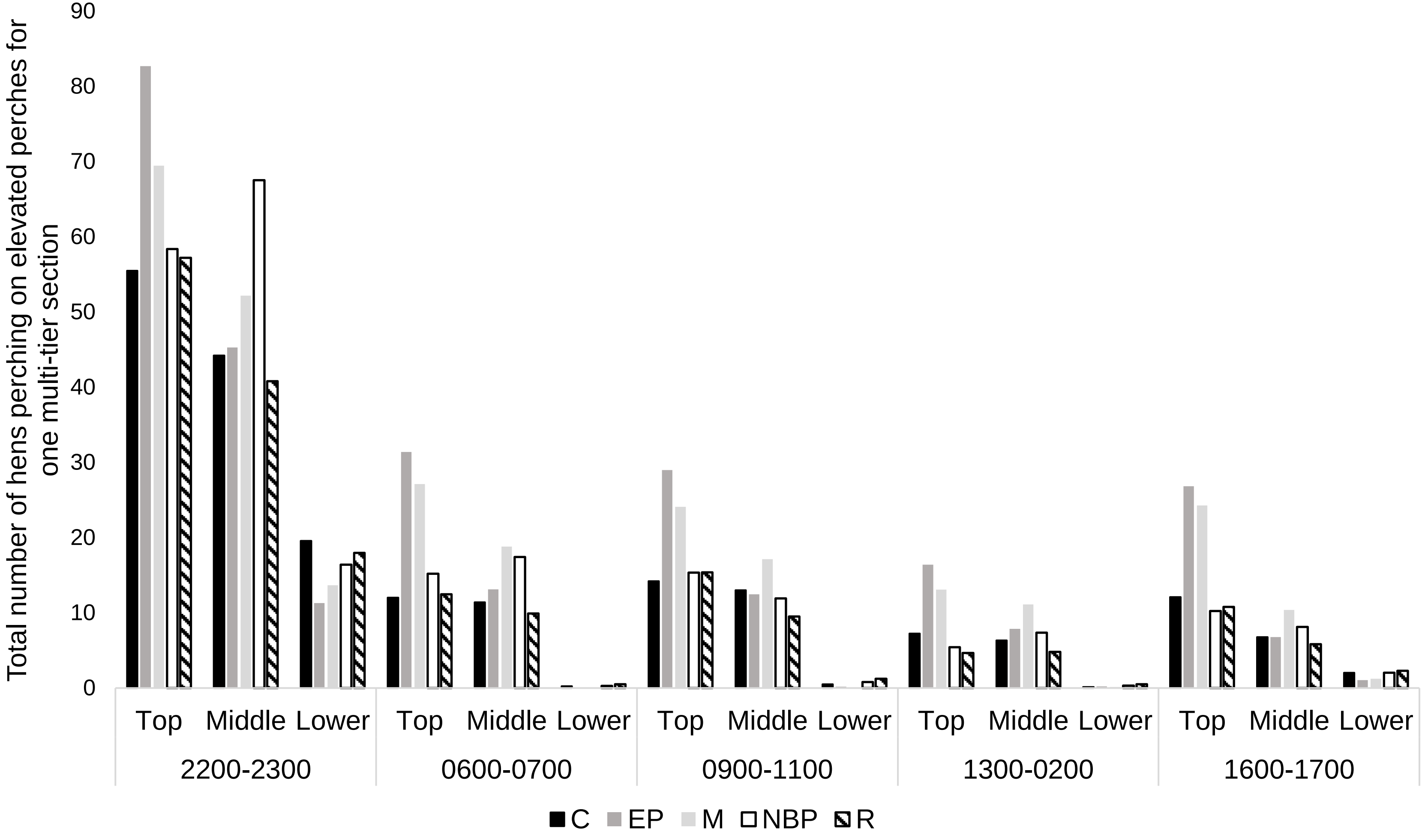

Figure 4.12: *The total number of hens perching on elevated perches for one multi-tier section across different times of day*

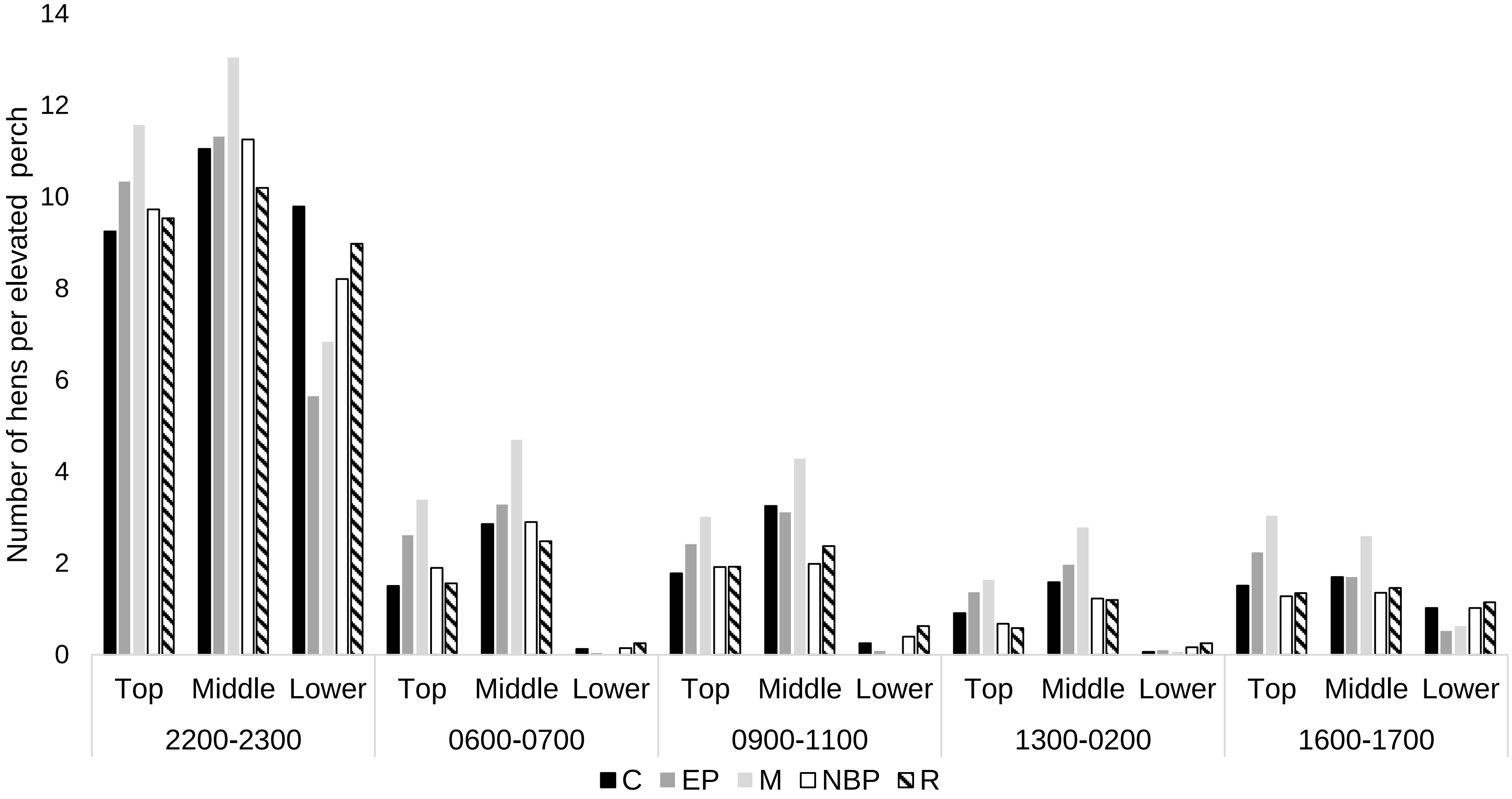

Figure 4.13: *The number of hens per elevated perch for one multi-tier section across different times of day*

4.3.9. Maximum summed acceleration vector (AV)

For the maximum acceleration AV on the keel, there was no overall effect of treatment (F=2.29, P=0.196) but there was an effect of the multiple comparisons when comparing each treatment to the control (Figure 4.14). The EP treatment group had a lower maximum summed AV on the keel compared with the C treatment (Z-value=-2.785, P=0.0193). The M treatment tended to have a lower maximum summed AV on the keel (Z-value=-2.247, P=0.0812). There was an effect on the number of outputs recorded on the accelerometer (F=10.52, P=0.0015) and the body mass of the hens (F=4.28, P=0.0404). As the number of outputs from the accelerometer increases, the maximum summed AV on the keel decreases and heavier hens had higher maximum summed AVs compared to lighter hens.

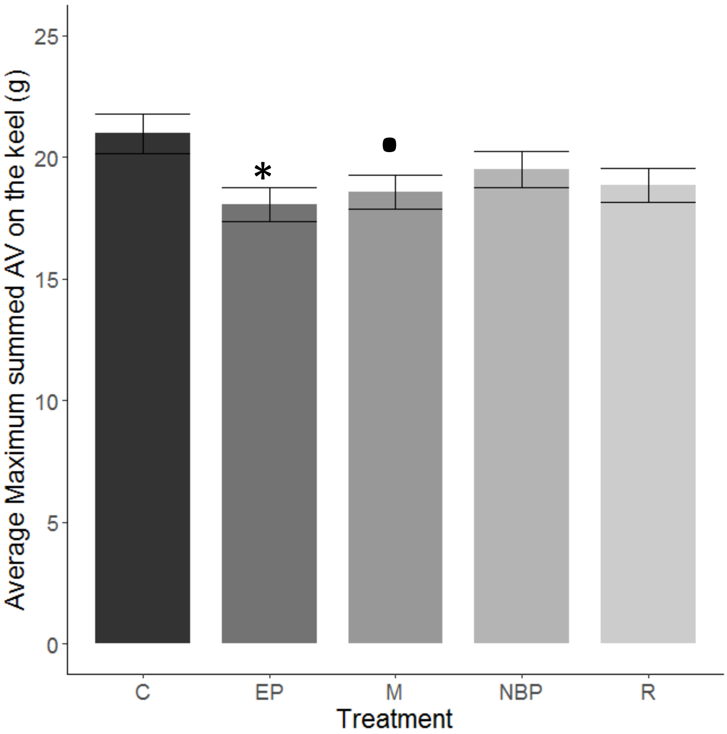

Figure 4.14: *Maximum summed AV on the keel*

C = control, EP = Extra mushroom-shaped perches, M = Mushroom-shaped perches, NBP = Extra nestbox perch and R = Ramp

**** = P<0.001, **=P<0.01, *=P<0.05, · =P<0.1: different from the control*

There were no overall differences between the treatment and the maximum summed AV on the body (F=0.82, P=0.5667) and there were no differences in the multiple comparison analysis (Figure 4.15). There was a difference in the number of outputs recorded on the accelerometer (F=13.11, P=0.0004), as the number of counts increase, the average maximum summed AV calculated from all the outputs on the body decreases.

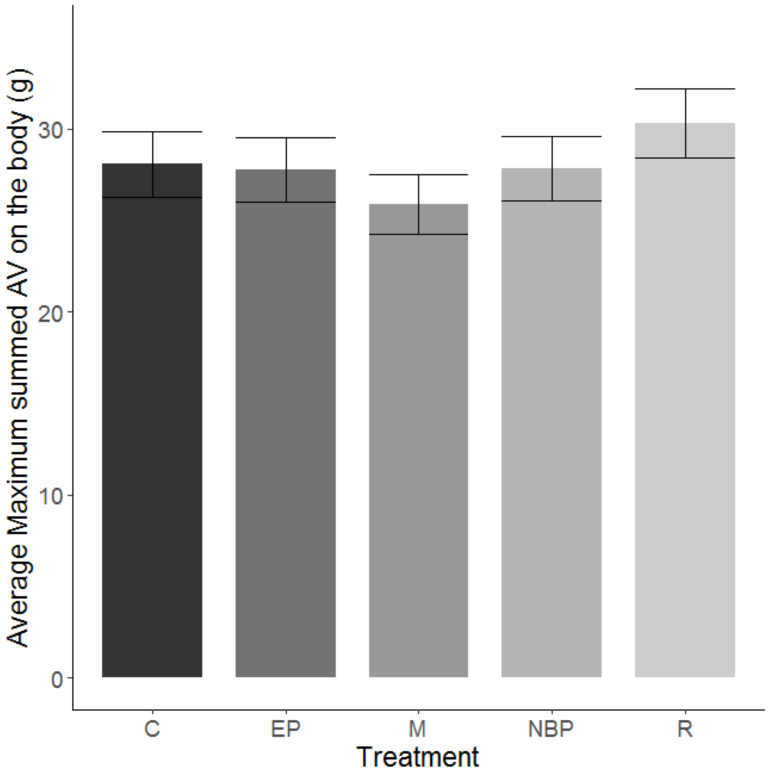

Figure 4.15: *Average maximum summed AV on the body*

C = control, EP = Extra mushroom-shaped perches, M = Mushroom-shaped perches, NBP = Extra nestbox perch and R = Ramp

**** = P<0.001, **=P<0.01, *=P<0.05, · =P<0.1: different from the control*

The number of accelerometer readouts per hen per hour is shown in Figure 4.16. There are more accelerometer readouts in the control groups between 1400hr and 1900hr compared with all the other treatments. The general trend shows that readouts are not generated until 0500hr and tend to stop after 2100hr.

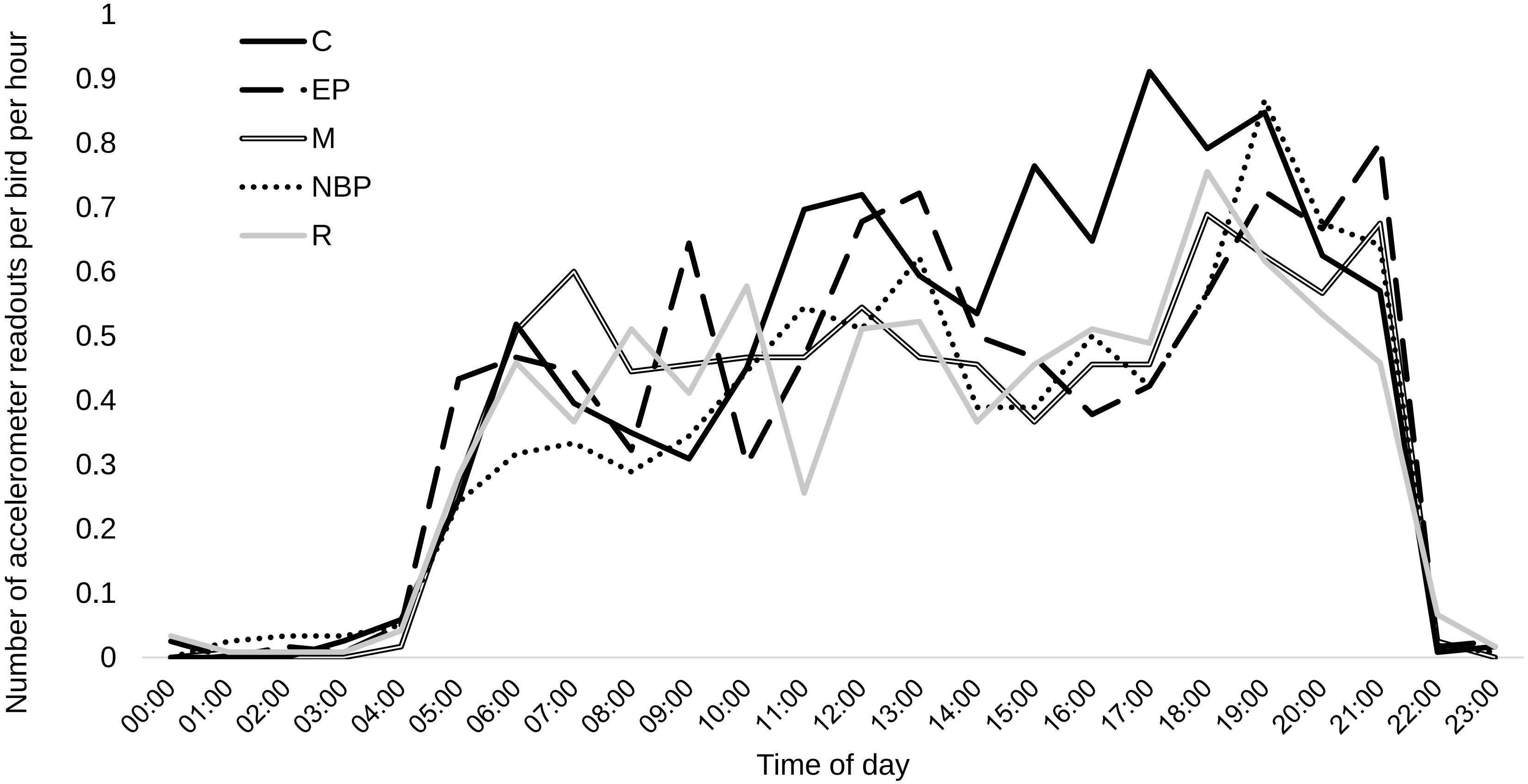

Figure 4.16: *The number of accelerometer readouts per hen per hour.*

C = control, EP = Extra mushroom-shaped perches, M = Mushroom-shaped perches, NBP = Extra nest box perch and R = Ramp

4.4. Discussion

4.4.1. Keel bone damage

The extra mushroom-shaped perch treatment (EP), the mushroom-shaped perch treatment (M) and the extra nestbox perch treatment (NBP) all had higher levels of keel bone damage compared with the control treatment (C). Although it was predicted that additional treatments would reduce the prevalence of keel bone fractures in comparison to the control (prediction **1**), this was not the result that was found.

The addition of extra perches around the nest box may have led to an additional hazard, causing hens to collide with the extra structure instead of the perch providing an added region for transitions to occur. The mushroom-shaped perches were also hypothesised to reduce keel bone fracture prevalence by reducing the number of falls and collisions, compared with standard metal perches by providing improved grip (Scholz *et al.*, 2014). However, mushroom-shaped perches did not reduce keel bone fracture prevalence. This may be due to an increased surface area compared with standard metal perches meaning that hens may be more likely to collide with these perches or that there is a larger contact area on the keel (Pickel *et al.*, 2011), which may lead to increased keel deformity. Another possible reason for the increase in keel bone fractures could be the greater use of elevated perches. The presence of elevated perches has been linked to increased keel bone fracture prevalence previously (Wilkins *et al.*, 2011). In the current study, there were more hens perching in both the mushroom-shaped perch treatments (EP and M) compared to the other treatments. This may mean that hens are moving to perches more frequently, which can increase the likelihood of having a fall or a collision and thus increasing keel bone fracture prevalence.

The addition of extra perches on the top level of the system led to an increased prevalence of keel bone fractures in the current study. Another study found that the addition of extra perches on the top tier increased keel bone fracture prevalence compared with a control treatment (Stratmann *et al.*, 2015a), which was in line with the results found in the current study. However, it was thought by adding in 4 extra perches instead of 2 (as in the study by Stratmann *et al.* (2015a), keel bone fracture prevalence would decrease due to reduced risk of displacement by conspecifics.

Previous studies have found that ramps reduce keel bone fractures prevalence throughout lay (Stratmann *et al.*, 2015a; Heerkens *et al.*, 2016a) but this was not found in the current study. Both previous studies used ramps that were orientated towards tiers or perches, whereas in the current study transverse ramps were used that connected the lower tier to the top tier through the nest box area. The previous studies were conducted in small experimental units (Stratmann *et al* 2015a: 225 birds/pen and Heerkens *et al* 2016a: 25 birds/pen) where ramps were present in all areas of the system (Stratmann *et al.*, 2015a; Heerkens *et al.*, 2016a). In the current study, ramps were placed intermittently (once in every two aviary units), transversely across the system, in commercial conditions (3,600 birds per pen). These results suggest that ramp orientation and the percentage of the house equipped with ramps may be important in the development of keel bone fractures. Another reason for the difference could be that 150 birds were palpated in each pen during the current study and only 6 and

20 birds per pen were palpated previously (Stratmann *et al.*, 2015a; Heerkens *et al.*, 2016a).

The NBP treatment had a higher prevalence of keel bone deviations in comparison to the C treatment. This may be because the additional round perch provides an additional area for more hens to rest and potentially bend their keel. Round, metal perches have been shown to be the perch with the highest pressure and mushroom-shaped perches had a lower pressure at the keel (Pickel *et al.*, 2011). This suggests that although hens perched more on the mushroom-shaped perches, in the current study, the pressure was lower compared with round perches, which may have led to lower levels of keel bone deviations. One study by Heerkens *et al.,* 2016a found that birds housed with ramps had a lower prevalence of mild keel bone deviations compared to birds that were not housed with ramps. There was a tendency for severe deviations to show the same pattern (Heerkens *et al.*, 2016a). There was no decrease in the prevalence of keel bone deviations in the ramp treatment group in the current study, corroborating the study by Stratmann *et al.,* 2015a that showed no difference in keel bone deviation prevalence across treatments (those provided with ramps, extra perches and platforms compared to a standard Bolegg terrace system).

It is important to note that future studies should aim at replacing existing structures instead of adding to the complexity of the environment. It may be that adding structures makes the environment more difficult for hens to navigate and provides extra structures for collisions to occur.

4.4.2. Footpad problems

In the current study, at 48 weeks of age, bumblefoot prevalence was higher in the EP treatment (extra mushroom perches on the top tier of the system) compared to the C treatment. This is contrary to prediction **2**, but in line with a previous study in which plastic mushroom-shaped perches increased the incidence of bumblefoot compared to wooden rectangular perches (Tauson and Abrahamsson, 1994). However, in that study, it is not known whether it is the mushroom shape or the plastic material that increases bumblefoot presence (Tauson and Abrahamsson, 1994). Nevertheless, in conjunction with the current study, there is a stronger likelihood that mushroom-shaped perches may be deleterious for foot pad health. In the current study, there was no difference between the C treatment and the M treatment, suggesting that the mushroom-shaped perches were only a problem for bumblefoot as the number of perches increased.

The results suggest that footpad dermatitis (FPD) was more prevalent in the EP treatment compared to the C treatment. This is also contrary to our prediction **2**, that mushroom-shaped perches should reduce foot pad problems. Previous research stated that the force exerted on the foot pad was potentially more damaging from a commercial round, metal perch compared mushroom perches (Pickel *et al.*, 2011) and thus better FPD scores were expected in the EP treatment. Previous research has stated that FPD can be aggravated by wet litter (Wang *et al.*, 1998). However, the litter quality in the EP treatment was not markedly different from the control group, and as such was likely not to be the cause for the observed differences in FPD. One reason for the increase in FPD in the EP treatment may be that the mushroom-shaped perches had small grooves along the surface (where the birds’ rested their foot pads). However, there was no difference in FPD and the M treatment (in which standard perches were replaced by mushroom-shaped perches) compared to the C group. The reason for this may be the same as that for bumblefoot. Mushroom-shaped perches may only pose a health risk as the number increases, potentially due to the increased number of hens perching as the availability of mushroom-shaped perches increase. However, the same issue presents itself in that we do not know whether it is the increase in the number of perches that is the problem or the fact that the perches are mushroom-shaped.

Nevertheless, the grooves on the mushroom-shaped perches accumulated faecal matter (personal observation). This would correspond to previous research stating that FPD prevalence was higher in treatment groups with perches made wet by submersion compared with dry perches (Wang *et al.*, 1998). It is important to determine if the removal of grooves from the mushroom-shaped perches (making the surface smooth) or changing the profile of the grooves, would reduce FPD.

If litter quality in the current study were to be the main causal factor for poor foot pad health, it would be expected that footpad problems would be more prevalent in the NBP treatment. Litter deterioration was more advanced in the NBP treatment than the control and poor litter quality is a risk factor for poor foot health (Wang *et al.*, 1998). But this was not the case, showing that other factors must be involved in foot pad health. It was expected that the NBP treatment would have poor litter quality compared to other treatments because the extra nestbox perch extended over the litter, leading to a faecal build up on the litter directly beneath the perches.

There was a decreased prevalence of FPD in the R treatment compared to the C treatment. This was similar to what has been found previously (Heerkens *et al.*, 2016a). One reason for this may be that as hens walk, any faecal build-up or dirt on their feet may be scraped off as they move along the ramp.

Hyperkeratosis was higher in the R treatment compared to the control. This may be an artefact due to hens in the R treatment having a lower prevalence of both bumble foot and FPD, making it easier to distinguish hyperkeratosis. It may also be that hens walk on the ramps losing litter from their feet and making their feet drier compared to hens in the control. Hyperkeratosis is thought to be caused by an increased pressure on wire flooring, in particular, sloping wire floors have been suggested as the problem (Abrahamsson and Tauson, 1995; Tauson, 2002; Weitzenbürger *et al.*, 2006), the ramps in the current study were made from metal and could have caused increased compression on the foot pads. However, hyperkeratosis is not thought to be as great a welfare issue as bumble foot or FPD as it is thought to be less painful due to lack of inflammation (Tauson, 2002).

Overall, foot pad health was worse in the EP and M groups, one reason this may be that the increased levels of perching in the EP and M groups may prolong the time that individual bird’s foot pads are in contact with faecal matter. Further work on mushroom-shaped perch design would be advantageous because it appears that mushroom-shaped perches are preferred over standard round perch, which is indicated by more birds perching in the M group compared to the C group in the current study.

4.4.3 Feather condition

The reason for poorer feather condition in NBP treatment compared to the control may be that the proximity of the additional perches to the nest box results in a high incidence of feather pecking behaviour. Previous work has shown that severe feather pecking is high around the nest box (Nicol *et al.*, 1999). The closeness to the nest box could potentially result in hens pecking feathers of other hens. However, it is important to note that the feather condition of hens was very good, and the data were analysed as perfect or not perfect. Thus, most hens had perfect feather condition and it is most likely not a welfare issue if few hens have marginally less than perfect feather condition.

4.4.3. Body mass

Hens from the NBP treatment had lower average weights, even though the result was significant, the absolute difference was very slight and is not likely to be a welfare issue. It was not possible in the current study to determine if there was a correlation between hens with poor feather cover and their body mass because different hens were weighed, and feather scored. However, previous work on broiler breeders indicates that as body mass increases, feather condition improves (Renema *et al.*, 2007). Hens that were aggressively feather pecked (leading to an increased incidence of feather damage) had lower body weights than conspecifics that had a lower incidence of aggressive feather pecking directed towards them (Bilcik and Keeling, 1999). However, the link between body mass and feather condition in the current study can only be speculated because the same individuals were not scored for both parameters.

4.4.5. Tibia bone analysis

Prediction **4** stated that the addition of extra perches would result in increased bone strength due to an increase in loading on the bones through exercise when jumping onto perches (Knowles and Broom, 1990; Casey-Trott *et al.*, 2017). However, there was no difference between the treatments for any of the bone properties. This could be because the hens were all reared in aviary systems with the ability to fly and jump around the system and potentially the treatment groups were not different enough to influence bone health.

There was an effect of keel bone fracture presence on tibia bone properties but only as tendencies for bone mineral density, bone mineral content, length, maximum load, custom flexure stress and maximum load. The data suggests that hens without fractures have stronger bones and this was shown previously (Toscano *et al.*, 2013). However, the opposite relationship was shown in another study as the prevalence of keel bone fractures increased on a farm, the strength of the tibia also increased (Wilkins *et al.*, 2011). It has been shown previously that the keel bones of hens with no fractures or slight deviations had a higher mineral content and calcium content within the keel bone compared to those with fractures (Gebhardt-Henrich *et al.*, 2017). However, hens were not checked to determine if they were still egg laying. Cessation of egg-laying would affect bone strength by limiting the release of oestrogen, allowing the structural cortical bone volume to increase and non-structural medullary bone to decrease (Whitehead, 2004). The number of hens with no keel bone fractures was low in the current study compared to those with fractures. Therefore, future studies investigating more hens with no keel bone fractures would be advantageous. Humeri were not analysed in the current study due to their non-uniform shape, but it may be worthwhile looking at differences in the humeri of hens when exposed to different housing environments because differences were seen when comparing different cage designs in the strength of the humerus but not the tibia (Vits *et al.*, 2005).

4.4.6. Perching Behaviour

Perching behaviour was not analysed statistically and any differences that are mentioned are done numerically. The mushroom perches appear to be utilised more for perching, this may be due to the mushroom-shaped perches being more comfortable compared with standard, round perches because it has been shown that peak forces on the feet of hens are lower when standing on mushroom perches (Pickel *et al.*, 2011). It is important to note that it is unclear whether the hens in the current study perch more on the mushroom-shaped perches because they are more comfortable, or because they are in more pain due to the increase in levels of keel bone fractures and footpad lesions. Due to it being difficult to collect accurate perching data at night, prediction **3** cannot be corroborated definitively in the current study but the mushroom-shaped perches did result in more hens perching overall.

4.4.7. Maximum summed acceleration vector (AV)

Both treatments with mushroom-shaped perches had lower levels of maximum summed acceleration vector (AVs) at the keel compared to the C group. This suggests that hens were potentially performing less hazardous activities (falls and collisions; from Chapter 2), as was stated in prediction **3**. However, video analysis was not paired with accelerometer output in the current study, so the actual behaviours hens were performing are unknown. Nonetheless, lower maximum summed AV were seen in the mushroom-shaped treatments suggesting movements were more controlled because results from Chapter 2 have shown that falls have higher maximum AV at the keel compared with controlled movements. However, this did not reflect in a decrease in keel bone fracture rates, on the contrary, there was an increase in keel bone fracture prevalence in the mushroom-shaped perch treatments.

However, there were limited differences, and this may be because of only a very small percentage of the total flock equipped with accelerometers. Seven/eight birds were used per flock and these hens were picked to match average weight for the breed of the hens used and to have limited keel bone, foot and feather condition problems. In comparison to Chapter 1 and 2 the accelerometer data was analysed slightly different here. This is partly due to the reduced sample size in the current study making it difficult to incorporate all the individual bird health characteristics. Also, the outputs for each hen were summed over the week for the current study. This created less variation in the data, meaning that if the data were not summed, no differences may have been detected due to the large variation.

4.5. Limitations

The main limitation of the study is that only two replicates per treatment were used. However, in all cases of keel bone damage (fractures and deviations) and footpad lesions (bumblefoot, dermatitis and hyperkeratosis) that were significant, the raw data also showed either an increase or a decrease in prevalence, matching the statistical model. This increases the likelihood that the results were true findings and not pen effects.

Another limitation is the inability to tag and re-catch the same hens at each time point to score for all health parameters. As well as this, 150 hens are approximately 4% of each pen. This is a low number to give a true estimate of a population, when using a free sample size calculator available at: (www.surveymonkey.com/mp/sample-size-calculator/). To obtain a 95% confidence interval with a margin of error of 10%, only 94 hens out of a sample of 3,600 hens would need to be palpated. However, if a 5% error is needed then the sample size rises to 348 hens for a flock of 3,600 hens. Due to the small difference was seen between pens and the prevalence of keel bone fractures in this study, it suggests that a sample size greater than 150 would be more accurate at determining the true prevalence of keel bone fractures. However, other studies looking at keel bone prevalence on the farm used a sample size of 100 hens per farm (Käppeli *et al.*, 2011; Wilkins *et al.*, 2011), whereas only 50 hens were scored in another study (Heerkens *et al.*, 2016b). Only one study reported flock sizes and they ranged from 12,000-120,000 hens (Käppeli *et al.*, 2011).

Although mentioned in the introduction and discussion; movements (falls and collisions) were not analysed in this chapter. Video data were collected during this study but was not fully analysed so is not presented here. All pens were video recorded for eight days in total across two-time points between 30-35 weeks of age and 45-50 weeks of age. Treatment groups were always recorded together with a control group. Video footage from the top tier to the litter on both sides of the systems (x2 days) and the top level of the system (x2 days). It would be optimal if future studies assessed the movement of hens in this system through the video data already collected or in any further studies in this area consider incorporating this type of behavioural analyses into their study design.

As in Chapter 2 and 3 a relatively small sample of hens from the total population were equipped with accelerometers. In future studies it would be beneficial to use more hens so that a wider range of behaviours and outputs could be shown.

4.6. Conclusion

In conclusion, the modifications made to the standard Bolegg terrace system did not improve laying hen health. Keel bone fracture prevalence increased in the M, NBP and R treatments. Bumblefoot and foot pad dermatitis prevalence increased in the EP treatment and hyperkeratosis increased in the R treatment. However, foot pad dermatitis decreased in the R treatment. This suggests that the addition of ramps may be a modification that warrants further study to aid in reducing painful footpad lesions in laying hens, but more is needed to be done to tackle reduction in keel bone fractures. Another avenue for future study would be altering existing structures instead of adding more structures to a system, this was explored in the current study by installing mushroom-shaped perches in the M treatment compared to the round perches in the C treatment. Other alterations could be providing softer perch material, outer movements on the system provided by ledges instead of perches, with perches still being provided in the system and on the top tier and possibly reducing the overall height of the multi-tier system.

Chapter 5

Effect of ramps on keel bone damage, related health parameters and behaviour of hens in commercial multi-tier systems

5.1. Introduction

Keel bone damage (fractures and deviations) and foot pad disorders are major welfare issues as they can cause pain (Gentle, 2011; Nasr *et al.*, 2012b; Kalaiselvi *et al.*, 2014; Malik and Valentine, 2018; Riber *et al.*, 2018). Keel bone fracture aetiology is multifactorial and includes; nutrition, genetics, environment, rearing conditions and bone strength (Harlander-Matauschek *et al.*, 2015). Whereas, keel bone deviations are thought to be caused by prolonged resting on perches and are not thought to be caused by the same factors as keel bone fractures (Scholz *et al.*, 2008).

Due to the EU ban on conventional cages (European Commision, 1999) alternative systems are being used and include a high proportion of multi-tier systems (Stadig *et al.*, 2016). However, raising the height and number of perches in systems leads to increased keel bone fracture prevalence due to an increased likelihood of having a fall or collision with a structure (Gregory and Wilkins, 1996; Moinard *et al.*, 2004a).

5.1.1. Keel bone damage

Addition of extra structures within systems, such as ramps, can give hens the option to walk between tiers instead of flying, which can prevent falls and collisions (Stratmann *et al.*, 2015a). Poultry companies now provide ramps to customers both at rearing and during lay for hens. Ramps provide a surface for hens to transition between different levels of a multi-tier system. Previous work by Heerkens *et al.* (2016a) showed that the provision of ramps can reduce keel bone fracture prevalence. The previous study was experimental and used small groups of birds, 16 pens housing 25 birds in each pen. However, results from this study have not yet been extrapolated to on-farm settings. Another study showed that the presence of ramps throughout lay reduced the prevalence of keel bone fractures (Stratmann *et al.*, 2015a). However, the Stratmann et al. (2015a) study was also experimental but with a larger number of birds. Farm-level studies are needed to understand how furnishings influence keel bone fracture prevalence and other issues because hens may move differently in larger groups compared to small groups. A review by Rodenburg and Koene (2007) looked at multiple studies and the effect of housing poultry in large and small groups. The main conclusion was that large groups show more fear, stress and feather damaging behaviour (Rodenburg and Koene, 2007). Therefore, it is possible that hens from controlled studies may act differently than those from commercial studies.

Hybrid differences in keel bone damage prevalence are well documented and multiple studies have been conducted examining the keel bone damage (fractures and deviations) prevalence in different laying hen hybrids (Stratmann *et al.*, 2015b; Heerkens *et al.*, 2016a; Candelotto *et al.*, 2017). Heerkens et al. (2016a) found that Delkalb White hybrid had more keel bone damage (fractures and deviations) in comparison to the ISA brown hybrid. Candelotto *et al.* (2017) found that White hybrids (Delkalb White hybrids and experimental white hybrids) had a higher prevalence of keel bone fractures in comparison to brown hybrids (Bovan Brown, ISA Dual Brown and an experimental Brown hybrid). However, a study by Stratmann *et al.* (2015b) found that ISA brown hybrids had a higher prevalence of keel bone fractures compared to Delkalb White hybrids. These rather contradictory studies were carried out in experimental conditions, and future studies should determine how hybrid influences keel damage in a commercial setting. The reason that hybrid could affect keel bone fractures and deviations may be the differences in their behaviour. Delkalb white hens have been shown to have lower levels of serotonin and are more fearful of a stationary person compared with ISA brown hybrids (de Haas *et al.*, 2013) and are more fearful and active compared to brown hybrids that tend to be more docile and perform fewer active behaviours. These differences have never been looked at regarding how hens more around a housing system.

Prevalence of medial fractures compared to caudal fractures was also investigated in the current study because caudal fractures (those at the tip of the keel bone) often occur dorsally on the keel and it is has been discussed whether caudal fractures are of less concern than medial fractures (Casey-Trott *et al.*, 2015). This study will only look at the prevalence of medial fractures as a total of all fractures, the welfare concerns of medial fractures will not be studied in this thesis.

5.1.2. Footpad disorders

Another problem that is related to use of perches and other housing conditions and structures is footpad health (Wang *et al.*, 1998; Shepherd and Fairchild, 2010; Heerkens *et al.*, 2016b). Footpad lesions are well studied in broiler chickens showing that nociceptors are present in the scaly skin of their feet giving them the potential to feel pain (Gentle *et al.*, 2001). Hyperkeratosis, footpad dermatitis and bumblefoot are the three main foot pad disorders in laying hens. Hyperkeratosis is the presence of dry, scaly feet where the underlying dermis becomes exposed with the likely risk factors being wire flooring and perching (Weitzenburger *et al.*, 2006). Footpad dermatitis is the presence of ulcers and dead tissue on the plantar surface of the foot and is thought to be caused by warm, wet conditions (Wang *et al.*, 1998). Footpad dermatitis can develop into bumblefoot, which is a severe swelling of the base of the foot and is caused by Staphylococcal bacterial infection (Gwatkin, 1940).

Previous work has shown that footpad dermatitis and bumblefoot were both higher in the ramp treatment groups compared to the non-ramp treatment groups at 29, 39 and 49 weeks of age (Heerkens *et al.*, 2016a). The prevalence of hyperkeratosis was higher in only 49-week-old hens in the ramp treatment group compared to the non-ramp treatment groups (Heerkens *et al.*, 2016a). The accumulation of these results indicate that ramps have the potential to improve foot pad health and potentially welfare.

Different hybrids have been shown to have different levels of foot pad lesions prevalence; with ISA brown hybrids having fewer food pad problems compared to Delkalb white hybrids (Heerkens *et al.*, 2016a). Footpad dermatitis was higher in the Delkalb white compared to the ISA brown and bumblefoot was only present in white hybrids and was absent in the brown hybrids. However, hyperkeratosis prevalence was higher in the brown hybrids compared to the white hybrids (Heerkens *et al.*, 2016a). This suggests that when on-farm studies into the prevalence of foot pad lesions are carried out, it is important to acknowledge that brown vs white hybrids may have different levels of foot pad lesion prevalence.

5.1.3. Other health parameters

Feather condition, wound presence and comb health may be important factors when determining whether a modification, such as the inclusion of ramps, have the potential to improve or decrease the welfare of hens in the system.

Different hybrids may have predispositions to different health problems, and it may be important to include this in any analysis when looking into the costs and benefits of housing systems.

5.1.4. Behaviour differences

Chapter 3 shows that a higher proportion of falls occurred during the dimming period (dusk) compared to during the day, it also shows that the number of movements in general is higher during dusk compared with during the day. White hybrids have shown in previous studies to have longer durations of tonic immobility compared to brown hybrids (Fraisse and Cockrem, 2006). This may indicate that white hybrids are more fearful and may potentially move more compared to brown hybrids.

5.1.4. Aims and predictions

The main aim of the study was to determine whether ramp provision and genetic hybrid influenced the prevalence of keel bone damage (deviations and fractures) and/or footpad problems (hyperkeratosis, footpad dermatitis and bumblefoot) on farms with multi-tier systems. The current study also looked at the general health parameters of hens on-farm (feather condition, comb health and wound presence). It was important to look at differences in other health parameters between different hybrids and between farms with and without ramps. Different hybrids and ramp access (or lack of access) may lead to other health detriments, other than keel bone damage, and it is important to acknowledge these (Blokhuis *et al.*, 2007).

Behavioural observations were important to determine the effect of ramp presence and hybrid on how hens moved around the system because movements in complex housing systems may increase keel bone fracture prevalence within a flock (Wilkins *et al.*, 2011; Stratmann *et al.*, 2015b). The behavioural movements in this study focused on the number of times hens fly/jump upwards, downwards and uncontrolled movements (falls and collisions) within the system.

The main predictions are:

1. Ramp provision will reduce keel bone fracture prevalence.
2. White hybrids will have a higher prevalence of keel bone fractures compared to brown hybrids.
3. Ramp provision will reduce the presence of all types foot pad lesions.
4. White hybrids will have a higher prevalence of foot pad lesions compared to brown hybrids.
5. White hybrids will have a larger number of movements compared with brown hybrids.
6. There will be more movements during dimming compared to during the day.

5.2. Methods

A total of 18 aviary farms were visited across Belgium and the Netherlands (Table 5.1). One farm per day was visited and the order of visit is shown in Table 5.1. Farmers were asked by phone and/or email if they would like to take part in the study. Questionnaires (as part of a project contributing to a master's thesis) were provided to farmers about personal preference of ramps as well as details about their farm. Selection criteria for farms were that hens had to be over 40 weeks of age and under 100 weeks of age. Hens between 42-85 weeks of age were used in the study.

Table 5.1*: All 18 farms used in the study along with a breakdown of data for each farm (table continues overleaf)*

Visit order	Hybrid	Ramp	Aviary System	Flock Size	Week of Age at time of visit	Outdoor Access	The maximum height of the system	Dimming period (minutes)
1	Lohmann Classic Brown	No	Jansen Comfort	18,000	56	Yes	2.7m	45
2	Bovan Brown	No	Jansen Comfort	17,000	65	No	2.5m	10
3	Lohmann Classic Brown	No	Venocomatic RED-L	40,000	55	Wintergarden	2.9m	45
4	Lohmann Classic Brown	No	Vencomatic Bollegg Terrace	18,000	44	Wintergarden	2.7m	30
7	Lohmann Classic Brown	No	Vencomatic Bollegg Terrace	48,000	58	Yes	2.7m	35
14	Lohmann Classic Brown	No	Vencomatic Bollegg Terrace	9,000	62	Yes	2.8m	45
16	Bovan Brown	No	Vencomatic Bollegg Terrace	29,000	67	No	2.8m	30
6	Lohmann Classic Brown	Yes	Vencomatic Bollegg Terrace	36,000	71	No	3.2m	40
8	ISA Brown	Yes	Vencomatic Bollegg Terrace	19,500	85	No	2.7m	45
10	ISA Brown	Yes	Jansen Comfort	19,500	85	No	2.7m	60
11	Bovan Brown	Yes	Jansen Comfort	29,800	69	Wintergarden	2.6m	50
12	Lohmann Classic Brown	Yes	Big Dutchman Natura	30,000	67	Wintergarden	2.7m	20
13	NOVOgen Brown Classic	Yes	Jansen Comfort	42,000	42	No	2.7m	15
15	NOVOgen Brown Classic	Yes	Jansen Comfort	42,000	43	No	2.7m	15
9	Lohmann LSL Classic White	No	Venocomatic RED-L	40,900	47	No	3.4m	10

5	Dekalb White	No	Big Dutchman Natura	41,000	72	No	2.7m	30
17	Dekalb White	No	Vencomatic Bollegg Terrace	20,000	54	No	2.9m	90
18	Dekalb White	No	Vencomatic Bollegg Terrace	29,000	67	No	2.8m	60

5.2.1. Individual bird scoring

Approximately 70 birds per farm were randomly selected and scored by the same one observer for each parameter, except Farm 1 where 100 birds were scored for keel bone fractures and deviations, 50 birds were scored for footpad lesions and 10 were scored for all other health parameters. This was due to a time restriction on that one farm. Hens were selected in a stratified manner from each tier and equally from each section on the farm. Hens were selected during the dark period when pop-holes were closed. Head torches were used to locate hens. Due to the limited time on farm, no measures were taken for reliability of health parameters. However, the same observer scored all hens, limiting any bias between observers. The observer was not blinded to treatment (brown vs white or ramp presence/absence) due to having scored behavioural measures.

Keel bone fractures were scored as 0 = no break, 1 = slight break, 2 = severe break (Wilkins *et al.*, 2004). The region of the keel where the break was detected was also recorded and scored as either caudal or medial (anything that was not caudal). The bottom 1cm of the keel bone was classed as caudal. Deviations were also scored as present or absent. Deviations were only scored when an obvious (>0.5cm) bend in the bone was present. This bend was a deviation from the straight plane of the keel, smaller bends were not summed, therefore, one >0.5cm had to be present to be classed as a deviation (Heerkens *et al.*, 2016a). If there was also a fracture present at both ends of the deviation, only the fracture was scored due to uncertainty into whether the deviations were caused by the break. Dermatitis (0= no dermatitis to 4 = severe dermatitis) (Butterworth, 2013), hyperkeratosis (none, slight and severe) and bumblefoot (0 = no bumblefoot to 3 = severe bumble foot) were all scored (Tauson *et al.*, 2005).

The bumblefoot score was like that of Tauson et al (2005) except there were two intermediate bumblefoot scores; 0=no bumblefoot, 1=slight swelling of the foot, 2=moderate swelling of the foot and 3=severe swelling of the foot.

Feather condition (neck, tail and back) and presence of wounds were scored (Tauson *et al.*, 2005). Only these regions were scored because they were visibly easy to identify when the hen was held. The feather scoring system was: 1= severe feather damage, 2 = moderate feather damage and 3 = mild feather damage and 4 = almost perfect feather coverage. Comb condition was also recorded, whether the comb was red or anemic (pale comb), this was scored visually with the headlamp as the source of light. Photographs were provided by Heerkens, J. and used as a reference.

5.2.2. Behaviour scoring

The number of hens present in all visible areas of the system (perches, tiers, litter) within a middle 2m section was recorded. A 2m section was chosen because it was used as a reference in another study looking at behaviours (Pettersson *et al.*, 2017a) and 2m is similar to the length of one aviary unit on most farms. The 2m section was measured and then masking tape was used to mark the start and end of the section. Recordings were carried out by 2 observers, with one near the wall and the other in the central area of the system. Counts were carried out during the day and when the lights began to dim. All farms had dimming periods (Table 5.1) but the dimming periods varied in length and start time. Beginning of the dimming period was monitored closely with a lux meter and using the timing specifications provided by the farmer during the visit. Therefore, the beginning of the dimming period was chosen to begin behavioural monitoring. Then, the number of transitions (upward and downward flights/jumps, falls and collisions) by all birds in the same section was recorded during a 15-minute period at both time points. On farms with ramps, a section of the system containing a ramp was chosen. This was because some farms had a ramp in every section of the system. Ramp use (walking up and down or sitting on the ramp) was scored separately from upward and downward transitions and was not included in the analysis of transitions.

A movement was considered a fall when the bird intended to move but missed the landing area, was pushed by a conspecific or had slipped. A behaviour was classed as a collision when there was a visible crash into a structure or another bird in the system. Stopwatches and tally counters were used to assist the two observers to time the observation period and count the number of movements. Before going onto farm the protocol was discussed between both observers and while on the first farm, practice runs were carried out to make sure that behaviours could be detected by both observers. On last two farms the same 2m section was scored for 15 minutes by both observers. The similarity of controlled movements was 91% ((total number of controlled movements observer 1/total number of controlled movements observer 2) x 100). The exact time each behaviour occurred was not noted, meaning it was not possible to compare the exact timings of the movements between observers. On the first farm used to test reliability, one scorer determined a collision while the other did not see a collision and on the second farm checked for reliability, one scorer determined two collisions while the other determined none. This may be due to the angle of each observer being slightly different and collisions being instantaneous, making them difficult to detect. The lack of reliability may also be because the number of occurrences was very low. No fall was detected by either of the observers on either of the farms used to check for reliability.

To calculate the number of movements per hen in the system the number of movements was divided by the number of hens counted in each 2m section of the system that was observed (2 x day and 2 x night per farm). This number was used in the statistical analysis.

5.2.3. Statistical analysis

To analyse the prevalence of each health parameter, the data were analysed as either present or absent, thus all the severity scores were pooled together to represent a binary presence of the condition. Keel bone fractures, keel bone deviations, footpad dermatitis, bumblefoot, hyperkeratosis, anaemic/red comb colour and wound presence/absence were all analysed using generalized linear mixed models in R with a binomial distribution (R Core Team, 2017). In each model farm was used as a random factor (farm was the experimental unit) and hybrid (brown vs white), ramp (presence vs absence) and age (continuous and as a covariate) were used in the model. Feather condition was split into good (scores 3-4) and poor (scored 1-2) and was analysed in the same way.

When analysing severity scores only hens that did have the condition e.g. those with keel bone fractures, were used to determine the prevalence of medial fractures compared with caudal fractures. Those with foot pad dermatitis were split into scores 1-2 (slight problem) and scores 3-4 (severe problem). Hens with score 1-2 bumble foot were classed as slight and those with score 3 were classed as severe. Hens with hyperkeratosis were split into slight and severe. All were analysed again as a generalized linear model in R with a binomial distribution. In each model, farm was used as a random factor and hybrid (brown vs white), ramp (presence vs absence) and age (continuous and as a covariate) were used in the model. Age was used as a covariate because it was important to consider the difference in age across flocks as it is known age has an effect on health parameters; particularly keel bone prevalence (Petrik *et al.*, 2015) and feather condition (Chapter 4). The LmerTest was used so that p-values were generated (Kuznetsova *et al.*, 2017a) and when needed, models were optimized to account for any convergence issues using the optimx package (Nash, 2014).

The total feather score (all feather scores were added together) was square root transformed and the same random factors and fixed effects were used as before.

In all cases of behaviour, the number of movements was divided by the number of birds in the 2m section. This gave an approximation of the number of times each hen moved, allowing the data to be analyzed using linear mixed effect models because the Poisson regression model was overdispersed. All responses were subjected to a square root transformation before analysis to select the best fitting model. In each case Q-Q plots and histograms of the residuals and the AIC were determined to check the model fit, non-transformed data models were also run, and the square root transformed model was a better fit. When analysing the behaviour data a linear mixed-effect model was used for upward, downward and uncontrolled movements (falls and collisions grouped together). In each model, farm was used as a random factor (farm was the experimental unit) and hybrid (brown vs white), ramp presence/absence, time of day (daylight or dimming) and age (continuous and as a covariate) were used.

5.2.4. Ethical Statement

All experimental procedures were approved by the University of Bristol’s Animal Welfare Research Board: UIN: UB/17/001.

5.3. Results

5.3.1 Health Parameters

All data are presented as least square means with standard errors. For the health parameter data, it was not possible to add in interaction terms because there were not enough observations in the dataset. All data for health parameters is displayed in Table 5.2.

Table 5.2: *Prevalence and significance of health parameters in flocks with/without ramps and either white/brown hybrids.*

Health parameter	**Prevalence (%)**				**Test statistic and P values**		
	Ramp (N)	Ramp (Y)	Brown	White	Ramp	Hybrid	Age
Total keel bone fractures	72.9 ± 3^{a}	63.7 ± 5.4^{b}	65.8 ± 2.8	71 ± 5.6	Z= -1.648, P=0.099	Z= 0.812, P=0.417	Z= 1.895, P=0.058
Medial keel bone fractures	64.4 ± 3.7^{a}	52.9 ± 6.3^{b}	61.3 ± 3.2	56.1 ± 7	Z= -1.695, P=0.090	Z= -0.684, P=0.494	Z= 0.794, P=0.427
Severe keel bone fractures	33.6 ± 3.6	24.6 ± 4.8	32.1 ± 3	25.9 ± 5.5	Z= -1.542, P=0.123	Z= -0.963, P=0.336	Z= 1.343, P=0.179
Keel bone deviations	30.5 ± 2.7	31.9 ± 4.4	28.6 ± 2.2	33.9 ± 5.1	Z= 0.291, P=0.771	Z= 0.996, P=0.319	Z= 2.047, P=0.041
Foot pad dermatitis	29.1 ± 4	25.4 ± 5.9	19.8 ± 2.7^{a}	36.1 ± 8^{b}	Z= -0.541, P=0.589	Z= 2.158, P=0.031	Z= -2.957, P=0.003
Severe foot pad dermatitis	17.1 ± 2.7	7.3 ± 3.7	6 ± 1.7^{a}	20.2 ± 5.9^{b}	Z= -1.574, P=0.116	Z= 3.622, P<0.001	Z= -1.411, P=0.158
Bumble foot	13.1 ± 2.6^{a}	4.9 ± 1.9^{b}	6 ± 1.3	10.8 ± 4	Z= -2.408, P=0.016	Z= 1.421, P=0.156	Z= 1.725, P=0.085
Severe bumble foot	42.8 ± 4.9	25.8 ± 10.7	32 ± 6.5	35.7 ± 9.4	Z= -1.300, P=0.194	Z= 0.421, P=0.674	Z= -1.182, P=0.237
Hyperkeratosis	60.8 ± 8.1	61.6 ± 12.7	67.5 ± 6.3	54.6 ± 15	Z= 0.057, P=0.954	Z= 0.811, P=0.417	Z= 0.319, P=0.750
Severe hyperkeratosis	11.2 ± 3.3	5.4 ± 2.8	6.5 ± 1.8	9.3 ± 5	Z=-1.272, P=0.203	Z= 0.591, P=0.555	Z= 2.409, P=0.016
Anaemic comb	7 ± 2.9	3.7 ± 23.8	10.3 ± 3.3^{a}	2.4 ± 1.9^{b}	Z=-930, P=0.352	Z= 1.752, P=0.080	Z= 1.703, P=0.089
Wounds	2.9 ± 1.3	0.9 ± 0.7	2.6 ± 1	1 ± 0.9	Z= -1.448, P=0.147	Z= -1.052, P=0.293	Z= 2.000, P=0.045
Severe feather damage (back)	5.7 ± 4	2 ± 2.2	12.2 ± 6.4^{a}	0.9 ± 1.2^{b}	Z= -0.907, P=0.365	Z= -1.925, P=0.054	Z= 3.576, P<0.001

Severe feather damage (neck)	6.7 ± 4.2	1.2 ± 1.4	2.6 ± 1	1 ± 0.9	Z= -2.055, P=0.150	Z= -1.962, P=0.168	Z= 3.815, P=0.005
Severe feather damage (tail)	13 ± 7.4^{a}	1.1 ± 1.3^{b}	12.8 ± 6.5^{a}	1.1 ± 1.4^{b}	Z= -1.441, P=0.040	Z= -1.380, P=0.050	Z= 2.809, P<0.001
Total feather condition	9.0 ± 0.4	10.2 ±0.7	8.7 ± 0.4^{a}	10.5 ±0.8^{b}	T=1.501, P=0.133	T= 2.104, P=0.035	T= -4.307, P<0.001

Data are presented as lsmeans (± SE). Any tendencies or significant differences within the ramp treatment or hybrid have been denote with different letters

5.3.1.1. Keel bone damage

Keel bone fracture prevalence tended to be higher in flocks without access to ramps, compared to those with access to ramps (P=0.099; Table 2; Figure 5.1). Of hens with keel bone fractures, those without ramp access tended to have a higher prevalence of medial fractures (64.4% ± 3.7%) compared to those with ramp access (52.9% ± 6.3%; P=0.090; Table 5.2). However, this does not consider the overall higher fracture prevalence in the non-ramp treatment group. There was no significant difference between hybrids and prevalence of keel bone fractures. Keel bone fracture prevalence tended to increase with age (possibly due to an accumulation of old fractures) (P=0.058; Table 5.2). There were no significant differences between keel bone deviation prevalence and the presence of ramps or hybrid. Keel bone deviations increased with flock age (P=0.041; Table 5.2).

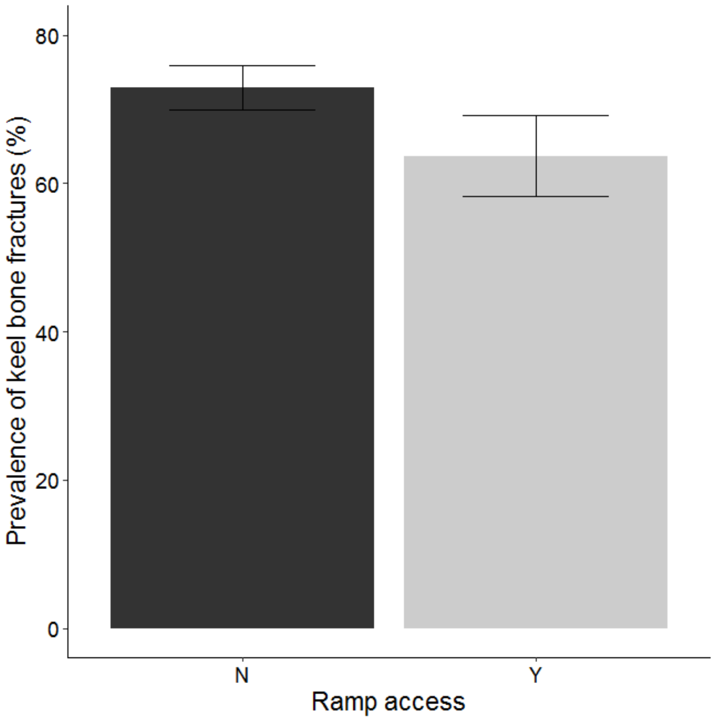

Figure 5.1: *Prevalence of keel bone fractures and whether farms had ramp access (presented as lsmeans ± SE)*

5.3.1.2. Foot pad disorders

There was no significant difference in footpad dermatitis prevalence and the presence of ramps. Prevalence of foot pad dermatitis, within flocks, was higher in white flocks (36.1% ± 8%) compared with brown flocks (19.8% ± 2.7%; P=0.031) and decreased with age (P=0.003; Table 5.2). Of hens with footpad dermatitis, white hens were more likely to have severe dermatitis (20.2% ± 5.9%) compared with brown hens (6% ± 1.7%; P<0.001; Table 5.2). However, this does not consider the increased total prevalence of foot pad dermatitis in white hybrids.

Prevalence of bumblefoot decreased was higher in flocks without ramp access compared to flocks with ramp access (P=0.016, Figure 5.2; Table 5.2).

There was no difference between the prevalence of bumblefoot and hybrid. Bumblefoot prevalence tended to increase with age (P=0.085; Table 5.2).

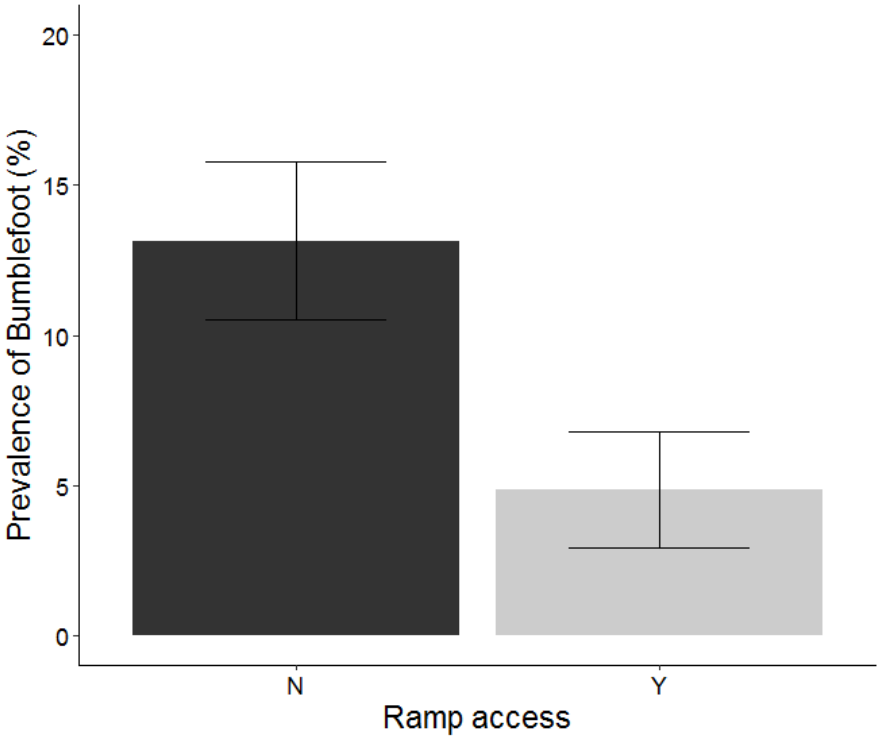

Figure 5.2: *Prevalence of bumblefoot and whether farms had ramp access (presented as lsmeans ± SE)*

There was no difference between the prevalence of hyperkeratosis and the presence or absence of ramps and white or brown hybrids. Hyperkeratosis prevalence did not change with age. Of hens that had hyperkeratosis, there was no different in ramp presence or hybrid in the prevalence of severe cases of bumblefoot. The prevalence of severe cases of hyperkeratosis increased with age (P=0.016; Table 5.2).

5.3.1.3. Other health parameters

The prevalence of anaemic combs was not affected by ramp presence, but brown flocks tended to have a higher prevalence (10.3% ± 3.3%) than white flocks (2.4% ± 1.9%; P=0.080; Table 5.2). Anaemic comb prevalence tended to increase with age (P=0.089; Table 5.2). The prevalence of wounds was not affected by the presence of ramps or hybrid. As age increased, the prevalence of wounds increased (P=0.045; Table 5.2). There was no difference in severe feather damage on the back of hens and ramp access. Severe feather damage on the back of hens tended to be higher in brown hen flocks (12.2% ± 6.4%) compared to white flocks (0.9% ± 1.2%; P=0.045; Table 5.2). Severe feather damage to the tail was higher in flocks without ramps compared to those with ramps (P=0.040, Figure 5.3; Table 5.2) and tended to be higher in brown hybrids (12.8% ± 6.5%) compared to white hybrids (1.1% ± 1.4%; P=0.050; Table 5.2). There was no difference in the prevalence of severe feather damage on the neck on farms with or without ramp access or on farms with brown or white hybrids. In all cases of feather damage (back, neck and tail), as flock age increased, the prevalence of severe feather damage increased (P<0.001, P=0.005 and P<0.001 respectively; Table 5.2).

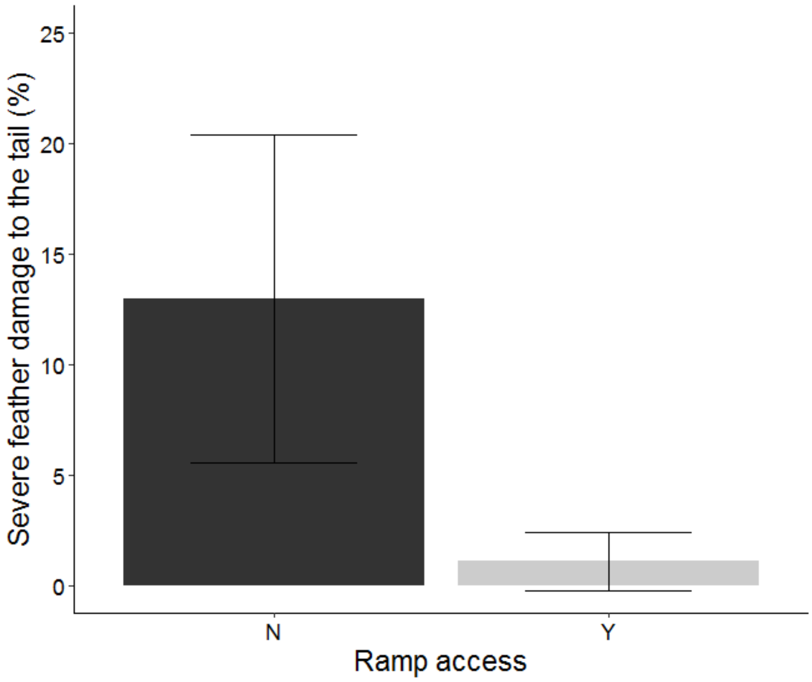

Figure 5.3: *Prevalence of feather damage to the tail and whether farms had ramp access (presented as lsmeans ± SE)*

5.4.1. Behavioural Parameters

All data are presented as least square means with standard errors with test statistics and significance levels in Table 5.3. All data are shown as the number of times moved per hen (number of times moved/ number of hens in a section).

Table 5.3: *Prevalence and significance of movement behaviour in flocks with/without ramps, with white/brown hybrids and during the day or dimming period.*

Movement Behaviour	**Prevalence**						**Test statistics and P values**				
	Ramp (N)	Ramp (Y)	Brown	White	Day	Dimming	Ramp	Hybrid	Age	Time	Age x Time
Upward transitions	1.70 ± 0.25	1.61 ± 0.39	1.35 ± 0.19	1.99 ± 0.49	1.31 ± 0.24^{a}	2.04 ± 0.30^{b}	T= -0.209, P=0.834	T= 1.316, P=0.188	T= -1.414, P=0.157	T= 3.499, P<0.001	-
Downward transitions	1.00 ± 0.14	0.91 ± 0.21	0.61 ± 0.09^{a}	1.38 ± 0.30^{b}	0.97 ± 0.16	0.93 ± 0.15	T= -0.384, P=0.701	T= 2.818, P=0.005	T= -0.961, P=0.337	T= -0.268, P=0.789	-
Total transitions	2.74 ± 0.37	2.58 ± 0.57	1.99 ± 0.27^{a}	3.43 ± 0.75^{b}	2.30 ± 0.37^{a}	3.05 ± 0.42^{b}	T= -0.243, P=0.807	T= 1.973, P=0.049	T= -1.399, P=0.162	T= 2.549, P=0.011	-
Uncontrolled transitions	0.035 ± 0.09	0.036 ± 0.014	0.034 ± 0.007	0.037 ± 0.016	0.017 ± 0.007^{a}	0.061 ± 0.013^{b}	T= 0.065, P=0.948	T= 0.134, P=0.893	T= 0.253, P=0.800	T= 3.219, P=0.001	T= -2.443, P=0.015

Data are presented as lsmeans (± SE). Any tendencies and significant differences within the ramp treatment, hybrid or the time of day have been denote with different letter

There was no difference between ramp access, hybrid or age on the number of upward transitions per bird. There were more upward transitions during the dimming period (2.04 ± 0.30) compared with during the day (1.31 ± 0.24; P<0.001; Table 5.3). There was no difference between ramp access and the number of downward transitions per hen. White birds performed more downward transitions (1.38 ± 0.30) compared with brown birds (0.61 ± 0.09; P=0.005; Table 5.3). However, there was no difference in age of the flock or time of day on the number of downward movements per hen. There was no significant difference between the total number of movements and whether hens had access to ramps. Overall, white birds tended to perform more transitions (3.43 ± 0.75) compared with brown birds (1.99 ± 0.27; P=0.049; Table 5.3). Flock age had no effect on the total number of movements per hen. A greater number of total movements occurred during the dimming period (3.05 ± 0.42) compared to during the day (2.30 ± 0.37; P=0.011; Table 5.3). There was no effect of ramp access and hybrid on the number of uncontrolled movements. For uncontrolled movements (falls and collisions together) there was an interaction effect between age x time: during daylight (P=0.015; Table 5.3). There was a consistent number of uncontrolled movements as flock age increased but uncontrolled movements within the dimming period decreased with age. Descriptive statistics for the overall ramp use were: 0.79 ± 0.16 times per hen.

5.4. Discussion

5.4.1. Keel bone damage

Flocks with ramp access tended to have a lower prevalence of keel bone fractures compared to those without access to ramps. However, these treatment differences are not as strong as in other studies (Stratmann *et al.*, 2015a; Heerkens *et al.*, 2016a). Stratmann *et al.* (2015a) found the prevalence of keel bone fractures in the ramp group at 60 weeks of age to be 29% compared to the control group being 52.6%, while Heerkens *et al.* (2016a) found a prevalence of approximately 82% in the ramp treatment at 29 weeks ad 97% in the control. The current study found that a 64% prevalence on farms with ramp access and 73% in farms with no ramp access. One reason for the smaller difference may be that the current study was conducted over various ages. Keel bone fractures and deviations increased with age in the current study and this finding concurs with other research (Petrik *et al.*, 2015; Stratmann *et al.*, 2015a; Heerkens *et al.*, 2016a).

There was no difference between white vs brown flocks with respect to keel bone fracture prevalence. The reason that no statistical difference was seen may be due to sample size (n=4 farms with white birds). The lack of significant difference could be because there is no difference but can also be due to the variability on farms. In previous experimental studies, all treatment groups were housed in the same conditions with the only alteration being the factor that was being studied (Stratmann *et al.*, 2015a; Heerkens *et al.*, 2016a). For example hens were either housed with or without ramps (Stratmann *et al.*, 2015a) or a factorial design: white hybrids with/without ramps and brown hybrids with/without ramps (Heerkens *et al.*, 2016a).

Deviations are thought to be caused by compression on the keel when resting on perches and other structures (Scholz *et al.*, 2008; Harlander-Matauschek *et al.*, 2015). Due to all systems monitored containing perches, hybrid (brown vs white) and ramp access, this would potentially have limited influence on the prevalence of keel bone deviations. Some farmers did not know what system their hens were reared and therefore, it was not possible to analyse the effect of rearing condition on the presence of deviations. It can be assumed that if hens are exposed to perches at a young age, they would be more likely to have keel bone deviations due to pressure on the keel before full ossification (Riber *et al.*, 2018).

5.4.2. Foot pad disorders

Unlike the Heerkens *et al.* (2016a) study there was no difference in the prevalence of foot pad dermatitis on farms with and without ramps in the current study. The lack of statistical significance could be due to the large variation in age in the current study. As hens age, they may be exposed to wet litter for a longer period of time, which is linked to footpad lesions (Wang *et al.*, 1998). In the current study, litter quality checks were not carried out so the direct effect of litter on footpad lesions could not be measured. Flocks with access to ramps had a lower prevalence of bumblefoot compared to flocks that did not have ramp access. This confirms what was found by Heerkens *et al.* (2016a). The lower prevalence of bumblefoot on farms with ramp access may be explained by hens walking on the ramps and any excess faeces being scraped off the feet of the hens as they walk. Ramps in the current study where either plastic or metal grids.

This would result in a decreased contact time with faeces or wet litter and any open wounds present on the feet of hens.

White birds had a higher prevalence of foot pad dermatitis compared to brown birds. Previous studies have also drawn the same conclusions (Abrahamsson and Tauson, 1995; Heerkens *et al.*, 2016a). There was no difference in the prevalence of hens with hyperkeratosis. However, the prevalence of hyperkeratosis increased as the age of the flock increased. Hyperkeratosis can be a result of continued localised pressure on the foot pads of laying hens, potentially from metal wires or perches (Weitzenbürger *et al.*, 2006; Ronchen *et al.*, 2008). There was no difference detected in the current study and this may be due to all farms having perches and wired gridded areas. Some of the ramps present on the farms were also made from wire mesh. The increased prevalence of severe hyperkeratosis at older ages in this study may be due to prolonged use of perches and wire gridded areas.

5.4.3. Other health parameters

In the current study, all other health parameters (wounds, comb health, feather condition) deteriorate with age. This suggests that irrespective of ramp provision and hybrid the overall health of hens will diminish over time. Feather damage in hens has shown to increase with age due to an increase in feather pecking behaviour (Bilcik and Keeling, 1999). In the current study, the increase in wounds on the body would also corroborate that feather pecking behaviour increases with age, although feather pecking behaviour was not monitored directly in this study.

White hens tended to have a lower prevalence of anaemic combs and better feather condition in comparison to brown hens. However, it must be noted that comb health was assessed with reference to photographs on farms when lights were off or dimmed. Reduced lighting may have hindered the ability to detect pale combs. Also, the combs of white hens may have appeared a deeper red due to the white pigment of their feathers. Therefore, comparison of comb health between white and brown hens may be slightly bias. Hens with access to ramps had better feather condition on their tails compared to those without ramps access. This may be caused by hens being able to escape conspecifics more easily when ramps are present, leading to less tail feather pecking. Previous work has shown that hens show less hesitancy behaviour in the presence of ramps (Pettersson *et al.*, 2017a). However, there is no significant difference in feather condition on any other body part.

5.4.4. Behaviour

To our knowledge this is the first-time movements between tiers within commercial aviary systems have been studied with the aim to determine whether having access to ramps or genetic hybrid affects movement (but not the interaction of the two). However, hybrid was confounded within ramp treatment, with no white hybrids having ramp access, meaning that the interaction between ramp access and hybrid could not be analysed. One study has looked at the movement of laying hens around a multi-tier system but this was only on one farm and therefore did not take into account any differences in genetic hybrid or ramp presence (Campbell *et al.*, 2016c). This means that although ramps only tended to reduce keel bone fractures in the current study and did not reduce uncontrolled movements, they may allow hens to perform fewer hesitancy behaviours when transitioning tiers (Pettersson *et al.*, 2017a). This is something that could be looked at in more detail because allowing hens to move more confidently in a system may result in increased welfare.

In the current study, differences were found mainly between white vs brown hybrids and time of day. White birds moved more in general compared to brown birds. Total movements and upward movements increased in frequency during the dimming period compared to during the day. This finding has been mirrored in other studies that suggest hens move more during dimming because they are trying to roost on the top level (Schrader and Müller, 2009; Stratmann *et al.*, 2015a). Due to there being no difference in downward movements but a difference in total movements and upward movements, this suggests hens are moving to perches and tiers higher in the systems. When looking at hybrid, white hybrid chicks tended to use higher structures than brown hybrid chicks (Kozak *et al.*, 2016a).

Through personal observation, the white hybrids appeared to move further from the observer initially during behavioural observations, which may have resulted in the increased movement in white hybrids. Although there was a 5-minute waiting time before beginning the observations, white hybrids may need more time than this to adjust to the presence of an observer. Previous work has shown that white hybrids have longer durations during a tonic immobility test compared with brown hybrids, suggesting that white hybrids are potentially more fearful (Fraisse and Cockrem, 2006). Uncontrolled movements were difficult to assess accurately because they occurred rarely making an accurate reliability assessment difficult. To encourage the normal behaviour of hens and increase the reliability of results, future studies would benefit from video recording behaviours and ramp use on-farm instead of relying on live observations.

5.4.5. Limitations

Due to the high variability of ramp design within and between farms, it is difficult to produce sound conclusions from the study. Future studies, on-farm should focus on separating different ramp types to make conditions more homogenous. A previous study found hens preferred a grid ramp compared to a ladder ramp (Pettersson *et al.*, 2017b). Birds had a higher proportion of failed landings from the ladder ramp (Pettersson *et al.*, 2017b) and the same pattern may be seen on-farm. It would also be beneficial to look at different types of ramp in a controlled environment between different hybrids and then use the findings on farm. The variation in the number, placement and design of ramps could influence how the birds on each farm moved.

Future studies would benefit from understanding the way that white laying hens interact with ramps. In the current study farms with white hybrids and ramps were not visited because no farms could be recruited. The reason for this may be three-fold; either there are not many farms in Belgium and the Netherlands that house white hybrids with ramps together, there aren't many farms with white hybrids or there aren't many farms with ramps.

Farms were only visited once during the study due to time restraints, but future studies would benefit from visiting farms across different ages. One benefit of this would be to filter out any unwanted effects of the specific day. It was unknown whether an event occurred on the farms that specific day that may influence hen behaviour and therefore, potentially skew the results.

Another point is that movements were only monitored for a short period of time (15 minutes at each time point), very few falls and collisions occurred within this time, indicating that 15 minutes may not be long enough to observe these behaviours. Reliability between the 2 observers when detecting collisions and falls was very low and may increase if the number of falls and collisions observed within the observation period increased. These results should be interpreted with caution due to the low reliability between observers.

The number of locations that behavioural recordings were carried may not be representative of the whole farm. Although two locations in the middle of the system (e.g. away from walls and fences) and then another two locations that was close to walls and fences were chosen, this may not be representative of each farm. Some of the farms contained tens of thousands of hens and these farms were split into areas separated by fences, effectively creating a separate pen. Therefore, the hens in each section may behave differently from hens located in another section. Also, although the hens were given time to acclimatise the presence of humans, they still may not be carrying out their normal behaviours. One way to prevent this behavioural change would be to set up video recording equipment throughout the farm.

5.5. Conclusion

By confirming that ramps tend to reduce keel bone fracture prevalence, reduce bumble foot prevalence and improve tail feather condition it can be confirmed that ramps have the potential to improve laying hen welfare. Genetic hybrid may influence a variety of health parameters. In the current study, foot pad dermatitis was worse in white hybrids. Whereas, feather condition on the back of hens, total feather condition score and comb health was better in white hybrids. However, the addition of ramps did not reduce the welfare of laying hens at all. Therefore, it should be recommended that ramps are provided on farms.

5.6. Acknowledgements

I would like to thank Sofie De Knibber for help collecting the behavioural data and for help catching the hens. Sofie wrote a Master's thesis (De Knibber, 2018) based on the questionnaires given to the farmers and some of the welfare parameters of the hens. Sofie only provided descriptive statistics and did not use all the data. What is presented in this thesis is the complete analysed data.

Chapter 6

General discussion

6.1. Aim

The main aim of this PhD was to understand the characteristics of movements within a multi-tier system and to define and mitigate hazards in this system to reduce keel bone fracture prevalence. This information would be used to alter the design of a multi-tier housing system. To determine whether any region of a Bolegg terrace multi-tier system (a common system used for laying hens) was hazardous, accelerometer outputs and behavioural analysis were first used (Chapter 2 and 3). Then the results from the first set of experiments were used as a starting point for another study where modifications of a standard Bolegg terrace system were monitored, again using accelerometer output and behavioural analysis, but also keel bone fracture and deviation prevalence, footpad lesion prevalence, feather condition and tibia bone mechanics (Chapter 4). The next study was conducted on-farm looking at health parameters but only focussing on the presence or absence of ramps (Chapter 5).

6.2. Topics of investigation

6.2.1. Accelerometry output in a multi-tier system

The maximum summed acceleration vector (AV) at the keel, the average summed AV at keel, the readout duration at the keel, the average AV x readout duration at the keel and the maximum summed AV at the body were all analysed. The maximum summed AV represents the mechanical hazard of a movement with a higher value representing a more hazardous movement compared to a movement with a lower summed AV. The average summed AV represents the total energy associated with a movement and therefore it is postulated that as the average summed AV increases so does the hazard and possible likelihood of fracture from that movement. The readout duration is the time, within the same movement, that the 15g threshold was reached on the accelerometer. The longer the readout duration, it was hypothesised that the behaviour would be more hazardous and would have a greater likelihood of leading to fracture because of the prolonged exposure to a high acceleration vector. If multiplying the maximum summed AV by the readout duration results in a high value output, this can either indicate that the maximum acceleration was extremely high, or the readout duration is long. However, if the value is low then either the maximum acceleration and/or the readout duration was short. A high numerical value for the maximum AV x readout duration is likely to be more hazardous and lead to greater risk of a keel bone fracture compared to a movement with a low numerical value. However, all these factors were used as proxies and have not been directly linked to fracture occurrence.

It could be argued that the potential for keel bone fractures to occur increases with access to higher perching structures (Gregory and Wilkins, 1996). This is corroborated by the increased prevalence of keel bone fractures in multi-tier systems compared to single tier and cage systems (Rodenburg *et al.*, 2008; Wilkins *et al.*, 2011; Petrik *et al.*, 2015). The most important finding, in Chapter 2, was that falls have higher summed AV at the keel compared to non-falls. Chapter 2 also showed that maximum summed AV readings increase as the total height (vertical distance) of a fall increases, although not statistically significant in the post-hoc analysis. Therefore, it can be speculated that maximum summed AVs can be used as a proxy for the likelihood of a system to cause keel bone fractures, as greater impact increases the likelihood of sustaining a keel bone fracture (Toscano *et al.*, 2018).

The data presented in this thesis is particularly important because it shows maximum summed AVs that relate directly to the keel bone of laying hens. One of the accelerometer sensors was placed directly over the keel bone and collected data relating to acceleration exposure specific to the keel bone. This is a novel methodology as previous studies have mainly looked at attaching devices onto the back of laying hens (Quwaider *et al.*, 2010; Daigle *et al.*, 2012; Banerjee *et al.*, 2014). The placement of accelerometers on the back of hens can be informative, particularly if looking at behaviours involving the whole body. However, if the behaviours of interest are falls or collisions, it may be more accurate to use a sensor on the keel bone of a hen because this is the outermost point of the hen and is the most likely region to meet a structure upon collision (Gregory and Wilkins, 1996). It was shown in Chapter 2 that when looking at the maximum AV, the keel sensor was more appropriate than the body sensor for picking up differences between fall and non-fall events and collisions and non-collisions events, with respect to the maximum summed AV reading. The keel sensor showed an interaction effect between the total height of the movement and whether the hen had a fall or not, with falls always having higher values than non-falls. The maximum AV of falls also increases as the total height increases but non-falls showed a decrease from 0-1m to 1.5-2.0m. Collisions had higher maximum summed AVs compared to non-collisions on the keel sensor. Falls had higher maximum AVs on the body accelerometer but there was no effect of collision. This may be because collisions can occur directly on the keel bone, such that extreme values of AVs may be easier to distinguish on the keel accelerometer than the body accelerometer.

It was found in Chapter 2 that readout durations of accelerometry data were longer for behaviours that were falls compared to those that were non-falls. This may indicate that there are other behaviours happening that are prolonging the accelerometry readout. One possible behaviour is wing flapping; hens that fall tend to show wing flapping behaviour to aid them in recovering control (observation from video data). Hens try to grab a perch or tier with their claws to prevent them from falling further. Due to the placement of the accelerometry sensors on the back and on the keel bone of laying hens, the wing flapping may produce an increase in summed AV on both sensors. This is because accelerometers were placed in a vest and as the hen flaps her wings the vest may move vigorously. Therefore, if the hen was falling and wing flapping was prolonged, when trying stop the fall by landing on a structure, this could lead to the readout duration for a fall being longer than that of a non-fall.

There was a three-way interaction between fall presence (Y/N), collision presence (Y/N) and the total height of the movement on the readout duration. Movements that were non-falls/non-collisions had the shortest readout durations, these were controlled movements and suggest that these movements were easily executed by the hens. This was followed by non-fall/ collision movements. Non-falls resulted in lower readout-durations than falls, more than likely because the movement was controlled and was not difficult for the hen to execute. This was followed by falls/non-collisions with the overall second longest readout durations and falls/ collisions having the longest readout durations. These were the most uncontrolled types of movements and falls/collisions probably represent uncontrolled movements that the hens could not recover from and this made the readout duration longer because of continued efforts to recover (e.g. a prolonged duration of wing flapping). Readout durations for total heights >1.5m were longer for all behaviours except falls/non-collisions, which peaked at 1.0-1.5m. Again, this may be because hens try to recover from falls at heights 1.0-1.5m but as the height of the falls increase, recovery may be easier and there may be less wing flapping, resulting in a decrease in the readout duration.

The average summed AV is more difficult to interpret, in part due to the way it was calculated, as some instances are possibly exaggerated. This was because there were sometimes missing data files when the movements lasted longer than one second. In these instances, the missing average summed AVs were not known. This means that the average summed AVs in the missing data files was most likely lower than in the files that were present because the files that were present produced a readout (meaning that the pre-set threshold was reached). As is like the readout duration, there was a three-way interaction between the fall presence (Y/N), collision presence (Y/N) and the total height moved. Movements that were non-fall/ collisions between 0.5-1m had an immediate peak in average summed AV compared with other movement types. This may be due to the small sample size of non-fall/ collisions in the dataset. In comparison to the readout duration, non-falls tended to have higher average summed AVs compared to falls. One reason for this may be that as readout duration decreased, the number of acceleration outputs in the readout decreased, and this decrease would result in an increase in the average summed AV. This will particularly be the case if there is a high maximum summed AV because the readout duration being short will inflate the average summed AV. There appeared to be an inverse relationship between the readout duration and the average summed AV, values that were high for one were low in other.

When the average summed AV was multiplied by the readout duration values were higher for falls and collisions, this was expected but was mainly because falls and collisions had longer readout durations whereas there was limited difference in the average summed AV (Chapter 2).

6.1.2. Falls within a multi-tier system

Falls occur in different regions of the Bolegg terrace multi-tier system. The regions of importance were determined by how often a navigation path was used and the percentage of falls compared with total movements in that navigation path. This is the first time that navigation paths were looked at in such detail with the aim to pinpoint hazardous navigation paths with the aim to alter them to become less hazardous. The top tier and nest box regions were found to have the highest percentage of falls of the data generated from hens wearing accelerometers. This may be due to them being highly used areas. As high percentage of falls in the top tier of the system may be due to hens being motivated to roost high in the system causing overcrowding (Olsson and Keeling, 2000; Schrader and Müller, 2009). There was also a higher percentage of falls around the nest box area compared to other areas in the system. This may be due to overcrowding, as nest boxes are a highly used resource, and they are in the centre of the multi-tier system, meaning the hens must move past them to move up the system. Hens may use the same nest box and enter the nest boxes at the same time of day (Riber, 2010), which would heighten the problems around the nest box. However, most falls occurred around the nest box during dusk, which suggests it is the movement to the top of the system and overcrowding that causes most of the falls.

It was also found that specific navigation paths were more hazardous than others (they resulted in a higher percentage of falls) (Chapter 3). For example, when hens had a missed landing, it is the movement from the starting region to the intended landing region that is difficult. Most of the missed landings happened when moving to or from a perch (Chapter 3). Perches have been shown in previous work to result in a high proportion of falls (Campbell *et al.*, 2016a). Slips occurred most frequently when moving from the top perch to the top tier (Chapter 3) and may be a result of overcrowding on the top perches. Previous work has shown that the time required to achieve balance increased as the landing area between obstructions decreased (Moinard *et al.*, 2005), suggesting slips may be more likely when perches are full. A push may suggest that the starting area is hazardous. A push tends to suggest that the fall is occurring in a region with a high bird volume. A small number of falls around the nest box were from pushes and interactions with other conspecifics (Chapter 3) and may be due to aggression from conspecifics around the nest box (Freire *et al.*, 1998). Most pushes occurred from the 3rd perch to litter and 2nd perch to lower tier (Chapter 3). This suggests that hens are being displaced as conspecifics move to these regions. This may indicate that other ways to navigate these regions are needed. It is important to note that the 2nd perch and 3rd perch are those perches surrounding the nest box.

There was also a link between time of day, with a greater percentage of falls during the night compared to other times of the day. However, what was also found was that the actual number of total movements and falls per hour was higher during the dusk-phase compared to all other phases (Chapter 3). The absolute number of movements were low at night but the percentage of falls during the night was high. This result suggested that it is important to look at the number of times movements occur and not just what the percentage of falls are in each time of day or navigation path. Hazard within a system is a function of the frequency of use of a given pathway, as well as the risk of injury on any one occasion. Dusk has been considered a hazardous time of day because of the high proportion of bird movement and the high number of falls (Stratmann *et al.*, 2015a). Hens are highly motivated to perch at night (Olsson and Keeling, 2002) and prefer high roosting locations (Schrader and Müller, 2009), so the increase in the percentage of falls at this time of day is most likely linked to the competition to perch high at night.

6.1.3. Modifications to multi-tier systems

Modifications in Chapter 4 were based on results from Chapter 2 and 3. The addition of mushroom-shaped perches in the place of standard perches was used with the expectation that mushroom-shaped perches would provide better grip compared to standard round, metal perches (Pickel *et al.*, 2011). Round perches have been shown to cause unstable movements while feeding compared to rectangular perches and platforms (Duncan *et al.*, 1992; Sirovnik *et al.*, 2018). One aim in Chapter 4 was to reduce falls from perches because movements from perches tended to have higher maximum and average summed AVs on at the keel (Chapter 2). Another predicted outcome of mushroom-shaped perches was that they would provide better grip when hens were pushed (pushes from perches occurred more that pushes from any other structure; Chapter 3). It was thought that these alterations would reduce keel bone fracture prevalence. However, keel bone fracture prevalence increased in the mushroom-shaped perch treatments (Chapter 4). More hens perched in the treatments with mushroom-shaped perches, which may explain the increased keel bone fracture prevalence. Hens may move more to access the perches and it is known that systems that provide greater movements often have increased prevalence of keel bone fractures (Rodenburg *et al.*, 2008; Wilkins *et al.*, 2011; Petrik *et al.*, 2015). Hens in Chapter 4 were perching more in the mushroom shaped perch treatments compared to the control and were therefore potentially moving more.

Keel bone deviation prevalence was not reduced in the mushroom-shaped perch treatments, despite what was postulated to be a more favourable profile, and the prevalence of bumblefoot and foot pad dermatitis increased in the extra mushroom-shaped perch treatment on the top tier of the system compared with the control (Chapter 4). This may be because of the extra perching in the mushroom-shaped treatments that may have counteracted the effects of the more favourable profile (increased surface area) of perches expected to reduce keel bone deviations. Food pad dermatitis and bumblefoot may have increased because of the grooves on the surface of the mushroom-shaped perches and this would lead to prolonged contact with faecal matter.

The addition of an extra round perch in the nest box perch (NBP) treatment increased keel bone deviations (Chapter 4). Round perches were found in a previous study to have the highest pressure on the keel bone (Pickel *et al.*, 2011), which may explain the increase in deviation prevalence because hens perched more in the middle tier of the system in the NBP treatment. However, it is unlikely that the addition of one perch was the only reason for the increase in keel bone deviations in the NBP treatment. It is important to note that hens were only counted on the perches and the length of time perching was not recorded, so it is unknown whether hens' perch for longer in the NBP treatment compared to the control. It is also worth noting that the addition of an extra perch around the nest box did not lead to an increase in footpad lesions compared to the control, even though there was slightly more perching in the NBP treatment compared to the control. There was also a slightly decreased percentage of perfect feather cover and a slight decrease in body mass in the NBP treatment compared to the control, which may be explained by the close proximity of the extra perch to the nest box (Nicol *et al.*, 1999) (Chapter 4). There was deterioration of the litter quality in the NBP treatment, suggesting that an extra perch in this region should not be recommended on-farm for hygiene reasons. There was an increase in perching behaviour in the NBP treatment compared to the control (Chapter 4). This suggests that the extra perches would be used by laying hens, but the placement of these perches would need some further investigation to determine optimal usage and increased benefit to hens.

Some of the results in this thesis are contradictory, such as in Chapter 4 ramps did not affect the prevalence of keel bone fractures, whereas farms with ramps tended to have a lower prevalence of keel bone fractures in Chapter 5. One reason for this difference between studies could be that ramps in Chapter 4 were transverse across the system and they ran from the top tier to the lower tier. Different ramp arrangements were present both within and between farms in Chapter 5. Ramps were both transverse to the system, acting as a pathway directly onto the system, either leading from the litter to the lower tier or from the lower tier to the nest box (as examples). It may be that different ramps affect movement in different ways, with some leading to better quality transitions than others, and perhaps some ramp configurations contributing to a more "cluttered" environment. Previous work has shown that grid ramps were easier for pullets to transition compared with rung ramps (Pettersson *et al.*, 2017b). Metal grid ramps were used in Chapter 4. Additionally, it could be that certain hybrids react to ramps differently, with some more readily using them or it may be that their previous experience of ramps affects transition ability as adults (Norman *et al.*, 2018).

A possible solution may be that ramps need to be localised to the areas within the system with the highest percentage of falls. From Chapter 3, a high proportion of falls occurred along the top tier of the system and the nest box area. Ramps were transverse across the whole system in Chapter 4 but ramps may have a greater impact on bird health if they are placed inwards towards the system, similar in design to previous work (Stratmann *et al.*, 2015a). As stated above, ramp placement was different between farms in Chapter 5, with some being transverse and others orientated towards the systems, with some farms having more than one type of ramp placement. Transverse ramps may not be the optimal orientation for ramp placement and a study comparing different ramp materials and placement is needed.

Chapter 4 showed that ramps increased the prevalence of hyperkeratosis, but the same result was not found in Chapter 5. The reason for this finding is unknown but may be due to hyperkeratosis being scored at multiple time points (ages) in Chapter 4, but only at one-time point (with variable ages) in Chapter 5. It is known that footpad dermatitis increases in the presence of wet litter, which in turn is affected by the time of year (Wang *et al.*, 1998). Chapter 5 was carried out for 5 weeks during the summer, whereas foot scoring in Chapter 4 was carried out through the autumn and spring, giving two different weather conditions. This may suggest that the colder weather can increase hyperkeratosis prevalence. Therefore, age and/or weather could be contributing factors affecting footpad lesions development.

The ramp treatment had a slight improvement in tail feather score in Chapter 5. The percentage of hens with perfect feather cover was not statistically lower in the ramp treatment compared to the control but it was numerically lower (Chapter 4). However, feather cover was accumulated over three separate body areas and most of the hens had perfect feather condition, making it difficult to make meaningful comparisons between different body areas (Chapter 4). The way that information on feather cover was collected was also variable. In Chapter 4, feather cover was scored without handling the hens, only walking through each pen. Whereas in Chapter 5, each hen was held and scored during handling. The difference in data collection may make it difficult to compare the two studies.

6.2. Limitations

One of the limitations of the study was that Chapter 2 and 3 were only conducted in a single multi-tier design, meaning the results cannot be directly extrapolated to represent other multi-tier systems. Hence, the modifications in Chapter 4 were only directly relevant to the Bolegg terrace multi-tier system, though this is a commonly used multi-tier system. However, the aim was to assess elements common between systems, and it is possible, therefore, that this study can aid in determining the starting points for improving other multi-tier systems. It would be sensible to begin studies of modifications at the nest box and top tier area in other multi-tier systems.

Another limitation is that the accelerometry output from the falls and collisions detected in Chapter 2 could not be linked to a precise event or behaviour. For example, it is not known whether a hen falling and then colliding with a perch generated the maximum summed AV at the point of collision or from the fall preceding the collision. This is partly due to the sampling rate of the accelerometers used being high (500HZ on each sensor), meaning that there are 500 readouts per second on each sensor. This level of accuracy makes it difficult to exactly match behaviours to video outputs, where the video recorded at fewer frames per second than the accelerometers. However, if a reduced sampling frequency was used on the accelerometers it is likely many events may have been missed.

Another limitation of the accelerometer analysis is that the data generated and used in Chapter 2 always corresponded to the acceleration vector (this is the sum of all 3-axis). If all the axes were looked at separately, this would have only been appropriate for the body sensor as the orientation was known. For the keel sensor it was not possible to determine the orientation of the sensor once fitted to the hen because it was able to move somewhat within the vest. However, the body sensor was always orientated in a known direction. The body sensor could have indicated whether the movement was a transition between tiers and perches or whether the output was generated when the hen was not moving (such as preening and dustbathing). Different levels of classification accuracy were found between different axes from cows (Watanabe *et al.*, 2008) and different axes were important when classifying behaviours of badgers (Graf *et al.*, 2015). Future work would benefit from analysing each axis in turn to classify behaviour.

One of the greatest limitations in this PhD thesis was the sample size in Chapter 4 (n=2 per treatment). This has already been discussed in Chapter 4, but it is important to reiterate that further research into mushroom-shaped perches would be needed before a conclusive decision is made about their suitability for use in laying hen housing systems. Another study could look at the use of mushroom-shaped perches but remove the grooves that were present on the surface, reducing faecal build-up and possibly reducing the prevalence of foot pad lesions.

6.3. Implications of the results

Research within this thesis was conducted in several countries within Europe (Switzerland, the Netherlands and Belgium) and the results obtained are important for the future development of laying hen multi-tier systems. Although the studies in Chapter 2, 3 and 4 were solely focussed on the Bolegg terrace multi-tier, the results indicate that ramps may have a benefit to reducing keel bone fracture prevalence (Chapter 5 only) and reduce footpad dermatitis and bumblefoot prevalence (Chapter 4 and 5).

This was the first body of evidence that the greater the total height hens are moving within a system the more likely they are to have high maximum summed AV at their keel bone. This adds to the body of evidence that falls and collisions in multi-tier systems are the cause of the increased levels of keel bone fractures compared with cages and single-tier systems (Gregory and Wilkins, 1996; Moinard *et al.*, 2004a; Wilkins *et al.*, 2011). Up until now, experimental studies have shown that increased forces on the keel of euthanised hens are more likely to result in keel bone fractures and that fractures were more severe, compared to lower forces (Toscano *et al.*, 2018). However, this is the first time this data has been quantified on living hens.

This was also the first in-depth study into areas within a multi-tier system that result in the highest and lowest percentage of falls. The body of research presented in this thesis shows the first possible step in identifying these key areas where falls and collisions occur and customising these areas to improve the mobility of laying hens; potentially reducing falls, collisions and keel bone fractures.

One of the advantages of this study is that there is a rich dataset of accelerometry outputs that are linked to a coded behaviour. Algorithms and machine learning tools can potentially link an accelerometry output to a behaviour. It may be possible to use this dataset to teach a machine learning algorithm what is a non-fall/ non-collision, non-fall/ collision, fall/ non-collision and a fall/ collision. This tool could then be used on farms equipped with multi-tier systems to determine the number of falls and collisions that are occurring on a farm. Once the data are collected from farms, it may then be possible to create bespoke modifications for that farm; either remove or adding in additional furnishings or recommending different rearing conditions that provide a more challenging environment to aid pullets in developing navigational skills for a multi-tier farm as adults.

Future avenue of study should be the direct monitoring of behaviours using wavelet analysis (Preece *et al.*, 2009) or machine learning techniques (Mannini and Sabatini, 2010). This study went some way in developing these techniques, but not to the extent that could be reported in this thesis. Algorithms have been developed for humans to identify falls and collisions (Chaudhuri *et al.*,

2014) and developing the data in the current study further would be beneficial to laying hen welfare because it may be possible to determine how many falls occur in housing systems and make a comparison between different systems. With the right data inputs and analysis, it may even be possible to distinguish detailed behaviours. Although, this data would have been incredibly useful, much more time and preparation of the data set was needed. Training was also needed to learn how to properly use the software and analyse the data. Future work should collaborate with computational experts to develop this data further.

Accelerometer outputs were looked at in excel and it could be seen that patterns could potentially be identified in the data between non-falls and falls as well as non-collisions and collisions. With the help of collaborators, (Andrew Dowsey; Figure 6.1 and Piotr Slowinski; Figure 6.2) preliminary analysis using a machine learning and wavelet transform were attempted. Figures below were created used R statistical software (machine learning) and Matlab (Mathworks, 2014) using a package described in previous work (Grinsted *et al.*, 2004). The data is Figure 6.1 is categorising falls and non-falls movements and the data from Figure 6.2 is using both a combination of Fourier transform and wavelet analysis to generate differences between collisions and non-collisions.

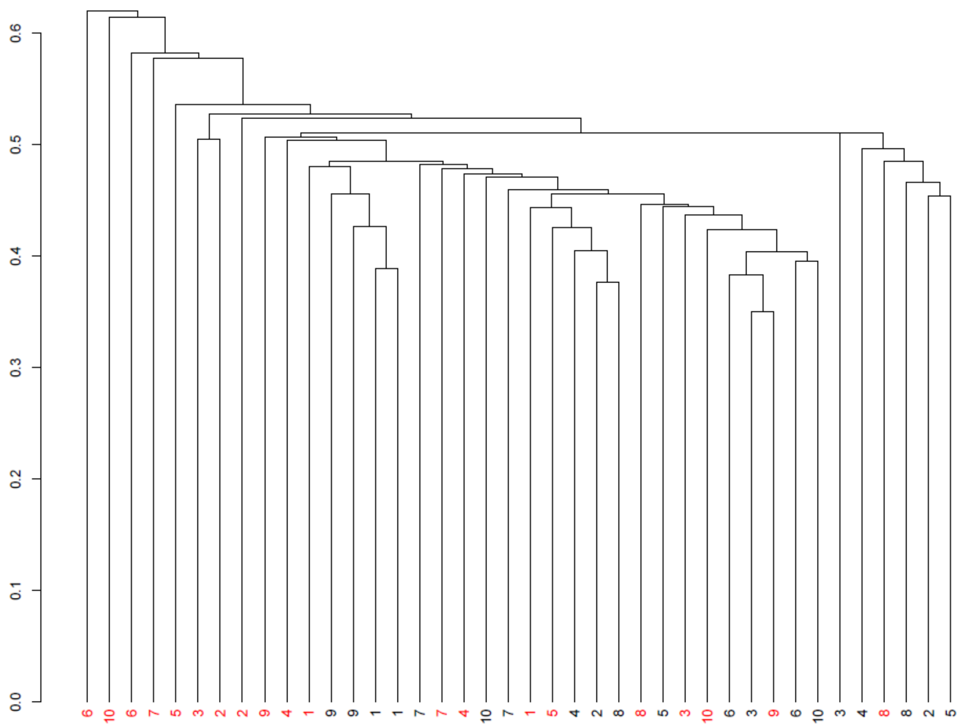

Figure 6.1: *Machine learning output showing classification of falls (red) and non-falls (black).*

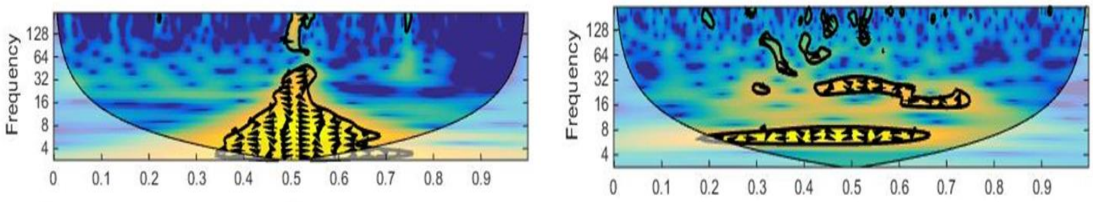

Figure 6.2: *Wavelet analysis of a collision (left) and a non-collision (right)*

This thesis was also partly funded by industry, so the results can potentially be developed by the industrial partner and used in the designing of future systems. The results should aid industry and researchers in the future when selecting housing design aspects to focus on for improving laying hen welfare. It is crucial that the effects of the mushroom-shaped perches, perch placement and ramps are investigated in more detail, with a focus on different designs (e.g. smoothing the surface of mushroom-shaped perches) and materials (e.g. plastic vs metal for perches and ramps).

6.4. Conclusions

Overall, the thesis adds to the existing body of knowledge about multi-tier systems for laying hens and how hens interact with their environment. The main results conclude that falls and collisions have higher loads at the keel and therefore pose a threat to keel bone health. The top tier area and the region around the nest box lead to more falls compared to other areas within the system and ramps have the potential to improve keel bone fracture prevalence and foot pad health.

6.5 References

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