OPPORTUNITIES FOR EXERCISE DURING PULLET REARING: EFFECTS ON BONE HEALTH AND KEEL BONE DAMAGE IN LAYING HENS

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This thesis is an investigation of the effect of providing opportunities for exercise on long term bone health of laying hens, with an emphasis on keel bone damage. Osteoporosis in laying hens is a welfare concern as it increases the risk of bone fractures. The keel bone is especially susceptible to fracture during the laying period. Providing opportunities for exercise during pullet rearing, a period of substantial musculoskeletal growth, offers a proactive approach to reducing osteoporosis by stimulating osteogenesis. The main objective was to determine whether rearing environments that offer different opportunities for exercise have long term effects on bone health and keel bone damage of laying hens. A secondary objective was to determine whether adult housing systems that offer different opportunities for exercise have an effect on bone health and keel bone damage of laying hens. Additionally, behavioural differences between hens with or without keel bone fractures were assessed, and an accelerometer to measure inactivity in laying hens was validated. Aviary-reared pullets had greater values for bone cross-sectional area, bone mineral density, bone mineral content, and bone breaking strength in wing and leg bones compared to bones of conventionally-reared pullets. Rearing effects on several bone composition measures were maintained through the end-of-lay, with aviary-reared hens having greater values for bone cross-sectional area and bone mineral content compared to conventionally-reared hens; however, bone mineral density was greater in conventionally-reared hens. Hens in large furnished cages had greater values for bone mineral density and content compared to adult hens housed in conventional cages. Aviary-reared hens had a lower prevalence of keel bone fractures throughout the laying period.
period compared to conventionally-reared hens, with no effect of adult housing. Hens with keel fractures perched more and stood less than hens without keel fractures. Further investigation into whether these behavioural differences cause keel damage, or are a coping strategy resulting from keel damage is warranted. Future studies using the accelerometer validated within this thesis to quantify inactivity levels in laying hens may be useful in measuring changes in activity as a result of keel damage or other pain related conditions in laying hens.
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The following quote has stuck with me over the last several years, and it represents my current state of mind (however idealistic it may be) as I approach my next exciting, but unknown endeavors:

“The future belongs to those who believe in the beauty of their dreams”

-Eleanor Roosevelt

So here we go…
### TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgements</td>
<td>iv</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>vi</td>
</tr>
<tr>
<td>List of Tables</td>
<td>viii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>x</td>
</tr>
</tbody>
</table>

#### Chapter 1: Introduction

INTRODUCTION

1.1 North American Egg Production ......................................................... 1
1.2 Societal Concern for Animal Welfare .................................................. 1

#### Chapter 2: Literature Review

LITERATURE REVIEW

2.1 Historical Review of Osteoporosis in Laying Hens .................................. 4
2.2 Welfare Concerns Related to Osteoporosis and Pain Related to Fractures .......... 6
2.3 Keel Bone Damage ...................................................................................... 8
2.4 Keel Bone Form and Function ...................................................................... 9
2.5 Avian Bone Physiology and Calcium Mobilization for Egg Production .......... 11
2.6 Strategies to Prevent or Reduce Osteoporosis and Bone Fractures .......... 14
   2.6.1 Nutrition ............................................................................................. 14
   2.6.2 Genetics ............................................................................................... 15
   2.6.3 Exercise ............................................................................................... 16

2.7 Importance of Targeting the Pullet Rearing Phase .................................... 20
2.8 Research Objectives ................................................................................... 34

#### Chapter 3: Long Term Effects of Rearing System on Bone Characteristics of Pullets Through to the End-of-Lay in Adult Laying Hens

3.1 Abstract .................................................................................................... 36
3.2 Introduction ................................................................................................ 38
3.3 Methods ........................................................................................................ 43
3.4 Results ......................................................................................................... 53
3.5 Discussion ................................................................................................... 57
Chapter 4 .................................................................86
Rearing system affects prevalence of keel bone damage in laying hens: a longitudinal study of four consecutive flocks
4.1 Abstract ........................................................................86
4.2 Introduction ....................................................................87
4.3 Methods ......................................................................90
4.4 Results ......................................................................98
4.5 Discussion ..................................................................101

Chapter 5 ........................................................................112
Behavioural differences of laying hens with fractured keel bones within furnished cages
5.1 Abstract ......................................................................112
5.2 Introduction ...................................................................113
5.3 Methods .....................................................................116
5.4 Results ....................................................................122
5.5 Discussion ..................................................................124

Chapter 6 ........................................................................136
Validation of an accelerometer to quantify inactivity in laying hens with or without keel bone fractures
6.1 Abstract ......................................................................136
6.2 Introduction ...................................................................137
6.3 Methods .....................................................................141
6.4 Results ....................................................................147
6.5 Discussion ..................................................................148

Chapter 7 ........................................................................161
GENERAL DISCUSSION
7.1 General Discussion of Main Findings ..........................161
7.2 Future Directions for Improving Skeletal Health in Laying Hens .................................166
7.3 Defining Animal Welfare ..............................................169

Literature Cited ................................................................173
LIST OF TABLES

Table 3.1........................................................................................................................................68
Comparison of muscle weights between aviary-reared (Avi) and conventionally-reared (Conv) pullets at 16 wks of age. All muscles weights were adjusted for pullet body weight.

Table 3.2 ........................................................................................................................................69
Comparison of keel bone growth between aviary-reared (Avi) and conventionally-reared (Conv) pullets at 16 wks of age. All keel bone skeletal characteristics were adjusted for body weight.

Table 3.3 ........................................................................................................................................70
Comparison of Quantitative Computed Tomography (QCT) bone measures between aviary-reared (Avi) and conventionally-reared (Conv) pullets at 16 wks of age.

Table 3.4 ........................................................................................................................................71
Comparison of bone breaking strength between aviary-reared (Avi) and conventionally-reared (Conv) pullets at 16 wks of age.

Table 3.5 ........................................................................................................................................72
Quantitative Computed Tomography (QCT) bone measures of the radius in adult laying hens at 73 wks of age.

Table 3.6 ........................................................................................................................................73
Quantitative Computed Tomography (QCT) bone measures of the humerus in adult laying hens at 73 wks of age.

Table 3.7 ........................................................................................................................................74
Quantitative Computed Tomography (QCT) bone measures of the tibia in adult laying hens at 73 wks of age.

Table 3.8 ........................................................................................................................................75
Comparison of bone breaking strength in adult laying hens at 73 wks of age.

Table 4.1..........................................................................................................................................109
Main effects of age, rearing system, and adult housing system on the percentages of keel bone fractures and deviations.

Table 5.1 ..........................................................................................................................................132
Ethogram used for behaviour observations.
Table 5.2 .................................................................................................................................................. 133
Description of each behaviour as a percentage of the total 90 min observation period.

Table 5.3 .................................................................................................................................................. 134
Relationship between fracture severity and the percentage of time resting on the floor vs perch.

Table 6.1 .................................................................................................................................................. 155
Ethogram used for focal behaviour observations
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Examples of various, commercially available pullet housing systems</td>
<td>25</td>
</tr>
<tr>
<td>2.2</td>
<td>Example of a standard, conventional rearing cage offered in various forms by several poultry equipment companies</td>
<td>28</td>
</tr>
<tr>
<td>2.3</td>
<td>Farmer Automatic Combi Pullet System</td>
<td>29</td>
</tr>
<tr>
<td>2.4</td>
<td>Big Dutchman Natura Rearing System</td>
<td>30</td>
</tr>
<tr>
<td>2.5</td>
<td>Barn floor rearing system with perches added</td>
<td>31</td>
</tr>
<tr>
<td>2.6</td>
<td>Farmer Automatic Pullet Portal Rearing System</td>
<td>32</td>
</tr>
<tr>
<td>2.7</td>
<td>Wing-assisted running exhibited by fowl-like Galliformes</td>
<td>33</td>
</tr>
<tr>
<td>3.1</td>
<td>Images of the Farmer Automatic Logia Pullet Portal aviary rearing system and conventional cage rearing system</td>
<td>76</td>
</tr>
<tr>
<td>3.2</td>
<td>Quantitative Computed Tomography (QCT) measurements comparing the effect of rearing environment on total bone measures of the radius at 16 wks (Pullet Reference Value) and 73 wks from hens housed in furnished cage-large (FC-L), furnished cage-small (FC-S), and conventional cages (CC)</td>
<td>77</td>
</tr>
<tr>
<td>3.3</td>
<td>Quantitative Computed Tomography (QCT) measurements comparing the effect of rearing environment on cortical bone measures of the radius at 16 wks (Pullet Reference Value) and 73 wks from hens housed in furnished cage-large (FC-L), furnished cage-small (FC-S), and conventional cages (CC)</td>
<td>79</td>
</tr>
<tr>
<td>3.4</td>
<td>Quantitative Computed Tomography (QCT) measurements comparing the effect of rearing environment on total bone measures of the tibia at 16 wks (Pullet Reference Value) and 73 wks</td>
<td>81</td>
</tr>
</tbody>
</table>
from hens housed in furnished cage-large (FC-L), furnished cage-small (FC-S), and conventional cages (CC).

**Figure 3.5**
Quantitative Computed Tomography (QCT) measurements comparing the effect of rearing environment on cortical bone measures of the tibia at 16 wks (Pullet Reference Value) and 73 wks from hens housed in furnished cage-large (FC-L), furnished cage-small (FC-S), and conventional cages (CC).

**Figure 3.6**
The state of cortical and medullary bone (MB) growth, prior to sexual maturity at 16 wks, at initiation of 1st egg, and at 67 wks of age.

**Figure 4.1**
Effect of age and rearing system on the percentage of keel bone fractures and deviations.

**Figure 4.2**
Distribution of keel bone fracture (FR) and deviation (DEV) prevalence at different ages during the laying period as scored by the simplified keel assessment protocol (SKAP) method.

**Figure 5.1**
Mean bout length of sitting, standing, and sleeping behaviours for hens with varied keel status.

**Figure 6.1**
Actical necklace attached to hen (A) and image of Actical accelerometer attached to beaded chain necklace with a size comparison for reference (B).

**Figure 6.2**
Correlation between Stationary Inactivity (SI), as determined by focal, live bird observation, and Actical Inactivity (AI) zero count

**Figure 6.3**
Comparison of the Actical Actogram graph output (A) and focal behaviour observation output (B) of a single bird over a concurrent one hour period.

**Figure 6.4**
Distribution of mean Actical Inactivity zero count by keel bone status for each individual bird

**Figure 6.5**
Example of Actical Actogram activity graph.
CHAPTER 1
INTRODUCTION

1.1 North American Egg Production

The North American egg industry raises more than 330 million laying hens each year, with approximately 90% of the production carried out in conventional cages (Statistics Canada, 2012; USDA, 2016). Approximately 27 million laying hens are raised for egg production annually in Canada, with the largest proportion of hens raised in Ontario (9.9 million hens) followed by Québec (5.1 million hens; Statistics Canada, 2012). Average flock size in Canada is 20,000 hens (ranging up to > 400,000 hens/flock), with each hen producing an average of 305 eggs/hen/year (AAFC, 2015). In the United States, approximately 302 million egg laying hens are raised yearly, producing an average of 288 egg/hen/year. The states with the largest egg production are Iowa (47.9 million hens), Ohio (31.5 million hens), and Indiana (29.3 million hens). US flock sizes range from an average of > 75,000 hens/flock to > 1 million hens/flock with a small proportion of farms raising > 5 million hens/flock (USDA, 2016).

1.2 Societal Concern for Animal Welfare

The welfare of laying hens has been at the forefront of public concern over the last few decades in North America, with the first notable change initiated by the passage of Proposition 2 in 2008 by the state of California, effectively banning the use of conventional cages (Mench et al., 2011). Several states (Michigan and Ohio) followed suit, followed by the first attempt at federal legislative changes initiated by the Humane Society of the United States (HSUS) and the United Egg Producers (UEP) in 2013. The “Egg Products Inspection Act Amendments of 2013”
was the first initiative to ban the use of conventional cages at a national level, moving toward a transition to enriched colony housing (furnished cages). Although the Bill failed to pass, the campaign to change the way laying hens are raised in North America continues to be fueled by pressure from public advocacy groups and pledges from retailers such as McDonald’s, Burger King, Tim Hortons, A&W, Walmart, and several Canadian grocery chains (Loblaw, Sobeys and Metro) to shift to selling products from cage-free hens only.

At a national level, Canada has recently surpassed the United States in progressive changes to animal welfare guidelines when Egg Farmers of Canada committed to a voluntary ban on installation of new conventional cage housing starting July 1, 2016. Additionally, an updated *Code of Practice for the Care and Handling of Pullets and Laying Hens*, currently in the final stage of review with an expected release date of 2017, calls for a transition to enriched colony or non-cage housing over the next 15 years, with 50% of the shift completed within the next 8 years. For the *Codes of Practice*, the *Review of Scientific Research on Priority Issues* (Widowski et al., 2013) clearly outlines six Priority Issues considered to be areas of concern for hen welfare:

- Rearing Methods
- Housing: Conventional, Furnished, and Non-Cage Systems
- Space Allowance and Group Size for Different Housing Systems
- Bone Health
- Feather Pecking and Cannibalism
- Beak Treatment

Three of the six Priority Issues were addressed in this Thesis, with the greatest emphasis on bone health, followed by information regarding rearing methods and housing of adult hens.
With the transition to alternative housing systems, the development of a sound bird is essential. Pullet rearing has the greatest potential to positively impact both the physical and cognitive capacity of the hens so that they can thrive in the adult housing system of their laying phase.
CHAPTER 2

LITERATURE REVIEW

2.1 Historical Review of Osteoporosis in Laying Hens

Poor skeletal health in commercial laying hens has been well documented as a production issue since the 1950s, a welfare issue since the 1980s, and as the topic of several extensive scientific reviews over the last several decades (Whitehead and Wilson, 1992; Thorp, 1994; Knowles and Wilkins, 1998; Newman and Leeson, 1997; Rath et al., 2000; Webster, 2004, Beck and Hansen, 2004; Fleming, 2008). Initially investigated as bone mineralization deficiency, signs of osteopenia (bone loss) began appearing shortly after implementation of battery cages in the 1940s and 1950s. The symptoms typically manifested in the form of spontaneous fractures within the spine or appendicular skeleton resulting in reduced mobility and increased risk of emaciation, starvation, or spontaneous mortality if left untreated (Couch, 1955; Grumbles, 1959; Urist and Deutsch, 1960; Bell and Siller, 1962; Riddell, 1968). With advances in nutritional strategies and improved understanding of critical metabolic pathways such as the calcium phosphorus ratio and vitamin D requirements (Taylor and Moore, 1954; Summers et al., 1976), cases of osteomalacia (the softening of bones as a result of improper bone mineralization or deficiencies in dietary calcium, phosphorus, or vitamin D), have become a relatively infrequent problem with practical management solutions with treatment options for commercial flocks.

While the incidence of osteomalacia can be effectively controlled by nutritional management strategies, another more complicated source of skeletal health concern is osteoporosis. Osteoporosis is the development of fragile, brittle bones from bone loss in the form
of a narrow trabecular framework and large pockets of empty space resulting from a combination of factors such as hormonal changes, calcium to phosphorus ratio, calcium metabolism, and genetics, as described thoroughly in several reviews (Knowles and Wilkins, 1998; Rath et al., 2000; Whitehead and Fleming, 2000; Webster, 2004; Whitehead, 2004). In laying hens, osteoporosis is believed to be a consequence of very effective selection for increased egg production over the last half century. The onset of osteoporosis is thought to begin between 16 and 31 wks of age when histological evidence demonstrates that the amount of cancellous (trabecular) bone volume declines by approximately 50% as shift towards production of medullary bone takes over. Sustained egg production exacerbates the problem leading to a dramatic increase in the prevalence of osteoporosis between 31-42 wks of age as the skeletal calcium reserves are substantially eroded (Cransberg et al., 2001), coinciding with reports of a dramatic increase in fractures around 40 wks of age (Fleming, 2008). The widespread problem of osteoporotic hens (Jendral et al., 2008) brings up the question of whether aspirations to achieve a “500 egg hen” is truly feasible or ethical.

In terms of production concerns, the effects of osteoporosis are in most cases only visible when the case is severe, first described as ‘cage layer fatigue’ (Couch, 1955), resulting in spontaneous immobility, mortalities, and poor egg shell quality. As the amount of structural mineralized bone tissues continues to decrease, the bones increase in fragility and are more susceptible to fractures (Whitehead, 2004). This brings up an ethical question relating to whether or not the welfare of the bird is compromised when it has a skeletal system with a high likelihood of incurring fractures during its production life.
2.2 Welfare Concerns Related to Osteoporosis and Pain Related to Fractures

Osteoporotic hens have been a prominent welfare concern since the 1980s because of the relationship between osteoporosis and high fracture incidence during the laying period (Thorp, 1994; Knowles and Wilkins, 1998; Fleming et al., 1998a; Sandilands et al., 2009), which is often further exacerbated during catching and handling for transport to slaughter (Gregory et al., 1992). Even when housing design and diet are held constant among groups, the lower breaking strength of osteoporotic bones was enough to significantly increase the hen’s risk of fracture (Knowles et al., 1993). The high prevalence of osteoporosis reported in commercial flocks around the world has led to a variety of research initiatives to attempt to eradicate this elusive health and welfare problem.

The prevalence of osteoporosis has been reported as responsible for 35% of the mortalities reported within commercial flocks (McCoy et al., 1996), and disorders related to calcium metabolism (osteoporosis, osteomalacia, osteopenia, and hypocalcimia) are still responsible for a high percentage of mortalities (51.2%) in current strains of commercial laying hens (Widowski, unpublished). Mortalities related to osteoporosis are believed to be a result of both paralysis from spinal degeneration and the development of improper muscle function due to lack of metabolic calcium reserves (Whitehead, 2004). The prevalence of old skeletal fractures (fractures occurring during the production life of the hen, prior to depopulation) in the femur, ischium, and pubis have been reported as high as 30%, increasing another 17% at depopulation with fractures due to catching, handling and transport (Gregory et al., 1990). The ischium, sternum, humerus, and pubis typically account for 50-78% of fractures (Knowles and Wilkins, 1998; Wilkens et al., 2004), with damage to the keel bone accounting for 90% of the fractures
reported (Wilkins et al., 2004). Similarities between mammalian and avian physiology suggest that all fractures present in poultry likely cause pain during the life of the hen (Gentle, 2011).

In laying hens, fractures have the potential to limit mobility and feed and water intake (Nasr et al., 2102a), increase pressure on the spinal cord (Riddell et al., 1968), restrict the normal respiratory process by having fresh or healed fractures of the ribs and keel bone (Duncker, 2000; Codd et al., 2005; Claessens, 2009), and are likely to cause acute and chronic pain (Nasr et al., 2012b).

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage (IASP, 1979 in Gentle 2011). In order for pain to be experienced by the animal, there are two main requirements: 1. the presence of nociceptors and 2. connections between those nociceptors and the cortex or analogous structure of the animal (Sneddon et al., 2014). Poultry have the presence of nociceptors as well as the connections to the pallium, their cortex equivalent (Gentle, 2011). The presence of these structures (nociceptors and C-fibers), coupled with the behavioural and physiological responses indicative of a painful experience provide evidence that poultry have the capacity to experience pain (Webster, 2004). The periosteum membrane present on the bone surface is very highly innervated in humans and sensitive to stretching and tearing (Talbert, 2010), and bone fractures in particular are commonly associated with a painful experience in humans due to the presence of nociceptors in the periosteum (Kessenich, 2000). The disruption of the neuroreceptor-rich periosteal layer due to fractures of the keel or other bones within the laying hen skeleton are highly likely to cause pain both at the time of fracture and during the healing process while the fracture is unstable (Fleming et al., 2004).
2.3 Keel Bone Damage

Recent research has highlighted the sternum of the hen (the keel bone) as a site particularly susceptible to fractures during the laying period with prevalence rates ranging from 10-68% in conventional cages (Sandilands and Sparks, unpublished as described in Sandilands et al., 2009; Sherwin et al., 2010; Petrik et al., 2015; Regmi et al., 2016a), 20-60% in furnished cages (Vits et al., 2005; Weitzenburger et al., 2006; Rodenburg et al., 2008; Sherwin et al., 2010; Wilkins et al., 2011), and approximately 50-90% in non-cage systems (Nicol et al., 2006; Sandilands and Sparks, unpublished as described in Sandilands et al., 2009; Sherwin et al., 2010; Kappeli et al., 2011a,b; Wilkins et al., 2011; Stratmann et al., 2015a; Regmi et al., 2016a). Although the prevalence of keel fractures is typically highest in non-cage systems, the presence of healed fractures occurring during the laying cycle are still detected in conventional cage systems, and the incidence of fresh fractures occurring during depopulation has been reported as nearly five times higher in hens from conventional cage systems compared to furnished and non-cage systems (Sherwin et al., 2010).

As reviewed by Harlander-Matauschek et al. (2015), the high rates of keel bone damage may be a result of a combination of factors such as increased risk of crashes in non-cage systems (Stratmann et al., 2015a), unequal wing-loading during wing-flapping, perch use (Tauson and Abrahamsson, 1994; Abrahamsson et al., 1996; Sandilands et al., 2009; Pickel et al., 2011; Hester et al., 2013; Stratmann et al., 2015b), compression fractures due to osteoporosis as seen in humans (Kondo, 2008), early onset of egg production (Gebhardt-Henrich and Frohlich, 2015), nutritional inadequacies (Whitehead, 2004; Fleming et al., 2006), reduced breast muscle mass of modern layers (Fleming et al., 2004), or genetic factors (Whitehead, 2004; Stratmann et al., 2016).
Keel bone damage typically occurs in the form of fractures and deformations along the spine of the keel as well as at the caudal tip (Casey-Trott et al., 2015). Fractures range from severe, complete fractures separating bone material, to less severe, incomplete fractures similar to ‘greenstick’ fractures as seen in human medicine (Chapter 5). Greenstick fractures are common in human pediatric cases as a result of bone mechanics. Pediatric bone has a lower elastic modulus than mature adult bones, and as a result, bends more in response to stress than adult bone, but fractures at lower loads resulting in incomplete greenstick fractures (Hefti, 2007). In human medicine, these types of fractures are known to be unstable and increase the potential for secondary angulation and further fracture several wks after the initial injury (Randsborg and Sivertsen, 2009).

2.4 Keel Bone Form and Function

The keel bone is an extension of the ventral surface of the sternum progressing along the midline of the sagittal plane. The keel spans from the cranial, Carina apex to the caudal tip, with the spine of the keel tapering off as it approaches the caudal portion of the keel (as described in Casey-Trott et al., 2015). The growth and ossification of the keel is a process that initiates in the cranial region of the keel progressing gradually toward the caudal tip. Ossification of the keel continues into the early stages of egg laying until approximately 28-40 wks of age (Buckner, 1949), well beyond the growth of the long bones which ceases at the onset of lay (Hurwitz, 1965; Hudson et al., 1993). Due to the slow ossification of the keel, the caudal portion of the keel is often still cartilaginous at the onset of lay (Chapter 3).

In avian species, the keel serves as an anchor to flight muscles and it is the development of adequate pectoral muscle growth on the keel bone that makes flight possible in birds. The
ratio between body size and pectoral muscle mass is imperative to successful flight attempts. Fowl-like birds of the *Galliformes* order are not designed for long-distance flight. Instead, they focus their anti-predator survival techniques on short bursts of direct-lifting flight with muscles comprised of anaerobic white fibres that fly strongly, but tire quickly (Duncker, 2000). Even though flight in fowl species is not sustained over great distances, the body weight to pectoral muscle mass ratio is still essential, requiring a flight muscle mass of at least 20% of the body weight for controlled lifting strokes during flight (Duncker, 2000). However, in attempts to maximize production efficiency in modern laying hens, the size of breast muscle mass has decreased (Fleming et al., 2004) and the overall body mass of commercial laying hen hybrids is roughly twice that of its ancestral jungle fowl (Jackson and Diamond, 1996); leaving the keel bone more exposed, and likely reducing the control and efficacy of flight attempts.

The keel bone also plays a pivotal role in expanding and contracting the thoracic cavity during inhalation and exhalation (Duncker, 2000; Powell, 2000; Codd et al., 2005; Claessens, 2009; Lambertx and Perry, 2015). Unlike mammals, avian species have extremely efficient, unidirectional respiratory function. The unidirectional flow of air through the lungs allows for constant absorption of oxygen into the blood stream; however, this anatomical design requires that airflow be driven by the presence of multiple air sacs acting as bellows ventilating the lungs, rather than a muscular diaphragm as seen in mammals (Powell, 2000). In order to draw air into the air sacs, the ribs swing cranially dropping the keel bone ventrally leading to an increased space within the thoracic cavity. Air then fills the air sacs, and subsequently, abdominal muscles attached to the keel bone contract, drawing the keel dorsally and forcing air out of the air sacs and into the parabronchials of the lungs (Zimmer, 1935 as cited in Powell, 2000; Codd et al., 2005; Claessens, 2009).
The role of the keel bone in both flight and respiratory efficiency, two processes that truly set avian species apart from all other land-bound species, highlights the fact that the keel bone is essential to the successful daily function of birds.

2.5 Avian Bone Physiology and Calcium Mobilization for Egg Production

Thorough descriptions of avian bone biology and calcium mobilization can be found in several review articles (Whitehead and Fleming, 2000; Whitehead, 2004; Beck and Hansen, 2004).

As an overview, birds possess three forms of bone, two of which are dedicated to structural support (cortical and trabecular bone) and one form, which is unique to birds (and crocodilians), is a type of bone known as medullary bone serving as a labile source of calcium used in egg shell formation (Mueller et al., 1964; Miller et al., 1984). During the chick and pullet phase, cartilage cells located at the growth plates divide and increase the length of the bones until the pullet approaches sexual maturity, at which point the cartilage is mineralized and long bone growth ceases (Whitehead, 2004). The majority of this longitudinal bone growth is dedicated to the formation of cortical bone, the hard, outer shell of the bone, and cancellous, trabecular bone, which forms a network of crossing struts that fill the internal cavity of the long bones (Fleming et al., 1998a).

As the bones grow, they undergo a constant process of modeling and remodeling which is driven by osteoblasts and osteoclasts. Osteoblasts are considered to be bone-forming cells, whereas osteoclasts have a bone-resorbing function. Both cell types work together in areas of active bone remodeling with the osteoclasts mobilizing the bone tissue to circulate calcium stores, followed by the osteoblasts depositing new bone tissue (Whitehead, 2004).
In the final wks before sexual maturity, the diameter of the long bones increases by approximately 20% (Riddell, 1992) with rapid accumulation of cortical bone on the outer surface of the bone accompanied by rapid resorption on the endosteal bone surface to accommodate the subsequent production of medullary bone (Whitehead, 2004).

During the transition into the laying phase, estrogen levels rise (Beck and Hansen, 2004) and shift the function of the osteoblasts to begin building medullary bone instead of structural bone (Whitehead, 2004). Unlike the structural components of bone (cortical and trabecular), which are composed of a highly organized network of collagen layers known as lamellar bone (Whitehead, 2004), medullary bone is a woven (non-structural) component that contributes only marginally to the overall strength of a bone (Fleming et al., 1998b; Whitehead, 2004). Medullary bone has such rapid turnover that it does not have enough time to form an organized collagen network in response to loading strains (Fleming et al., 1998b). Approximately 10-14 days prior to the onset sexual maturity (Simkiss, 1961), medullary bone begins to coat the trabecular bone within the interior cavity of the structural cortical bone as estrogen levels rise (Hurwitz 1964; Hudson et al., 1993; Beck and Hansen, 2004). Although cortical and trabecular bone growth ceases at the onset of lay (Hurwitz, 1965; Hudson et al., 1993), medullary bone mobilization and replenishment from dietary calcium is a continuous process as long as estrogen levels remain high for egg production (Fleming et al., 1998a,b).

To support this process, commercial layer diets include high levels of calcium, offering approximately 4% calcium/d for egg shell production and medullary bone renewal (Hurwitz, 1964; Summers et al., 1976); however, the rate of calcium deposition into the shell is limited by the gastrointestinal absorption of calcium (Tyler, 1940a,b; Simkiss, 1961; Al-Batshan et al., 1994), and subsequently, only 60-75% of the calcium required for the shell is derived from the
diet with the remainder drawn from the bone (Mueller et al., 1964). Osteoclasts mobilize calcium from the bone indiscriminately gathering calcium from medullary bone if it is readily available and structural cortical bone in areas where medullary bone is depleted (Fleming, 2008). The endosteal lining of structural bone in mature hens is covered in osteoclasts and is noticeably more scalloped and irregular in comparison to immature hens whose endosteal surfaces are completely coated in medullary bone with the presence of significantly fewer osteoclasts (Hudson et al., 1993). Since cortical and trabecular bone are not replenished unless estrogen levels drop and the hen stops laying eggs, the structure of the bone weakens over time in areas repeatedly degraded by osteoclasts.

While molting is a natural combative process to this progressive bone weakening that allows for restoration of structural bone stores (Gregory et al., 1991), the period of low estrogen and a pause in production contradict the maximum efficiency principles of intensive commercial production. In contrast to wild birds where medullary bone stores are temporary (Kyes and Potter, 1934), commercial laying hens are no longer seasonal breeders (Sandilands et al., 2009). The constant demand for calcium puts extreme pressure on a relatively small skeletal system to continuously provide enough calcium to support the roughly 2-2.5 g of calcium required for each egg shell (Johnson, 2000) comprised of 96% calcium carbonate (Wisedt, 2013). This problem is further exacerbated by the combination of high productivity with restrictive housing limiting the amount of bone growth stimulation by muscle strain forces on bone during exercise. When bone loss decreases the bone mass of a hen by greater than 2.5 standard deviations, the hen is considered to be in an osteoporotic state (Beck and Hansen, 2004).
2.6 Strategies to Prevent or Reduce Osteoporosis and Bone Fractures

2.6.1 Nutrition

Although osteoporosis has been at the forefront of laying hen research for more than half a century, the condition is far from eliminated from commercial flocks around the world. Several different research avenues have arisen to combat the problem. The initial approach of using nutritional strategies effectively eliminated osteomalacia, but had limited success with osteoporosis. Understanding the rate limiting step of gastrointestinal calcium absorption (Tyler, 1940a,b; Taylor and Moore, 1954) has shifted the research focus away from allocating increasing amounts of dietary calcium to instead applying feeding strategies that better incorporate the relationship between the physiology of the hen and the feeding process.

Understanding that the natural circadian rhythm of egg shell deposition occurs over the nighttime hours when the dietary calcium supply is traditionally low, led to the shift in the time and the method of delivery of calcium (Hughes, 1973). Administration of large particle calcium in the diet allows for the slow release of calcium to the hen throughout the night when it is needed for the shell deposition process. This feeding practice is frequently reported as improving bone strength and egg shell quality (Fleming et al., 1998a; Saunders-Blades et al., 2009; Cufadar et al., 2011). Supplying the growing pullet with fine ground limestone as a calcium source has also been shown to improve bone strength (Koutoulis et al., 2009), and unlike calcium supplementation during the laying period, supplying calcium during the pullet phase supports the growth of structural as well as medullary bone (Hurwitz, 1964; Fleming, 2008).

Another nutritional approach has been to aid in the absorption of calcium by supplying various dietary additives such as omega-3 fatty acids (Tarlton et al., 2013), vitamin D
metabolites, ascorbic acid, low crude protein, vitamin K, or low phosphorus diets (Fleming, 2008); however, even though several of the additives mentioned above successfully increased the amount of medullary bone, the overall loss of trabecular volume was not prevented, leaving the majority of hens in an osteoporotic state (Rennie et al., 1997). These results indicate that it is not the supply and use of dietary calcium that is to blame for the widespread occurrence of osteoporosis in commercial flocks.

2.6.2 Genetics

One area with possibly the greatest potential for improvement in skeletal health is genetics (Whitehead, 2004). Genetic variation in the incidence and recovery rates from cage layer fatigue were initially reported in the 1950s (Francis, 1956), and subsequently, several studies have reported strain differences in bone quality characteristics (Hocking et al., 2003; Riczu et al., 2004; Budgell and Silversides, 2004; Silversides et al., 2006; Silverides et al., 2012). Bishop et al. (2000) highlighted the potential for using genetic selection as a tool for improving skeletal health, by demonstrating that genetic selection for improved bone strength yields moderate to high heritability within only six generations. Fleming et al. (2004) further confirmed this finding by demonstrating that the high bone index line had improved keel bone integrity with significantly less fresh fractures, and fewer twisted and severely deformed keels. Using the same genetic lines, Stratmann et al (2016) also reported that hens from the high bone index line had a significant reduction in keel bone fractures compared to both the low index line and a commercially available hybrid. The study also highlighted the potential additive effects of pairing genetics and housing system as two styles of aviary design were also compared in conjunction with assessment of the effects of genetic bone index on keel bone damage.
Unlike improvements made by dietary changes, the studies involving genetic selection report both improvements to overall bone characteristics and composition as well as reduction of fractures (Fleming et al., 2006). Lower incidence of old fractures in less selected, heritage lines compared to high producing commercial lines also suggest a genetic component to osteoporosis (Budgell and Silversides, 2004). Even within high-producing lines, up to a 7-fold difference has been reported between groups (Clark et al., 2008). Using the relatively short incubation and maturation period of poultry in combination with identifying quantitative trait loci (QTL) to find genetic markers for the bone strength characteristics (Dunn et al., 2007; Rubin et al., 2007; Podisi et al., 2012) has the potential to bring about positive changes to skeletal health in a relatively short period of time.

Unfortunately selection for improved bone characteristics does have the potential to negatively impact production traits and reduce efficiency. Reports of an inverse relationship between shell quality and bone strength (Bishop et al., 2000) and production and egg quality traits from the high bone index line (Stratmann et al., 2016) complicate the attempt to reduce osteoporosis solely through genetics; however, contrasting results have also been reported. This suggests that there is not enough evidence of a relationship between bone quality parameters and egg quality (Jendrel et al., 2008), indicating improvement to bone health without reduced egg quality may be possible. This apparent contradiction warrants further research into the direct relationship between bone quality and egg quality.

2.6.3 Exercise

Dating back to the 1950s when osteoporosis was first acknowledge as a problem in commercial flocks, confinement to battery cages was suspected to be in part responsible for the
poor bone condition (McCoy et al., 1996; Webster, 2004). This was because the severe cases of osteoporosis (cage layer fatigue) were rarely seen in floor raised systems (Couch, 1955; Grumbles, 1959). When improvements made by nutritional strategies seemed to plateau in terms of effectiveness, the importance of load-bearing exercise began to be further investigated.

In human medicine, a theory developed in the 19th century, known as Wolff’s Law, postulated that bone structure adapts its form in response to external loading forces to sufficiently support future bone loading. This responsiveness of bone to the strain produced by muscle contractions during exercise is one of the key regulators in the process of bone modeling and remodeling to achieve homeostasis in bone tissue (Huiskes, 2000). As loads increase, so does bone mass; the opposite effect is also true – disuse or absence of loading leads to reduced bone mass (Shipov et al., 2010). This adaptive response of bones to loading strain induced by exercise is a key component of mammalian and avian bone physiology (Laynon, 1993), and the incredible ability of bones to accumulate bone strength and composition to withstand added loading makes the area of exercise a strong candidate for identifying strategies for combating osteoporosis in laying hens.

The addition of perches (Tauson and Abrahamsson, 1994; Abrahamsson et al., 1996; Jendral et al., 2008; Ennecking et al., 2012; Hester et al., 2013) and dust baths (Jendral et al., 2008), increased cage heights, larger space allowances, and opportunities for flight were all part of cage modifications used to assess whether opportunities for load bearing exercise stimulated bone growth (Whitehead and Wilson, 1992; Newman and Leeson, 1998). While these adjustments were met with measured success, it seemed that marked improvements to bone health were predominately seen in cases where the opportunities for load bearing exercise were substantially increased and diverse (Whitehead, 2004). Floor, free-range, and aviary systems
designed to allow flight as well as perching, jumping, walking, and dustbathing provided the gains in bone strength, typically in the form of increased bone mineral density, cross-sectional area, cortical area, trabecular thickness, and decreased trabecular space (Michel and Huonnic, 2003; Jendral et al., 2008; Shipov et al., 2010; Silversides et al., 2012; Freire and Cowling, 2013); however, the increase in space and furnishings also put these non-cage hens at the highest risk for fractures and injuries from crashes and falls (Newman and Leeson, 1998; Whitehead, 2002; Rodenburg et al., 2008; Wilkins et al., 2011).

While the cases and severity of osteoporosis are commonly reduced in non-cage systems by improving overall bone composition and strength, the welfare of the hen is not necessarily improved because the incidence of fractures typically increases in non-cage systems (Regmi et al., 2016a). Inability to effectively navigate the system design (Campbell et al., 2016) and poor perch placement (angles or distance between perches) can increase the number of crashes and falls contributing to a greater risk of keel bone damage (Wilkins et al., 2011; Heerkeens et al., 2016). Addition of ramps or ladders between vertical levels has been shown to reduce the number of keel bone fractures (Stratmann et al., 2015a) and the need to investigate the effect of other system design elements, such as lighting and flooring, as risk factors for keel bone damage have been suggested (Harlander-Matauschek et al., 2015). Aside from crashes and falls, overall perch use and perch design has been well documented as a potential cause of keel bone deformations (Tauson and Abrahamsson, 1994; Abrahamsson et al., 1996; Sandilands et al., 2009; Pickel et al., 2011; Hester et al., 2013). The constant loading pressure of the perch on the keel bone likely induces keel deviations and fractures over time. Unfortunately, even though housing in extensive or alternative systems provides many behavioural benefits, until genetic
lines and housing systems are designed to accommodate the modern laying hen, there will be a trade-off between behavioural freedoms and risks of injury.

Even though keel bone damage from crashes, falls, or perch use is still a concern, evidence suggests that the load-bearing exercise provided by increased space, diversity of movement, and perch use in alternative systems still has great potential to improve overall bone health of laying hens. Load bearing exercise is a critical part of the prevention and treatment of osteoporosis in human medicine, and it is widely accepted that prevention is more successful than treatment (Vincente-Rodriguez, 2006). Stimulating peak bone mass through routine load bearing exercise during pre-pubertal growth in humans, has become a key area of interest for preventing osteoporosis, especially in women (Burrows, 2007). Although osteoporosis manifests in adulthood, the precursors begin in early childhood (Bailey et al., 1999).

Recently, the same concept has now shifted the focus to critical periods of exercise during the rearing phase of pullets. It is possible that building a stronger skeleton with a greater capacity for structural bone growth and medullary bone calcium reserves prior to sexual maturity can add to structural bone strength and reduce depletion later in life. There is some evidence that rearing in non-cage systems improved the overall bone composition of pullets at 16 wks (Regmi et al., 2015) and reduced overall keel bone damage (Vits et al., 2005). The addition of perches during rearing in conventional cages also provided some long term benefits to bone health in 71 wk old hens (Hester et al., 2013). Regmi et al. (2015) reported a positive effect on bone composition at 16 wks that was maintained during the laying phase. Although rearing effects on keel bone damage are less understood, results from Hester et al. (2013) indicated that the improvements in bone strength initiated by the minor addition of perches in conventional cages were not enough to prevent fracture and deformation of the keel bone.
2.7 Importance of Targeting the Pullet Rearing Phase

The pullet rearing phase offers a unique opportunity to influence the success of the adult laying hen in a variety of ways. Not only does exercise during rearing have the potential to improve physical characteristics such as bone composition and musculoskeletal growth, but environmental complexity has also been shown to improve cognitive abilities. Growing chicks and pullets undergo critical periods of age-related learning and development (Appleby and Duncan, 1989) that can affect how they navigate the space provided and utilize enrichments within the environment later in life. Rearing with perches improves perch use in adult hens (Gunnarsson et al., 2000) and the greater complexity of rearing environment provided by aviary-rearing compared to floor-rearing has lasting positive effects on overall mobility and space use, especially in terms of use of vertical levels in adult hens (Colson et al., 2005). Aviary-reared pullets also exhibit better use of three-dimensional space (Brantsaeter et al., 2016) and have a better working memory and ability to complete spatial tasks than conventionally-reared pullets (Tahamtani et al., 2015).

Enrichments and environmental complexity during rearing can also reduce fear responses. Chicks exposed to visual (Broom, 1969) and auditory enrichment (Candland et al., 1963), or a variety of objects in contrast to barren environments (Jones, 1982) showed reduced fear responses and performed better in tests of timidity. Recent research confirmed that complex non-cage rearing and additions of visual, auditory, gentle handling, and nutritional enrichments reduced fear of humans and novel environments (Morris, 2009).

With transitions toward the exclusive use of alternative housing systems for adult laying hens, it is critical to consider the importance of preparing pullets, both physically and
cognitively, for the demands required by their adult housing system. Matching the complexity of the rearing system as closely as possible to the adult housing system is recommended based on practical experience, and at minimum encouraging use of vertical space with provisions of perches and platforms is essential to reducing the risk of emaciation, dehydration, and floor eggs when pullets are moved into adult housing systems where amenities are located on various levels (Tauson, 2005).

The pullet housing systems currently available include various versions of standard, conventional cages (Figure 2.1A; Figure 2.2), larger, single-level cages with perches, such as the Natura rearing system, (Figure 2.1B; Figure 2.4; Big Dutchman, Holland, MI, USA) that can be opened up to allow for floor and vertical access at a later stage, multi-level Combi Pullet cages (Figure 2.1C; Figure 2.3; Farmer Automatic GmBH, Laer, Germany) that offer a raised platform and various perch heights while also providing the opportunity to open the cages to floor and vertical access, and finally open systems such as barn floor or aviary rearing systems. Barn floor rearing offers an entirely open space for locomotion, drinking, and feeding within the barn. Perches or platforms can be added to encourage use of vertical space (Figure 2.1D; Figure 2.5). Aviary rearing systems, such as the Pullet Portal (Farmer Automatic GmBH, Laer, Germany) or Jumpstart (Vencomatic, Cochrane, AB, Canada), provide a large open floor space within an enclosed system and, in addition, provide adjustable perches, platforms and drinking lines that can be raised in accordance with pullet growth and opened up to floor access (Figure 2.1E; Figure 2.6).

Each available system offers a different degree of complexity, especially at 1 day-of-age. Standard cages offer minimal opportunities for load bearing exercise and diversity of movement throughout the rearing phase. Single-level cages with perches and subsequent floor access offer
incrementally greater space for locomotion and perching; however substantial use of three-dimensional space is not permitted until the system is opened to the floor access, typically after six wks of age, potentially bypassing critical periods for learning and musculoskeletal development. Multi-level rearing cages encourage the use of vertical space with the raised platform and various perch heights, and chicks have been reported as using the platform and perches in these Combi Pullet systems as early as one and two wks of age, respectively (Habinski et al., in press).

Unlike the previously described cage systems, open floor plan systems such as barn floor and aviary rearing (Pullet Portal or Jumpstart) offer large floor areas that encourage walking and running locomotion. Unique to these systems is the opportunity for wing-assisted running (Figure 2.7) which involves motion of both the wings and legs, stimulating muscle and structural growth. Fowl species differ from long distance flight or soaring avian species in that their capacity for flight is very limited, and they are typically described as ‘a more terrestrial based, “running” bird’ (Duncker, 2000). Wing-assisted running is a naturally occurring behaviour in fowl species used for periods of rapid locomotion and predator escape (Duncker, 2000; Dial and Jackson, 2011). Young chicks frequently demonstrating running or walking with the assistance of their wings during rapid motion and escape attempts (Collias and Collias, 1967), and currently available commercial lines of pullets and laying hens have been shown to utilize wing-assisted incline running to access enrichments and rewards (LeBlanc et al., 2016). This type of high-intensity running and wing-flapping in pullets likely provides significant loading-strain on the leg, wing, and keel bones, stimulating bone growth. Although wing-stretching, stationary wing flapping, and flight are the most commonly discussed behaviours that impact wing and keel bone
growth, wing-assisted running likely also plays an important role and its value during the rearing period in relation to subsequent bone health should be further quantified.

Although both the barn and aviary systems provide an open environment to support such high intensity locomotion, the aviary rearing systems (Pullet Portal or Jumpstart) offer important cognitive and spatial training, in addition to increased opportunity for physical activity. The ability to raise platforms, perches, and drinking lines as the pullet grows encourages gradual increases in the use of vertical space. Especially if the pullets are going to be placed into an adult aviary system, resource allocation on multiple levels during the pullet phase is essential to preparing the birds to navigate complex systems to find feed, water and nests in the adult systems (Tauson, 2005).

It is common practice to place cage-reared pullets into cages as adults, and aviary-reared pullets into adult aviaries. As the transition is made toward alternative or non-cage adult systems, the building and modification of pullet-rearing systems will also be required to better accommodate transitions into alternative adult systems, a reality that has yet to be addressed and requires further research. In addition, it is still unclear which type of pullet rearing should be paired with housing adult hens in furnished cages; this area also requires further research.

Osteoporosis and the high prevalence of fractures in laying hens is a production-related problem. Unfortunately, as evident by nearly 80 years of research attempting to solve this worldwide problem, as long as laying hens are raised for egg production, it is not likely that the problem of osteoporosis and fractures in laying hens will be entirely eradicated. How the damage manifests itself and impacts the hen depends heavily on the housing system provided. Providing housing systems that encourage regular, diverse exercise has the potential to improve
bone composition and strength through load-bearing exercise, helping to reduce the prevalence of osteoporosis and fractures. The largely unexplored research avenue of targeting exercise during the pullet rearing phase in particular has the potential to stimulate physical and cognitive function, improve navigational capacities, and create a sound bird better prepared for lasting egg production and placement into complex adult systems.
Figure 2.1 Examples of various, commercially available pullet housing systems.

**Image A.** Farmer Automatic Classic Pullet as an example of a commercially available standard, conventional pullet rearing system with the feed trough and drinking line provided within the cage. Many other standard, conventional rearing systems are commercially available.


**Image B.** Big Dutchman Natura provides perches down the centre of the cage along with a feed trough, and drinking line. The cages are typically opened at approximately 6 wks to allow for access to the floor litter area. Side perches and ramps (right image) outside the cage aid in re-entry into system. The first two levels include a stationary feed trough, drinking line, and perch. The top level offers a high perch that is accessible after the cage units are opened to allow for floor and vertical access.

**Image C.** Combi Pullet system providing various perch heights, raised platform, winched drinking line, and a feed trough inside the compartment with side perches for access from the floor area. Unlike the Natura system above (Image B), the Combi system offers multiple levels of floor space within the compartment with the addition of the raised platform.

Image courtesy of Clark Ag Systems (Ontario, Canada):

**Image D.** Example of a barn floor rearing system. Chicks have access to entire barn space with litter, feed troughs, and drinking lines provided in the centre. Perches and platforms can be added to encourage the use of three-dimensional space.

Image courtesy of The Poultry Guide:
**Image E.** Farmer Automatic Pullet Portal (top image) includes a winched centre platform, perches, and drinking lines that can be raised in accordance with pullet growth. A feed trough is also located inside the system enclosure. Side platforms fold down to open the system at approximately 6 wks of age to allow for access to the floor litter area. A similar system known as Jumpstart is provided by Vencomatic (bottom image). Both systems include features that can be raised to grow with the pullets and allow large open floor areas for walking, running, perching, flight, and wing-assisted running from 1 day-of-age.

Images courtesy of Farmer Automatic (top image) and Vencomatic (bottom image):


Figure 2.2. Example of a standard, conventional rearing cage offered in various forms by several poultry equipment companies. Cages include a feed trough at the front and drinking line running down the centre. Total floor space depends on the equipment used. Pullets are housed inside rearing cages until placement into adult housing systems typically between 16-18 wks.

The standard, conventional cages pictured here were used to house all conventionally-reared pullets used experimentally as part of this thesis.
Figure 2.3 Farmer Automatic Combi Pullet System

**Image A.** View from inside the Combi system with the cage/compartment walls closed preventing access to the floor litter area. Cage enclosure offers perches at various heights, a winched drinking line, and a raised platform.

**Image B.** View from outside the Combi system with the cage/compartment walls opened allowing for access to the floor litter area. Systems walls can be opened at approximately 6 wks of age. Ramps can be supplied to encourage re-entry into the system from the floor area.

Figure 2.4 Big Dutchman Natura Rearing System

**Image A.** View from inside the system with the cage/compartment. Walls are typically closed until approximately 6 wks of age preventing access to the floor litter area (cage walls opened for sake of clearer picture). Cage enclosure offers perches running down the centre, drinking line, and a feed trough.

**Image B.** View from outside the system with the system opened allowing for access to the floor litter area. Systems walls can be opened at approximately 6 wks of age. Ramps can be supplied to encourage re-entry into the system from the floor area.

Images courtesy of Big Dutchman:

http://www.bigdutchman.co.za/Natura_rearing.pdf
Figure 2.5 Barn floor rearing system with perches added. Views inside a barn floor rearing system with access to litter, feed troughs, drinking lines, and perches. Perches (above) and platforms (not pictured) can be added to floor barn rearing systems to encourage use of three-dimensional space.

Images courtesy of Iran Oliveira Piracicaba
Figure 2.6 Farmer Automatic Pullet Portal Rearing System

**Image A.** View from inside the system with the side platforms folded up to create a system enclosure until approximately 6 wks of age preventing access to the floor litter area. System enclosure offers a feed trough along with a winched centre platform, perches, and drinking lines that can be raised in accordance with the growth of the pullet. The system provides a large open floor area for walking, running, perching, flight, and wing-assisted running from 1 day-of-age. Chicks in the image are 1 day-of-age.

**Image B.** Demonstrates the gradual height increase of the platform, perch, and drinking line to encourage use of vertical space in growing pullets. Side platforms are open to encourage use of vertical space and allow for access to the floor litter area. Pullets in the image are 12 wks of age.

**Image C.** View from outside the system with the side platforms opened allowing for access to the floor litter area. Side platforms are typically folded down to open the system at approximately 6 wks of age. Ramps can be supplied to encourage re-entry into the system from the floor area. Pullets in the image are 12 wks of age.

The Pullet Portal images pictured here (A, B, and C) were used to house all aviary-reared pullets used experimentally as part of this thesis.
Figure 2.7 Wing-assisted running exhibited by fowl-like Galliformes during rapid motion and anti-predator escape attempts. Example features a six wk old pullet.
2.8 Research Objectives

The research presented in this PhD Thesis was designed to address the effect of exercise during the pullet rearing phase on the long term bone health of laying hens, with an emphasis on rearing exercise effects on the prevalence of keel bone damage throughout the laying phase. The bone quality of the wing and leg bones, as well as the prevalence of keel bone fractures and deviations were quantified in housing systems that provided different opportunities for exercise during rearing (aviary rearing system vs. standard, conventional rearing) and during the adult laying phase (furnished cages vs. conventional cages). Behavioural and general activity implications of keel bone damage of hens in furnished cages were also addressed.

1. Determine whether different opportunities for exercise during the pullet rearing phase, as accomplished by housing pullets in contrasting rearing systems (aviary vs. standard, conventional cages), influences the overall bone quality and keel bone status of adult hens throughout the laying phase. I hypothesized that the contrasting levels of exercise allowed by different rearing systems would affect overall bone health and keel bone damage, and I predicted that aviary-reared pullets would show improved bone composition and reduced keel bone damage.

2. Determine whether different opportunities for exercise during the adult laying phase, as accomplished by housing adult hens in furnished cages compared to conventional cages, influences the overall bone quality and keel bone status of adult hens throughout the laying phase. I hypothesized that the contrasting levels of exercise allowed by different adult housing systems would affect overall bone health and keel bone damage, and I
predicted that hens housed in furnished cages would show improved bone composition but likely exhibit increased keel bone damage due to the presence of furnishings.

3. Report the prevalence of keel bone fractures and deviations throughout the adult laying phase of hens housed in furnished and conventional cages, and determine if there is an association between the two forms of keel bone damage. I hypothesized that keel bone damage would change during the life of the hen, that adult housing system would affect the prevalence of keel bone damage, and that deviations and fractures are associated with each other. I predicted that keel bone damage would increase with age, that hens housed in furnished cages would have a higher prevalence of keel bone fractures and deviations, and that there would be a strong association between the development of deviations and fractures.

4. Assess whether hens with keel bone fractures express behavioural differences or differences in overall activity compared to hens without keel bone fractures. I hypothesized that there would be behavioural and activity differences between hens with or without keel bone fractures, and I predicted that hens with keel bone fractures would perch more and have higher levels of inactivity, spending more time performing inactive behaviours related to a potentially painful state.
CHAPTER 3

Long term effects of rearing system on bone characteristics of pullets through to the end-of-lay in adult laying hens

3.1 ABSTRACT

The high incidence and severity of osteoporosis in laying hens has been a welfare concern for several decades. Increased load-bearing exercise improves bone quality characteristics in a variety of species, including laying hens. Providing increased opportunities for exercise during the pullet rearing phase, a period of substantial musculoskeletal growth, offers a proactive approach to reducing osteoporosis by improving bone composition. The main objective of this study was to determine whether differing opportunities for exercise during rearing influences pullet musculoskeletal characteristics and long term bone quality characteristics of end-of-lay hens. A secondary objective was to assess whether differing opportunities for exercise in the adult housing system alters bone quality characteristics in end-of-lay hens. Four flock replicates of 588 Lohmann Selected Leghorn-Lite pullets were reared in either standard, conventional cages (Conv) or an aviary rearing system (Avi) and placed into either conventional cages (CC), 30-bird furnished cages (FC-S), or 60-bird furnished cages (FC-L) for adult housing. The keel bone and the muscles and long bones of the wing and leg were

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1 This manuscript is in preparation for submission to *Poultry Science* with the following authors: T.M. Casey-Trott, D. R. Korver, M.T. Guerin, V. Sandilands, S. Torrey, and T. M. Widowski
collected at 16 wks to measure muscle growth differences between rearing treatments and quantify bone quality characteristics using quantitative computed tomography (QCT) and bone breaking strength (BBS) assessment. Wing and leg long bones were also collected at the end-of-lay for QCT and BBS. At 16 wks of age, rearing system had an effect on the majority of keel bone characteristics (P < 0.05). Wing and breast muscle weights of the Avi pullets were greater than the Conv pullets (P < 0.001), but leg muscle weights were greater in the Conv pullets (P = 0.026). Avi pullets had greater total bone density, total cross-sectional area, cortical cross-sectional area, total bone mineral content, and cortical bone mineral content than Conv pullets for the radius, humerus, and tibia (P < 0.001). Avi pullets had greater BBS compared to the Conv pullets for the radius, humerus, and tibia (P < 0.01). At the end-of-lay, aviary-reared hens had greater total and cortical cross-sectional area (P < 0.05) for the radius and tibiae, greater total bone mineral content of the radius (P < 0.001), and greater cortical bone mineral content of the tibia (P = 0.029) than the conventionally-reared hens; however total bone density of the radius (P < 0.001) and cortical bone density of the radius and tibia (P < 0.001) were greater in the conventionally-reared hens. Hens in the FC-L had greater total bone density for the radius and tibia (P < 0.05) and greater trabecular bone density for the radius (P = 0.027), compared to the FC-S and CC for the radius and tibia. Total bone mineral content of the tibia (P = 0.030) and cortical bone mineral content of the radius and tibia (P < 0.05) were greater in the FC-L and FC-S compared to the CC. The humerus of conventionally-reared hens had greater BBS than the aviary-reared hens (P < 0.001), and the FC-L and FC-S hens had greater BBS than the CC hens (P = 0.006). Increased opportunities for exercise offered by the aviary rearing system significantly improved bone quality characteristics in pullets and adult hens at the end-of-lay.
3.2 INTRODUCTION

Osteoporosis is described as the development of fragile, brittle bones from loss of bone mass and deterioration of the microarchitecture of the bone leading to a greater susceptibility to fractures (Saraff and Hogler, 2015). In laying hens, the widespread occurrence of osteoporosis is believed to be related to a combination of factors including hormonal changes, calcium to phosphorus ratio, calcium metabolism, confined housing, and genetics as described thoroughly in several reviews (Knowles and Wilkins, 1998; Thorp, 1994; Rath et al., 2000; Whitehead and Fleming, 2000; Webster, 2004; Beck and Hansen, 2004; Fleming, 2008).

Osteoporotic hens have been a prominent animal welfare concern since the 1980’s because of the relationship between osteoporosis and high fracture incidence during the laying period (Newman and Leeson, 1997; Knowles and Wilkins, 1998; Fleming et al., 1998a) which is often further exacerbated during catching and handling at the end of lay for transport to slaughter (Gregory et al., 1992). In the 1990’s, the high prevalence of osteoporosis was reported to be responsible for 35% of mortalities within commercial flocks (McCoy et al., 1996), and disorders related to calcium metabolism, (osteoporosis, osteomalacia, osteopenia, and hypocalcaemia) are still responsible for a high percentage of mortalities (51.2%) in current strains of commercial laying hens (Widowski, unpublished). Even when housing design and diet are held constant among groups, the lower breaking strength of osteoporotic bones is enough to significantly increase the hen’s risk of fracture (Knowles et al., 1993).

As part of a naturally occurring process, the skeletal frame of avian species routinely supplies calcium for production of the egg shell in the form of readily-available calcium stores in the medullary bone (Mueller et al., 1964; Miller et al., 1984). Unlike the structural (cortical and
trabecular) bone that is composed of lamellar bone, medullary bone is a type of non-structural woven bone that contributes only marginally to the overall strength of a bone (Whitehead, 2004). At the onset of sexual maturity in laying hens, and as long as estrogen levels remain high, these medullary stores are replenished daily by dietary calcium (Fleming et al., 1998a; Beck and Hansen, 2004), whereas structural cortical and trabecular bone ceases development prior to the onset of laying and are not replenished unless estrogen levels drop and the hen pauses egg production during a molt (Hurwitz, 1965; Hudson et al., 1993). Osteoclasts mobilize calcium from the medullary bone stores, however, osteoclasts are indiscriminate and they can also mobilize structural bone in areas where medullary bone is sparse (Fleming et al., 2006). Since structural bone is not replenished while the hen is in lay, areas of erosion in the structure can result in increased risk of fracture.

To combat the problem of osteoporotic hens, several research avenues have been investigated including the effect of dietary changes to calcium-phosphorous ratios (Tyler, 1940a,b; Summers et al., 1976; Rennie et al., 1997), supplemental large particle calcium strategies (Fleming et al., 1998a; Saunders-Blades et al., 2009; Koutoulis et al., 2009; Cufadar et al., 2011), genetic selection for high bone quality lines (Bishop et al., 2000; Hocking et al., 2003; Podisi et al., 2012), and increased opportunity for exercise in more complex adult housing systems (Newman and Leeson, 1998; Leyendecker et al., 2005; Fleming et al., 2006; Jendral et al., 2008; Shipov et al., 2010; Silversides et al., 2012; Enneking et al., 2012; Hester et al., 2013). In the 1950s, initial investigations into the causes of severe bone loss suggested the importance of exercise, noting that the problem was highly prevalent in hens housed in confined, conventional (i.e. battery) cages, yet was rarely seen in hens housed in open, barn floor systems (Couch, 1955; Grumbles, 1959). Although osteoporosis is present across all housing systems
with current commercial breeds, exercise still seems to be an essential component to reducing severe levels of osteoporosis (Knowles and Broom, 1990; Abrahamsson and Tauson, 1995; Fleming et al., 2006). Adaptation of bones in response to loading strain induced by exercise is a key component of mammalian and avian bone physiology (Layon, 1993), and the incredible ability of bones to accumulate bone strength and composition to withstand added loading makes the area of exercise a strong candidate for identifying strategies for combating osteoporosis in laying hens.

In human medicine, load-bearing exercise is frequently associated with higher levels of peak bone mass, bone size, and bone mineral density (Specker and Binkley, 2003; Linden et al., 2007) and load-bearing exercise is a critical part of the prevention and treatment program for osteoporosis in humans (Vincente-Rodriguez, 2006). Research on human athletes offers unique insight into the significant impact that load-bearing exercise can have on bone characteristics. For example, evidence from tennis and squash players showed that the bone mineral content of the playing arm consistently exceeded that of the non-playing arm by > 20% (Kontulainen et al., 2001), highlighting solely the role that loading strain has on osteogenesis. The effect of load-bearing exercise has also been assessed in a variety of animals, such as chickens, rats, and horses. Repeated high-impact drop jumps of 60 cm effectively increased periosteal and endocortical bone growth in young male chickens (Judex and Zernicke, 2000). In rats, consistent low-intensity exercise, and sudden impact exercise, increased cortical wall thickness (Jarvinen et al., 1998) and even minimal exercise of 5 impact jumps per day was enough to increase bone weight, maximum loads required for bone breaking analysis, and cortical area (Umemura et al., 1997). Horses, too, exhibited changes in bone characteristics and increased bone mineral density with consistent low-intensity loading exercise (Brama et al., 2009). Housing adult laying hens in
modified furnished cage systems (Jendral et al., 2008), aviaries (Leyendecker et al., 2005), or free-range systems (Shipov et al., 2010) compared to the relative confinement of conventional cages has also demonstrated beneficial effects on bone characteristics; however, marked improvements to bone health in laying hens are predominantly seen in cases where the opportunities for load-bearing exercise were substantially increased and diverse (Whitehead, 2002).

Since the detrimental effects of osteoporosis are most obvious after long periods of egg production, minimal research efforts have targeted the pullet rearing stage as an important part of the prevention of osteoporosis in laying hens. In humans, osteoporosis is increasingly considered to be a pediatric disease, as bones that do not develop adequate structural support during childhood and adolescence are especially susceptible to weakness later in life. Although osteoporosis manifests in adulthood, the precursors begin in early childhood (Bailey et al., 1999). Epidemiological determinants of osteoporosis in women include the amount of peak bone mass in adolescence, the maintenance of peak bone mass in middle age, and the overall rate of bone loss (Chesnut, 1989). Stimulating peak bone mass through routine load-bearing exercise during pre-pubertal growth in humans has become a key area of interest for preventing osteoporosis, especially for females (Burrows, 2007), and routine weight-bearing activity in adolescence is considered a key component to increasing peak bone mass in adulthood (Welten et al., 1994). The benefits of exercise in youth appear to be maintained in adults (Kontulainen et al., 2001).

The same approach might hold true for laying hens: building a stronger pre-lay skeleton may result in sufficient calcium reserves and structural bone strength to reduce depletion later in life. The pullet growing phase, prior to sexual maturity, represents a critical period for bone growth. In the last few wks before the onset of lay, the diameters of the long bones increase by
approximately 20% (Riddell, 1992), and then as estrogen levels begin to rise, structural bone deposition and longitudinal growth of long bones ceases. During this stage, cross-sectional growth occurs by periosteal apposition as osteoblasts add mineralized tissues to the outer periosteal surface (Rauch, 2007), and constant endosteal resorption allows for increased space for medullary bone production within the bone cavity (Whitehead, 2004). This increase in bone diameter in pullets may be critical to enhancing life-long bone strength, as the increase in bone diameter in humans has been shown to be positively correlated with the overall bending strength of a bone (Rauch, 2007).

To date, very few studies have addressed the effect of exercise in pullets on long term bone health (Enneking et al., 2012; Hester et al., 2013; Regmi et al., 2015, 2016). These studies either focused on minimal increases in opportunities for loading exercise with the addition of perches to conventional cages (Enneking et al., 2012, Hester et al., 2013) or the experimental design only allowed access to increased exercise at > 6 wks of age (Regmi et al., 2015).

The objective of this study was to assess the effect of housing that allows for exercise during the pullet rearing period, available in the form of running, jumping, perching, wing-flapping, and flight allowed starting at 1 day-of-age, on the long term bone quality characteristics of laying hens. A secondary objective was to determine if opportunities for exercise during rearing, coupled with an adult housing system with moderate opportunities for exercise (furnished cages) afforded greater increases in bone quality characteristics relative to an adult housing system with limited opportunities for load-bearing exercise (conventional cages). Placement of hens into furnished or conventional cages after rearing allowed for a 2 x 3 factorial arrangement with replication of rearing treatment in multiple cage units, and also allowed for the comparison of the effect of increased exercise in the form of greater total cage area on bone
quality. This is the first study to carry out a controlled longitudinal assessment of the effect of exercise during rearing on the long term bone quality characteristics of several consecutive flocks, of a single strain, raised from 1 day-of-age to the end-of-lay on a single site with identical feeding and lighting programs using a factorial experimental arrangement.

3.3 METHODS

The effects of rearing system (standard cage (Conv) or rearing aviary (Avi)) and adult housing system (conventional cage (CC), 30-bird furnished cage (FC-S) or 60-bird furnished cage (FC-L)) were tested using a 2 x 3 factorial arrangement with rearing flock replicated in 4 blocks over time. Each of the 4 rearing flocks contributed 3 replicate cages to each of the adult housing systems. Animal use was approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #1947).

3.3.1 Pullet management of Flocks 1-4

From 4 consecutive flocks, a group of 588 Lohmann Selected Leghorn Lite (LSL-Lite) pullets per flock were conveniently selected and reared from 1 day-of-age to 16 wks of age at the University of Guelph Arkell Poultry Research Station. For each consecutive flock, half of the pullets were conveniently selected for rearing in standard conventional cages (Ford Dickinson, Mitchel, Ontario, Canada; 16 pullets/cage during wks 0-6 with a space allowance of 145 cm²/pullet followed by 8 pullets/cage during wks 6-16 with a space allowance of 290 cm²/pullet; total cage area = 2,322 cm²) and half were conveniently selected for rearing in a Farmer Automatic Logia Pullet Portal (Clark Ag Systems, Caledonia, Ontario, Canada; 756 pullets/aviary enclosure; system space allowance of 285 cm²/pullet during wks 0-6; total system + outer platforms + litter space allowance of 754 cm²/pullet during wks 6-16). The aviary system
was selected to allow maximum opportunities for exercise starting at 1 day-of-age with access to
the floor area of the system (183,272 cm$^2$), perches, and a suspended platform (32,371 cm$^2$) that
was gradually raised vertically in accordance with the age of the pullet to encourage hopping and
flight to access vertical space (Total System Area: 215,643 cm$^2$). At 6 wks of age, additional
space was added by opening the sides of the system to allow for access to the litter area (235,767
cm$^2$) and 9 elevated terraces (118,887 cm$^2$) on the outer edge of the system, increasing the total
system area to 570,297 cm$^2$ (Figure 3.1).

Both the conventionally- and aviary-reared pullets for all 4 flocks were fed identical, 21%
crude protein, 1.06% calcium , 0.47% available phosphorus starter diets (as crumbles) from 0-6
wks and identical 18% crude protein, 1.01% calcium, 0.45% available phosphorus grower diets
(as crumbles) from 6-16 wks with the addition of granite chick grit to the Avi diet for Flock 1,
followed by the addition of granite chick grit to both the Conv and Avi diet for Flocks 2-4. Both
rearing treatments also followed identical vaccination and lighting programs. Lights were on for
16 hrs/d for wks 1 and 2, alternating 4 hrs on and 2 hrs off. At 3 wks, lights were on continuously
for 14 hrs/d starting at 05:00 hr, and subsequently reduced by 1 hr/wk until maintaining 8 hrs of
light from wks 8-15. The lights were set at 40 lux at placement, and reduced by 5 lux every 5 to
7 d until maintaining 10 lux from wks 4-16. In the aviary rearing system, water lines were
located in the middle of the enclosure with a chain feeder running along the perimeter. Water
lines with nipple drinkers and chain feeders were suspended from the ceiling and could be raised
vertically in accordance with pullet growth. In the standard cages, water lines with nipple
drinkers were located in the middle of the cages with the chain feeders running past the front of
the cage. At 1 day-of-age, all chicks were beak-trimmed at the hatchery using infrared treatment.
A subsample of 100 pullets/flock (Flock 3 and 4) from each rearing system were weighed biweekly and compared to the target weights outlined in the North American Edition of the LSL-Lite Management Guide Layers (Lohmann Tierzucht GmbH, Germany). Pullets from within the aviary rearing system were sampled from various areas on the floor and within the system. For the standard rearing system, 2 pullets were selected from each cage. In an attempt to achieve equal body weights between the conventionally-reared and aviary-reared pullets at placement into adult housing, the room temperatures were increased slightly ($\leq 2^\circ$C) in the conventional cage rearing room to reduce feeding behaviour when the biweekly weight of the conventionally-reared pullets exceeded that of the aviary-reared pullets by more than 10%. Aviary-reared pullets from Flock 4 remained on the starter diet for 1 additional wk in order to bring them up to a body weight matching that of the conventionally-reared pullets. All 4 flocks came into lay between 17 and 18 wks of age, and achieved 50% production at wk 19.

3.3.2 Adult laying hen management of Flocks 1-4

At 16 wks of age, 294 pullets from each rearing system (Avi and Conv) from each flock (1-4) were weighed and transferred to 2 adult housing rooms each holding 12 Farmer Automatic Enrichable (Furnished) Cages (Clark Ag Systems), and 1 adult housing room holding 90 standard conventional cages, of which 12 standard conventional cages (Ford Dickinson) were included in the study. In both the furnished cage rooms, and the conventional cage room, 2 flocks were housed simultaneously (Flock 1 and 2; Flock 3 and 4) with the placement into all cages equally balanced for all 4 flocks. In all rooms, a conveniently selected group of hens from a single rearing treatment (Avi or Conv) was placed into each cage, balancing both rearing treatments equally within each room. Each furnished cage room contained 6 large furnished cages (60 hens, total area = $41,296 \text{ cm}^2$, 688 cm$^2$/hen) and 6 small furnished cages (30 hens, total
area = 20,880 cm², 696 cm²/hen). Each bank of 6 cages had 3 tiers with 1 large and 1 small cage on each tier. The conventional room contained 12 standard conventional cages of equal size (8 hens/cage; total area = 4,025 cm², 503 cm²/hen), all on a single tier. The same rearing and adult rooms were used for each consecutive flock.

All flocks were fed identical, standard commercial layer crumbled pellet diets (18% crude protein, 4.22% calcium, 0.44% available phosphorus) with automatic feed chains running every 3 hrs commencing at the start of a 14 hr light period from 05:00-19:00 hrs with 15-min sunrise and sunset starting at 05:00 hr and 18:45 hr, respectively. In the furnished cage rooms, the light intensity varied among tiers, with the highest intensity recorded at the top tiers measuring 10-15 lux and the lowest intensity at the bottom tiers measuring 4-5 lux. Each rearing treatment was balanced for tier. Each furnished cage provided a curtained nest area proportional to cage size (94 cm²/hen), 10 cm high perches (15 cm²/hen) running parallel to the cage front throughout the middle area, and a smooth plastic scratch area (large: 42 cm²/hen; small: 83 cm²/hen). Nipple drinkers with cups were located above the feed auger down the middle of the cage. The feed troughs (12 cm/hen) were located on both outer sides of the cages. Conventional cages were equipped with a nipple drinker running down the middle of the cages, with the feed troughs (8 cm/hen) on the outer side of the cage. All rooms were sealed and entirely lit with artificial light (incandescent) with no natural, external light sources.

3.3.3 Pullet muscle and bone collection of Flocks 3 and 4

At 16 wks of age, a subset of 20 Avi and 20 Conv pullets from each of Flock 3 and Flock 4 (n = 40 Conv pullets; n = 40 Avi pullets) were euthanized by cervical dislocation and frozen at -20°C for later collection of muscle and bone tissues. Pullets from within the aviary rearing
system were sampled from various locations on the floor and within the system. For the standard rearing system, 2 pullets were selected from each cage. Tissues were not collected from Flocks 1 and 2 as the decision to collect pullet tissues was made after completion of the first 2 flocks.

The protocol for the collection of muscles was designed with the assistance of a veterinary avian pathologist (Dr. Emily Martin) to ensure consistent muscle specimen collection. After thawing the pullets, the bicep brachii, pectoralis major, pectoralis minor, and combination of all leg muscles (of the femur: iliotibialis, sartorius, semitendinosus, semimembranosus, quadriceps femoris, addiens, adductor longus; of the tibiotarsus: gastrocnemius, tibialis anterior, peroneus longus, flexor perforans et perforates II & III) were removed from the left side of each pullet. The bicep brachii was detached from the bone by severing the muscle at the site of tendon origin (proximal head of the humerus) and insertion (proximal anterior surface of the radius) to include only muscle tissue in the measurements and ensure a consistent visual identification of the site of removal. The pectoralis muscles were removed by severing the attachment at the origin (Carina sternum, furcula and sternal ribs) and insertion of the major (proximal ventral surface of the humerus) and insertion of the minor (proximal dorsal surface of the humerus) and gently freeing the muscles from any additional fascia tissue attachment to the sternum and rib cage. Due to the presence of multiple tendon and ligament bundles, the individual leg muscles could not be easily separated. To prevent inconsistent collection, the entire group of leg muscles, tendons, and ligaments were removed as a group and all included in the measurement. The leg muscle group was detached from the axial skeleton at the distal end of the tibia, severing the Achilles tendon between the tibial condyles and the proximal tarsometatarsus, followed by severing the fascia attachment of the thigh muscle group over the synsacrum at the midline of the ilium. All muscles were weighed immediately upon removal.
Following muscle collection, the right and left radius, humerus, tibia, and keel bone were removed. The bones from the right side were subsequently used for Quantitative Computed Tomography (QCT) analysis and the bones from the left side were used to test breaking strength. Only 1 freeze and thaw cycle was allowed for all bones. Immediately after the bones were extracted, the bones from the right side were placed into 10% formalin for > 7 d for QCT analysis. All the bones from the left side were placed in a moving air fume hood to air dry for > 7 d for analysis of breaking strength. If the bone on the left side was fractured, the right bone was used instead.

The keel bone was removed to assess the current state of growth of the keel. The total length (mm) of the keel metasternum, as well as the length of the cartilaginous caudal tip (mm) of the metasternum were measured with Fisher Science Education™ Traceable™ Digital Carbon Fiber Calipers (Fisher Scientific, Toronto, Ontario, Canada). The total length of the metasternum was measured on the dorsal metasternum surface parallel to the cranial region of the sternal notch, ending at the caudal border of the keel metasternum tip. The cartilaginous tip was measured on the dorsal metasternum, from the line of distinction between the end of ossified bone tissue and initiation of cartilage tissue, to the end of the caudal tip of the keel metasternum. The percentage of cartilage was calculated using the total length of the metasternum and the length of the cartilaginous region of the keel. The height of the keel was measured on the cranial region, from the ventral base of the metasternum to the Carina apex. The keel area was estimated using the equation for the area of a right triangle using the measurements of the total length of the metasternum and the height of the keel. The distance between the caudal-most tip of the keel and the left pubic bone, and the distance between the pubic bones themselves were also measured to estimate the proximity to initiation of egg production as the gap between pubic
bones increases and the keel bone gradually tilts ventrally, away from the pubic bones, at the onset of lay (Chapman, 1943).

3.3.4 Adult laying hen bone collection of Flocks 1-4

At 73 wks of age, the room lights were dimmed for ease of collection, and 10% of the hens from each furnished cage (FC-L: n = 6; FC-S: n = 3; Total N = 54/ flock) from all 4 flocks were conveniently-selected from various regions within the cage, weighed, euthanized by cervical dislocation, and frozen for later bone collection. All hens from each conventional cage (n = 8; Total N = 48/flock) were collected, weighed, euthanized by cervical dislocation, and frozen at -20°C for later bone collection.

To collect the bones, hens were thawed and the right and left radius, humerus, and tibia were removed. The bones from the right side were used for QCT analysis and the bones from the left side were used to test breaking strength. Only 1 freeze and thaw cycle was allowed for all bones. Immediately after the bones were extracted, a subset of 8 conveniently-selected bones per rearing by adult housing system treatment per flock (N = 48/flock: Conv/FC-L: n = 8; Conv/FC-S: n = 8; Conv/CC: n = 8; Avi/FC-L: n = 8; Avi/FC-S: n = 8; Avi/CC: n = 8), from the right side were placed into 10% formalin for > 7 d for later QCT analysis. All the bones from the left side were placed in a moving air fume hood to air dry for > 7 d for later analysis of breaking strength. If the bone on the left side was fractured, the right bone was used instead. No bones with visible fractures were used for either the QCT or breaking strength analysis.

3.3.5 Quantitative Computed Tomography (QCT)

A Stratec XCT³ scanner (Model 922010; Norland Medical Systems Inc., Wisconsin, USA) with XMENU software version 5.40C was used for analysis of bone density (mg/cm³) and
area (mm$^2$) of the total bone, cortical bone, and trabecular bone. Measurements of the trabecular space presumably include both medullary and trabecular bone (Korver et al., 2004a). A longitudinal scan of the bone was used to set the mid-points of the bone, and a 1-mm bone cross-section at a view of 30% from the distal end of each bone was used for analysis. Threshold density values of 400 and 500 mg/cm$^3$ were used for trabecular and cortical bone separation, respectively (Korver et al., 2004a). The density and area measures were used to calculate the total bone mineral content (mg/mm) of the total and cortical bone, and bone in the trabecular space within the 1 mm length of bone included in the scan. The QCT protocol was the same for the both pullet and adult laying hen bones.

3.3.6 Three-point breaking strength

An Instron Dynamic and Static Materials Test System (Model # 4204; Instron Corporation, Canton, Massachusetts, USA) with Automated Materials Test System software was used to assess bone breaking strength. A cradle support with posts 5 cm apart was used to support each bone. A 1 kN static load cell and speed of 100 mm/min was used to apply a shear plate (8 cm long by 3 mm wide) to the mid-point of the bone shaft. All bones were placed in the same orientation on the support cradle. The maximum voltage required to break the bone was recorded, which was then converted to kilograms (kg) using a slope equation of a calibration curve generated by calibrating the machine with 10, 20, 50, 100, and 500 g weights. All bone breaking strength results are presented as kg of force required to break each bone. The same protocol for the assessment of breaking strength was used for both the pullet and adult laying hen bones.
3.3.7 Statistical analysis

All statistical analyses were completed using SAS statistical software version 9.4 (SAS Institute, Cary, North Carolina, USA). The level for assessment of statistical significance of differences between means was set at $P < 0.05$.

3.3.7.1 Pullet muscle and bone analyses of Flocks 3 and 4 at 16 wks of age

The effect of rearing system (Avi vs Conv) on rearing body weight at the time of placement into the adult housing system was assessed using a general linear mixed model analysis (PROC MIXED command) to determine the relationship between the two variables. Since rearing system did not have a significant effect on rearing body weight for Flocks 3 and 4 ($P = 0.875$), rearing body weight was included as a covariate in subsequent analyses of pullet characteristics for long bones. Muscle weights and keel characteristics were adjusted for body weight.

To assess the effect of rearing system on the muscle and keel characteristics, a general linear mixed model analysis (PROC MIXED command) was performed with rearing system (Avi, Conv) as a fixed effect and Flock number (3, 4) as a random factor to account for any variation due to flock differences. All muscle weights were expressed relative to the body weight of the pullet (g/kg). Keel dimensions were also adjusted for pullet body weight (mm/kg). To assess the effect of rearing system on the long bones of the pullets (QCT and breaking strength outcome measures), a general linear mixed model analysis (PROC MIXED command) was performed with rearing system (Avi, Conv) as a fixed effect and rearing body weight as a covariate. Flock number (3, 4) was included as a random factor.
All data were tested for normality and normality of residuals using PROC UNIVARITE command and no data required transformation. Due to the lack of medullary bone content prior to the onset of lay (Whitehead, 2004), density and bone mineral content of bone in the trabecular space could not be assessed for pullets.

3.3.7.2 Adult laying hen bone analyses of Flocks 1-4 at 73 wks of age

Rearing and adult body weight at depopulation were analyzed separately to determine the relationship of rearing and adult housing system on body weight for Flocks 1-4. To assess rearing body weight, a general linear mixed model analysis (PROC MIXED command) was used with rearing system (Avi vs Conv) as a fixed effect and Flock number (1, 2, 3, 4) as a random effect. For adult body weight, PROC MIXED was used with rearing system (Avi, Conv), adult housing system (FC-L, FC-S, CC) and the interaction between the two included in the model as fixed effects and Flock number (1,2, 3, 4) as a random effect. Rearing and adult body weights were not included as covariates in the further analyses assessing adult laying hen bone characteristics as rearing system had a significant effect on rearing body weight ($P = 0.042$) and adult body weight ($P = 0.049$), and therefore rearing system was not considered to be independent from rearing or adult body weight.

Bone quality data were analyzed using a general linear mixed model analysis (PROC MIXED command). The QCT and breaking strength response variables were assessed with rearing system (Avi, Conv) and adult housing system (FC-L, FC-S, CC) and the interaction between the two as fixed effects. Flock number (1, 2, 3, 4) was included in the model as a random effect.
All data were tested for normality and normality of residuals using PROC UNIVARIATE command and no data required transformation. The trabecular density and trabecular bone mineral content of the humerus could not be assessed due to the presence of minimal to no trabecular or medullary bone present in the humeri as it is primarily a pneumatic bone.

3.4 RESULTS

3.4.1 Pullet muscle and bone characteristics of Flocks 3 and 4 at 16 wks of age

For Flocks 3 and 4, there was no difference in mean body weight ($P = 0.875$) between Avi (1204.6 g ± 18.1 SE) and Conv (1202.1 g ± 18.2 SE) pullets.

Rearing system had a significant effect on all muscle and keel characteristics, except for keel height (Table 3.1 and 3.2). The muscle weight of the bicep brachii, pectoralis major, and pectoralis minor was greater in the Avi pullets compared to Conv pullets ($P < 0.001$, $P < 0.001$, $P < 0.001$, respectively); however, the weight of the leg muscle group was greater in the Conv pullets compared to Avi pullets ($P = 0.026$: Table 3.1). The length of the metasternum was greater in the Avi pullets compared to the Conv pullets ($P = 0.003$). The length of the cartilage on the keel bone ($P < 0.001$), and percentage of the keel comprised of cartilage ($P < 0.001$), was greater in the Avi pullets compared to the Conv (Table 3.2). Keel area was greater in the Avi pullets compared to the Conv pullets ($P = 0.026$). The distance between the keel and pubic bone ($P < 0.001$) was greater in the Conv pullets compared to the Avi pullets; however, there was no significant difference in the distance between the pubic bones ($P = 0.717$) for the Avi and Conv pullets.

Rearing system had a significant effect on each of the QCT characteristics for the radii and humeri, and the majority of the QCT characteristics of the tibiae (Table 3.3). The total
density and cortical density of the radius and humerus of the Avi pullets was greater than that of the Conv pullets. For the tibia, the Avi pullets had a greater total density than the Conv pullets ($P < 0.001$), yet the cortical density was not different ($P = 0.989$). The total cross-sectional area, cortical cross-sectional area, and trabecular cross-sectional area of the radius and humerus were greater in the Avi pullets compared to the Conv pullets. For the tibia, the total cross-sectional area was greater in the Avi pullets compared to the Conv pullets ($P = 0.009$) and the same was true for the cortical cross-sectional area ($P = 0.001$); however, trabecular cross-sectional area of the tibia was not different between the rearing system groups ($P = 0.823$). The total bone mineral content and cortical bone mineral content was greater in the Avi pullets compared to the Conv pullets for the radius, humerus, and tibia. Rearing body weight did not have an effect on the total or cortical bone density for any of the bones; however, rearing body weight had a positive linear effect on the majority of QCT measurements for cross-sectional area and bone mineral content for all 3 bones. The $P$-values for rearing body weight are reported in Table 3.3.

The breaking strengths of the humerus, radius, and tibia were greater in the Avi pullets compared to the Conv pullets ($P = 0.014$ or less; Table 3.4). Rearing body weight did not have an effect on the breaking strength of the humerus ($P = 0.568$); however, rearing body weight did have a positive linear effect on the breaking strength of the radius ($P < 0.001$) and the tibia ($P = 0.038$; Table 3.4).

3.4.2 Adult laying hen characteristics of Flocks 1-4 at 73 wks of age

There was an effect of rearing system on rearing body weight, with the aviary-reared pullets having a lower mean body weight (1213.2 g ± 14.8 SE) at placement into the adult housing systems than conventionally-reared pullets (1240.7 g ± 14.7 SE; $P = 0.042$); however,
placement of pullets was balanced for body weight with no significant difference between adult housing treatments (FC-L: 1225.0 g ± 15.5 SE; FC-S: 1243.9 g ± 19.5 SE; CC: 1223.8 g ± 15.5 SE; \( P = 0.555 \)).

Rearing system also had an effect on the body weight of the adult hens at 73 wks of age. Hens that were reared in the pullet aviary had a higher mean adult body weight (1838.1 g ± 27.3 SE) compared to hens that were reared in conventional cages (1770.1 g ± 26.0 SE; \( P = 0.049 \)). Adult housing system did not have an effect on the adult body weight (FC-L: 1823.4 g ± 28.8 SE; FC-S: 1807.8 g ± 29.4 SE; CC: 1781.1 g ± 25.5 SE; \( P = 0.473 \)).

Egg production and mortality were recorded daily and will be reported elsewhere (Widowski, unpublished). All mortalities were sent for post mortem analysis and there were no outbreaks of disease, feather pecking, or cannibalism throughout the duration of the study.

3.4.3 Effect of rearing system on adult bone characteristics at 73 wks of age

The total density of the radius at 73 wks of age was greater in the conventionally-reared hens compared to the aviary-reared hens (\( P < 0.001 \); Table 3.5). The cortical density at 73 wks of age of the radius (\( P < 0.001 \); Table 3.5), humerus (\( P < 0.001 \); Table 3.6), and tibia (\( P < 0.001 \); Table 3.7) were significantly greater in the conventionally-reared hens than the aviary-reared hens.

Rearing system had the opposite effect on bone cross-sectional area. The total cross-sectional area at 73 wks of the radius (\( P < 0.001 \); Table 3.5), humerus (\( P < 0.001 \); Table 3.6), and tibia (\( P = 0.019 \); Table 3.7), was less in the conventionally-reared hens compared to the aviary-reared hens. The same pattern was found for the cortical cross-sectional area of the radius (\( P <
0.001; Table 3.5) and tibia ($P = 0.003; \text{Table 3.7}$), and the trabecular cross-sectional area of the radius ($P < 0.001; \text{Table 3.5}$) and humerus ($P < 0.001; \text{Table 3.6}$).

The radius of the aviary-reared hens had a greater total bone mineral content ($P < 0.001$) and greater trabecular bone mineral content ($P < 0.001$) than the conventionally-reared hens at 73 wks of age. The tibia of the aviary-reared hens had a greater cortical bone mineral content ($P = 0.029$), but a lower trabecular bone mineral content ($P = 0.039$) than the conventionally-reared hens at the end of lay. Rearing system did not affect any of the bone mineral content measures for the humerus of adult hens.

There were no significant interactions between rearing and adult housing systems for any of the QCT measures (Tables 3.5-3.7).

### 3.4.4 Effect of adult housing system on adult bone characteristics at 73 wks of age

Adult housing system had a significant effect on several QCT bone characteristics for the radius and tibia, but not the humerus. Total bone density was greatest in the FC-L compared to both the FC-S and CC for both the radius ($P = 0.013; \text{Table 3.5}$) and tibia ($P < 0.001; \text{Table 3.7}$). Trabecular bone density was also greatest in the FC-L with no difference between the FC-S and CC for the radius ($P = 0.027; \text{Table 3.5}$). There was no effect of adult housing system on total or cortical cross-sectional area for the radius, humerus, or tibia. Trabecular cross-sectional area of the tibia was least in the FC-L with no difference between the FC-S and CC ($P = 0.002; \text{Table 3.7}$). The total bone mineral content was greater in the FC-L compared to the CC for the tibia ($P = 0.030; \text{Table 3.7}$) with the FC-S having an intermediate value. The cortical bone mineral content of the radius ($P = 0.030; \text{Table 3.5}$) and tibia ($P = 0.013; \text{Table 3.7}$) followed the same
pattern with the greatest values reported in the FC-L compared to the CC, and FC-S with an intermediate value.

A graphical comparison of the pullet QCT results and adult QCT results of the total bone measures for the radius are shown in Figure 3.2 cortical bone measures of the radius in Figure 3.3, total bone measures of the tibia in Figure 3.4, and cortical bone measures of the tibia in Figure 3.5. The main effect of rearing environment (Avi, Conv) from pullets at 16 wks of age from Flocks 3 and 4 is presented as a reference value, followed by the rearing environment and adult housing effects at 73 wks of age as found in the adult QCT analysis for Flocks 1-4.

The results for the bone breaking strength of the humeri, radii, and tibiae are presented in Table 3.8. Rearing system only had an effect on the breaking strength of the humerus \((P < 0.001)\), with conventionally-reared hens exhibiting a greater breaking strength \((9.2 \, \text{kg} \pm 0.45 \, \text{SE})\) compared to aviary-reared hens \((6.4 \, \text{kg} \pm 0.44 \, \text{SE})\) at 73 wks of age. Adult housing system only had an effect on the breaking strength of the tibia \((P < 0.006)\), with a significantly higher breaking strength of FC-L and FC-S \((14.8 \, \text{kg} \pm 0.76 \, \text{and} \, 14.4 \, \text{kg} \pm 0.78 \, \text{SE})\) than CC \((13.5 \, \text{kg} \pm 0.77 \, \text{SE})\) at 73 wks of age.

### 3.5 DISCUSSION

The fairly consistent, statistically significant patterns identified by the QCT analysis of the bone characteristics for both pullets at 16 wks and adult hens at the end of lay indicates that an aviary rearing system that provides regular opportunities for varied load-bearing exercise has substantial, life-long effects on the bone characteristics of laying hens. This is the first study to clearly demonstrate that after adjusting for body weight, the musculoskeletal frame of growing pullets differ between Avi and Conv pullets, even when genetics, diet, and lighting are held
constant between the groups. Additionally, many of the differences in bone characteristics that developed during the rearing phase are maintained through to the end-of-lay, and additional bone mineral deposition can be stimulated by continued opportunities for exercise during the laying period.

3.5.1 Rearing system effects on muscle and bone characteristics at 16 wks of age

The musculoskeletal frame of Avi pullets surpassed that of Conv pullets in most muscle and bone growth characteristics. This suggests that opportunities for diverse load-bearing exercise at an early age stimulated muscle deposition and osteogenesis. Hester et al. (2013) demonstrated that allocation of perches in conventional cages during the pullet rearing phase had a positive effect on muscle growth compared to pullets in conventional cages without perches; however, these differences were only described in adult hens as the detailed pullet muscle weights were not measured. The results reported here clearly show a difference in muscle weights at 16 wks of age between the Avi and Conv rearing systems. Wing and breast muscle weights of the Avi pullets were greater than the Conv pullets. This is likely due to the allowance of flight, wing-flapping, and wing-assisted running in the aviary rearing system compared to the relative confinement of the conventional rearing cages. The leg muscle weights were greater in the Conv pullets compared to the Avi pullets. The opposite effect of rearing system on leg muscle weights compared to wing and breast muscles suggests that the constant standing in conventional cages also stimulates muscle growth, with perhaps more lean muscles developing in the Avi pullets due to more diverse, extensive exercise. Alternatively, our method of weighing the leg muscles as a group may not have been sensitive enough, and perhaps assessing individual muscles might yield a different result.
The longer metasternum and larger cartilage portion of the keel of Avi pullets at 16 wks compared to the Conv pullets indicates that ossification of the keel was slower in the Avi pullets. This result suggests that the overall skeletal development of the Avi pullets was slower than that of the Conv pullets; although this hypothesis requires further testing. Buckner et al. (1949) described the process of calcification of the keel as a slow progression of ossification from the cranial portion of the keel to the caudal tip of the metasternum, noting that calcification of the caudal tip was not complete until 28-40 wks of age, long after structural growth of the long bones is complete. The percentage of cartilage present in the current study at 16 wks of age was similar for both rearing treatments to the cartilage percentage reported by Buckner et al. (1949) for wks 22-26, although direct comparison is difficult due to the lack of thoroughly-described anatomical marker measurement details within that study. Alternatively, the greater proportion of cartilage found in the Avi pullets might be related to the larger area and greater length of the keels of the Avi pullets compared to the keels of the Conv pullets, suggesting that increased wing loading exercise is stimulating the growth of the keel to produce a larger keel bone overall. This may also explain the differences in distance between the keel and pubic bone, as a larger keel likely leads to a shorter distance to the pubic bone. Alternatively, the longer distance between the keel and pubic bone could indicate that the Conv pullets were closer to sexual maturity, as the distance between the keel and pubic bones has been used as a measure of proximity to lay (Chapman, 1943); however, both Flock 3 and 4 achieved 50% production by 19 wks. The lack of difference in the distance of the gap between the pubic bones at 16 wks of age indicates that neither group had entered the laying phase (Satterlee and Marin, 2004). Detailed assessment of keel measures in adult hens is warranted, as certain keel bone characteristics, such as length,
area, or degree of ossification at the onset of lay might be precursors to keel bone damage, a prominent welfare concern for laying hens.

In addition to the skeletal differences of the keel bone between Avi and Conv pullets, the long bones of the pullets in both the wing and leg were also affected by the opportunity for exercise during the rearing phase. The larger total cross-sectional area of the Avi pullet bones indicates greater bone width, which is a characteristic of increased bone growth in human adolescents (Rauch, 2007). Increased area of aviary-reared pullet bones was also reported by Regmi et al. (2015) for the humerus and tibia. A larger cortical area coupled with a larger trabecular area of the radii, humeri, and tibiae of Avi pullets (for all but the bone in the trabecular space of the tibiae) indicates that bone apposition occurred on the periosteal surfaces, which is also typically observed in human studies in which exercise programs target pre-pubertal and adolescent stages (Specker et al., 2015). In agreement with the current results, evidence of periosteal growth during the pullet phase was also reported by Bierwener and Bertham (1994) and Judex and Zurnicke (2002), whereas Regmi et al. (2015) reported periosteal growth of the humerus but endosteal growth of the tibia. With the exception of the cortical bone density of the tibia, the consistently higher values for bone density and bone mineral content in the Avi pullets reported here indicate that not only were the Avi pullet bones wider than Conv pullet bones, but the Avi bones were also undergoing greater mineral deposition. The bone mineral content of the humerus in aviary-reared pullets, as measured by bone ash, has also been reported as being greater than that of conventionally-reared pullets, whereas no differences were observed in the tibia (Regmi et al., 2015).
3.5.2 Rearing system effects on adult bone characteristics at 73 wks of age

The patterns of greater area and bone mineral content of the aviary-reared pullets also carried over into adulthood; however, bone density values in adult hens were typically higher in the conventionally-reared pullets. Significantly lesser bone areas of hens housed in conventional cages compared to furnished cages (Jendral et al., 2008) and free-range systems (Shipov et al., 2010), with less noticeable differences in bone density, have been reported. While these results initially give the impression that conventionally-reared hens produce more overall bone content, the greater adult bone density in conventionally-reared hens in the present study is more likely due to the same amount of bone mineral production, but in a bone with a smaller cross-sectional area compared to the larger cross-sectional area of the long bones of the aviary-reared hens. The typically higher total and cortical bone mineral content values for the adult radii and tibiae of the aviary-reared hens indicated that the same or more mineral content was spread out over a greater area in the aviary-reared hens.

The sequence of bone growth during development might also explain the greater density values of the conventionally-reared hens. As the long bones undergo their final growth in diameter, increasing by more than 20% in the final weeks before the onset of lay (Riddell, 1992), the growth outward is normally coupled with an incomplete bone framework (Figure 3.6) of channels in the cortical ring that are subsequently filled in when outward growth ceases (Whitehead, 2004). It is possible that the lesser adult bone density values of the aviary-reared hens were a result of greater growth in an area with lower density medullary bone integrating into the pores in the cortical bone. The accrual of medullary bone within the cortical space might have been exacerbated by the early lighting program and placement into adult housing at 16 wks in this experiment, which was carried out to encourage nest usage in furnished cages. It is
possible that the bones of the aviary-reared pullets had not entirely completed their structural growth at the time of the abrupt light stimulation, and in turn filled in the remaining channels with less dense medullary bone, thereby reducing the overall cortical density. Although a later initiation of the photo-stimulation for the aviary-reared pullets might have allowed for more complete bone growth, the study was designed to minimize any management differences between the aviary- and conventionally-reared pullets. Since the aviary-reared pullets are slightly slower growing, as seen by the lower rearing body weights of the aviary-reared pullets from Flocks 1-4 and the less ossified keel bones of the aviary-reared pullets from Flocks 3 and 4, they may have benefited from later photo-stimulation. Even with the lower cortical density reported for the aviary-reared hens, the increased bone width, which was seen here in the aviary-reared pullets at 16 wks of age and carried over into adulthood, has been shown to positively improve bone strength by increasing periosteal modeling and reducing endosteal resorption in humans (Burr and Martin, 1989). Not only this, but wider bones are also directly correlated with higher bending strength (Rauch, 2007).

It is possible that these characteristics could be attributed to the larger end-of-lay body weight of the aviary-reared hens, especially since body size is a predictor of peak bone mass as it adds to the loading strain placed on a bone (Cooper et al., 1995). However, it is important to emphasize that the larger bone areas of aviary-reared hens were present at the end of rearing and at the end-of-lay, even though the initial placement body weights of the aviary-reared hen were lower than those of conventionally-reared hens. This suggests that even though the aviary-reared pullets were smaller to start, their bones were larger than the conventionally-reared pullets since structural bone growth ceases at the onset of lay (Whitehead, 2004). The larger skeletal frames of the aviary-reared pullets compared to the conventionally-reared pullets
potentially offers the opportunity to provide a larger frame to store and mobilize calcium during the laying period.

3.5.3 Adult housing system effects on adult bone characteristics at 73 wks of age

Opportunities for exercise allowed by adult housing systems have been more extensively studied, and researchers frequently cite the positive effects of exercise on bone characteristics. Allocation of perches in conventional cages has been shown to increase bone mineralization (Hester et al., 2013), and housing in furnished cages (Jendral et al., 2008), aviaries (Leyendecker et al., 2005), or free-range systems (Shipov et al., 2008) during the laying phase allows for enough exercise to improve bone mass, bone mineral density, cortical area, and breaking strength compared to hens housed in conventional cages. The direct effects reported here of adult housing system on bone characteristics highlights the differences between the effect of exercise during adolescence and adulthood. In humans, bone growth during adolescence primarily alters the bone geometry by adding to the periosteal and endocortical bone layers, thereby increasing bone width, whereas bone formation in adults is driven by internal trabecular remodeling (Rauch, 2007). The same is true for laying hens, with structural growth only occurring prior to sexual maturity, followed by accrual of medullary bone filling in the trabecular cavity (Whitehead, 2004). The presence of rearing effects in both pullet and end-of-lay hens on measurements of total and cortical bone area, and the lack of adult housing system effects on the total or cortical area dimensions, fits this described sequence of bone growth and appears to confirm previous results that loading exercise prior to sexual maturity primarily alters the shape and size of the bone rather than substantially affecting mineral components (Regmi et al., 2015).
In adult housing, the radius and tibia were positively affected by furnished cage housing presumably due to the increased allowance of exercise, especially in the FC-L. For every significant or nearly significant result for density, area, and bone mineral content of the radius and tibia, the FC-L had the highest values compared to FC-S and CC, except for trabecular area and trabecular bone mineral content. This suggests that the increased strain from loading exercise as adults continues to increase total and cortical bone density and either enhances osteogenesis or helps preserve the beneficial effects of rearing exercise on the total and cortical bone area as the hens age. Jendral et al. (2008) demonstrated an increase in bone density and area with minor modifications to cages, such as increasing space and adding perches and nests to conventional cages or providing cages with or without dust bath access. The differences in bone density values of the radius and tibia observed between the FC-L and FC-S provides evidence that access to increased total area within a cage, even when furnishings and space allowance are held constant, encourages increased exercise and use of space significant enough to influence bone density in adult laying hens.

Trabecular area and trabecular bone mineral content had the opposite effect with FC-S or CC possessing the highest trabecular values and FC-L with the lowest (although this effect was only significant for the trabecular bone area of the tibia). This pattern is supported by previous research (Jendral et al., 2008) and potentially highlights a difference in calcium metabolism triggered by exercise levels, as it has been suggested that larger trabecular areas might be indicative of inadequate prevention of bone resorption on the endosteal surface of the cortical bone (Jendral et al., 2008). The greater trabecular density observed in FC-L (statistically greater for the radius only) might be a result of increased bone mineral present; however, considering
that the trabecular area was smaller for the FC-L group (statistically smaller for the tibia only), the difference in density is likely due to similar mineral content placed into a smaller area.

With all of the evidence of positive bone quality characteristics provided by the QCT analysis at both the pullet and end-of-lay stages, it brings up the question of why consistent differences between rearing systems were found at the end of rearing in the analysis of breaking strength, but not between rearing system or between adult housing system at the end-of-lay. In addition, although the tibiae breaking strength results are in line with the highest QCT bone values reported for the FC-L, it is unclear why the humeral breaking strength is higher in the conventionally-reared hens at 73 wks of age. It is possible that the bone mineral was more evenly distributed in the pullet bones, whereas the adult bones might have produced more varied breaking strength results due to inconsistent bone mineral distribution or areas of structural weakness. Or perhaps the breaking strength technique used here was not ideal for assessing the resiliency of adult bone. Techniques described by Crenshaw et al. (1981) and Newman and Leeson (1998) were used in the current study to minimize any variation from inadvertent drying of wet bone during testing; however, drying the bones might not have been appropriate for a robust understanding of breaking strength in the adult bones. Several studies of bone breaking strength in adult laying hens use a ‘wet’ technique to assess the bending strength (Knott et al., 1995; Fleming et al., 1998a; Silversides et al., 2006, 2012; Riczu et al., 2008; Jendral et al., 2008; Habig and Distl, 2013), instead of the drying method that was used here. Although bone geometry and content are critical components to the strength of a bone, collagen and the collagenous matrix of cross-linkages also play an important role in the resistance to bending and compression stresses (Knott et al., 1995), and the gene expression of collagen type I is known to increase in response to loading in chick tibiae (Zaman et al., 1992). More complex techniques for
the assessment of breaking and bending strength that include the consideration of collagen and complete bone components would be favourable in future research. Techniques described by Regmi et al. (2015) can provide much more detailed information. Although using a wet method, with more comprehensive testing is recommended for future studies assessing breaking strength, the consistency of techniques used for all bones handled here still allows for a valid comparison of the bones within the study.

Previous validation of QCT analysis acknowledges that bone quality values generated from QCT do not typically correlate strongly with traditional methods, such as bone ash or breaking strength (Korver et al., 2004a; Saraff and Hogler, 2015). This is because QCT provides very detailed information from a section of the bone rather than less detailed information involving the entire bone. Unlike traditional methods, QCT effectively distinguishes between bone tissues, allowing for the differentiation between cortical bone and trabecular bone (Korver et al., 2004a; Saraff and Hogler, 2015). Further, comparisons between histomorphometry and QCT have demonstrated that QCT is a suitable measure of changes in trabecular bone mineral density and area (Rosen et al., 1995). Quantitative computed tomography also accurately determines the true volumetric density distribution of the bone, which traditional x-ray and dual-energy x-ray absorptiometry (DEXA) methods cannot determine (Korver et al., 2004a; Saraff and Hogler, 2015). Although traditional techniques, such as bone ash, might be sufficient for quantifying bone material in pullets, bone ash can be misleading in adult hens; for example, an osteoporotic hen with extremely low levels of cortical bone, but high levels of medullary bone, can show nearly the same level of ash content as a hen with bones high in cortical bone and lower in medullary bone. Understanding the distribution of bone material provides a more holistic approach to understanding the type of bone mineral present, and the ability to distinguish
between cortical and trabecular bone allows for the measurement of changes to the actual structural bone loss over time, which is believed to be the underlying cause of osteoporosis.

Overall, the greater total and cortical bone area observed in the Avi pullets at 16 wks of age, and the fact that the effects of aviary-rearing were maintained through to the end of lay indicates that opportunities for diverse, loading exercise during the rearing phase substantially alters the geometry of growing pullet bones. This increase in size of the aviary-reared pullet skeleton potentially affords greater space for bone mineralization and medullary bone deposition. Perhaps other avenues of research targeting methods to increase the rate of calcium absorption or medullary bone deposition can be used in conjunction with this increased skeletal growth to capitalize on the newly available skeletal framework.
Table 3.1 Comparison of muscle weights between aviary-reared (Avi) and conventionally-reared (Conv) pullets at 16 wks of age. All muscles weights were adjusted for pullet body weight.

<table>
<thead>
<tr>
<th>Rearing</th>
<th>Bicep brachii (± SE)</th>
<th>Pectoralis major (± SE)</th>
<th>Pectoralis minor (± SE)</th>
<th>Leg Muscle Group (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conv</td>
<td>2.1 (0.05)</td>
<td>44.8 (0.69)</td>
<td>16.8 (0.20)</td>
<td>83.9 (1.28)</td>
</tr>
<tr>
<td>Avi</td>
<td>2.3 (0.05)</td>
<td>54.7 (0.68)</td>
<td>17.8 (0.19)</td>
<td>81.9 (1.27)</td>
</tr>
<tr>
<td>DF</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>F-Value</td>
<td>27.58</td>
<td>157.92</td>
<td>13.02</td>
<td>5.17</td>
</tr>
<tr>
<td>P-Value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.026</td>
</tr>
</tbody>
</table>

1 Leg muscle group comprised of all femur and tibiotarsus muscles (of the femur: iliotibialis, sartorius, semitendinosus, semimembranosus, quadriceps femoris, ambiens, adductor longus; of the tibiotarsus: gastrocnemius, tibialis anterior, peroneus longus, flexor perforans et perforates II & III)
Table 3.2 Comparison of keel bone growth between aviary-reared (Avi) and conventionally-reared (Conv) pullets at 16 wks of age. All keel bone skeletal characteristics were adjusted for body weight.

<table>
<thead>
<tr>
<th>Rearing</th>
<th>Metasternum Length (mm/kg)</th>
<th>Height (mm/kg)</th>
<th>Area (mm²/kg)</th>
<th>Cartilage Length (mm/kg)</th>
<th>Cartilage Percentage (%/kg)</th>
<th>Keel to Pubic (mm/kg)</th>
<th>Pubic Gap (mm/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conv</td>
<td>72.3 (1.30)</td>
<td>25.3 (0.35)</td>
<td>1097.2 (18.64)</td>
<td>12.6 (0.92)</td>
<td>14.4 (1.01)</td>
<td>33.2 (0.93)</td>
<td>15.7 (0.65)</td>
</tr>
<tr>
<td>Avi</td>
<td>75.5 (1.29)</td>
<td>25.1 (0.33)</td>
<td>1138.3 (18.41)</td>
<td>16.3 (0.90)</td>
<td>18.0 (0.91)</td>
<td>27.5 (0.90)</td>
<td>15.5 (0.64)</td>
</tr>
<tr>
<td>DF</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>F-Value</td>
<td>9.70</td>
<td>0.36</td>
<td>5.18</td>
<td>24.01</td>
<td>18.98</td>
<td>19.65</td>
<td>0.13</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.003</td>
<td>0.552</td>
<td>0.026</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.717</td>
</tr>
</tbody>
</table>

1 Length measured on the dorsal metatsternum surface parallel to the cranial region of the sternal notch ending at the caudal border of the keel metasternum tip
2 Height as measured from the ventral surface of the metasternum to the peak of the Carina apex
3 Area of the keel estimated using the formula for area of a right triangle: Area = (metasternum length x height) x ½
4 Cartilage length measured on the dorsal metatsternum from the line of distinction between the end of ossified bone tissue and initiation of cartilage tissue to the end of the caudal tip of the keel metasternum
5 Percentage cartilage = (cartilage length/metasternum length)x 100
6 Distance between the caudal tip of the keel metasternum to the tip of the left pubic bone
7 Distance between tips of pubic bones
### Table 3.3 Comparison of Quantitative Computed Tomography (QCT) bone measures between aviary-reared (Avi) and conventionally-reared (Conv) pullets at 16 wks of age.

<table>
<thead>
<tr>
<th>Bone &amp; Housing</th>
<th>Density mg/cm³ (± SE)</th>
<th>Cross-sectional Area mm² (± SE)</th>
<th>Bone Mineral Content mg/mm (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Cortical</td>
<td>Total</td>
</tr>
<tr>
<td><strong>Radius</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conv</td>
<td>523.4 (9.65)</td>
<td>898.5 (9.36)</td>
<td>5.0 (0.08)</td>
</tr>
<tr>
<td>Avi</td>
<td>615.5 (9.59)</td>
<td>972.3 (9.33)</td>
<td>7.3 (0.07)</td>
</tr>
<tr>
<td>DF</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>F-Value</td>
<td>87.91</td>
<td>122.08</td>
<td>437.21</td>
</tr>
<tr>
<td>P-Value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Rearing BW²</strong></td>
<td>P-Value</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.114</td>
<td>0.745</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Humerus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conv</td>
<td>71.9 (3.96)</td>
<td>887.1 (5.67)</td>
<td>53.3 (0.70)</td>
</tr>
<tr>
<td>Avi</td>
<td>126.6 (3.90)</td>
<td>928.7 (5.65)</td>
<td>65.9 (0.69)</td>
</tr>
<tr>
<td>DF</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>F-Value</td>
<td>96.49</td>
<td>102.13</td>
<td>233.01</td>
</tr>
<tr>
<td>P-Value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Rearing BW²</strong></td>
<td>P-Value</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.052</td>
<td>0.078</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Tibia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conv</td>
<td>429.9 (5.36)</td>
<td>1016.3 (3.04)</td>
<td>37.4 (0.35)</td>
</tr>
<tr>
<td>Avi</td>
<td>456.8 (5.30)</td>
<td>1016.2 (3.00)</td>
<td>38.8 (0.34)</td>
</tr>
<tr>
<td>DF</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>F-Value</td>
<td>13.17</td>
<td>0.00</td>
<td>7.03</td>
</tr>
<tr>
<td>P-Value</td>
<td>&lt;0.001</td>
<td>0.989</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Rearing BW²</strong></td>
<td>P-Value</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.052</td>
<td>0.717</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

---

1. Refers to the bone in the trabecular space
2. Rearing body weight (BW) was a covariate in the statistical model
Table 3.4 Comparison of bone breaking strength between aviary-reared (Avi) and conventionally-reared (Conv) pullets at 16 wks of age

<table>
<thead>
<tr>
<th>Rearing</th>
<th>Humerus (± SE)</th>
<th>Radius (± SE)</th>
<th>Tibia (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conv</td>
<td>9.8 (0.38)</td>
<td>3.9 (0.09)</td>
<td>15.5 (0.36)</td>
</tr>
<tr>
<td>Avi</td>
<td>17.5 (0.37)</td>
<td>7.2 (0.09)</td>
<td>15.7 (0.36)</td>
</tr>
<tr>
<td>DF</td>
<td>72</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>F-Value</td>
<td>206.50</td>
<td>617.32</td>
<td>6.46</td>
</tr>
<tr>
<td>P-Value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Rearing BW\(^1\)  
P-Value 0.5684 <0.001 0.039

\(^1\) Rearing body weight (BW) was a covariate in the statistical model
Table 3.5 Quantitative Computed Tomography (QCT) bone measures of the radius in adult laying hens at 73 wks of age

<table>
<thead>
<tr>
<th>Rearing1</th>
<th>Density mg/cm³ (± SE)</th>
<th>Cross-sectional Area mm² (± SE)</th>
<th>Bone Mineral Content mg/mm (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Cortical</td>
<td>Trabecular</td>
</tr>
<tr>
<td>Conv</td>
<td>730.1 (16.74)</td>
<td>223.9 (7.72)</td>
<td>19 (0.12)</td>
</tr>
<tr>
<td>Avi</td>
<td>616.7 (17.50)</td>
<td>220.8 (8.11)</td>
<td>17.30</td>
</tr>
<tr>
<td>DF</td>
<td>114</td>
<td>114</td>
<td>114</td>
</tr>
<tr>
<td>F-Value</td>
<td>0.14</td>
<td>0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>P-Value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult Housing2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC-L</td>
<td>708.5 (17.78)</td>
<td>237.3 (8.27)</td>
<td>6.6 (0.13)</td>
</tr>
<tr>
<td>FC-S</td>
<td>653.6 (22.77)</td>
<td>217.4 (10.91)</td>
<td>6.8 (0.17)</td>
</tr>
<tr>
<td>CC</td>
<td>658.2 (17.14)</td>
<td>212.5 (7.94)</td>
<td>6.7 (0.12)</td>
</tr>
<tr>
<td>DF</td>
<td>114</td>
<td>114</td>
<td>114</td>
</tr>
<tr>
<td>F-Value</td>
<td>4.50</td>
<td>3.73</td>
<td>0.33</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.013</td>
<td>0.027</td>
<td>0.027</td>
</tr>
<tr>
<td>Interaction R*A3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conv * FC-L</td>
<td>760.1 (23.39)</td>
<td>243.1 (11.25)</td>
<td>5.6 (0.17)</td>
</tr>
<tr>
<td>Conv * FC-S</td>
<td>708.1 (27.07)</td>
<td>214.4 (13.12)</td>
<td>5.7 (0.20)</td>
</tr>
<tr>
<td>Conv * CC</td>
<td>722.2 (21.18)</td>
<td>214.4 (10.21)</td>
<td>5.9 (0.15)</td>
</tr>
<tr>
<td>Avi * FC-L</td>
<td>656.9 (21.31)</td>
<td>231.5 (10.15)</td>
<td>7.6 (0.16)</td>
</tr>
<tr>
<td>Avi * FC-S</td>
<td>599.0 (32.76)</td>
<td>220.5 (16.01)</td>
<td>7.8 (0.25)</td>
</tr>
<tr>
<td>Avi * CC</td>
<td>594.2 (21.17)</td>
<td>210.6 (10.06)</td>
<td>7.5 (0.15)</td>
</tr>
<tr>
<td>DF</td>
<td>114</td>
<td>114</td>
<td>114</td>
</tr>
<tr>
<td>F-Value</td>
<td>0.24</td>
<td>0.28</td>
<td>1.69</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.784</td>
<td>0.584</td>
<td>0.754</td>
</tr>
</tbody>
</table>

1 Pullets housed in a conventional rearing system (Conv) or an aviary rearing system (Avi) until 16 weeks of age Flock 1-4
2 Adult hens placed into furnished cage-large (FC-L), furnished cage-small (FC-S), or conventional cages (CC) from 16-73 weeks of age Flock 1-4
3 Interaction between rearing housing system and adult housing system
Table 3.6 Quantitative Computed Tomography (QCT) bone measures of the humerus in adult laying hens at 73 wks of age

<table>
<thead>
<tr>
<th>Rearing¹</th>
<th>Density mg/cm³ (± SE)</th>
<th>Cross-sectional Area mm² (± SE)</th>
<th>Bone Mineral Content mg/mm (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Cortical</td>
<td>Trabecular</td>
</tr>
<tr>
<td>Conv</td>
<td>115.0 (14.78)</td>
<td>989.0 (6.65)</td>
<td>-</td>
</tr>
<tr>
<td>Avi</td>
<td>95.9 (17.20)</td>
<td>933.6 (8.06)</td>
<td>-</td>
</tr>
<tr>
<td>DF</td>
<td>83</td>
<td>84</td>
<td>-</td>
</tr>
<tr>
<td>F-Value</td>
<td>1.00</td>
<td>29.64</td>
<td>-</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.321</td>
<td>&lt;0.001</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adult Housing²</th>
<th>Density mg/cm³ (± SE)</th>
<th>Cross-sectional Area mm² (± SE)</th>
<th>Bone Mineral Content mg/mm (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC-L</td>
<td>121.9 (16.33)</td>
<td>968.8 (7.63)</td>
<td>-</td>
</tr>
<tr>
<td>FC-S</td>
<td>79.7 (22.61)</td>
<td>954.3 (11.25)</td>
<td>-</td>
</tr>
<tr>
<td>CC</td>
<td>114.8 (16.48)</td>
<td>960.8 (7.55)</td>
<td>-</td>
</tr>
<tr>
<td>DF</td>
<td>83</td>
<td>84</td>
<td>-</td>
</tr>
<tr>
<td>F-Value</td>
<td>1.46</td>
<td>0.65</td>
<td>-</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.237</td>
<td>0.525</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interaction R*A³</th>
<th>Density mg/cm³ (± SE)</th>
<th>Cross-sectional Area mm² (± SE)</th>
<th>Bone Mineral Content mg/mm (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conv * FC-L</td>
<td>123.9 (22.40)</td>
<td>991.5 (11.23)</td>
<td>-</td>
</tr>
<tr>
<td>Conv * FC-S</td>
<td>101.8 (24.41)</td>
<td>990.3 (12.95)</td>
<td>-</td>
</tr>
<tr>
<td>Conv * CC</td>
<td>119.2 (19.29)</td>
<td>985.3 (9.41)</td>
<td>-</td>
</tr>
<tr>
<td>Avi * FC-L</td>
<td>119.9 (20.40)</td>
<td>946.0 (10.07)</td>
<td>-</td>
</tr>
<tr>
<td>Avi * FC-S</td>
<td>57.5 (35.19)</td>
<td>918.2 (18.25)</td>
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</tr>
<tr>
<td>Avi * CC</td>
<td>110.4 (23.78)</td>
<td>936.4 (11.59)</td>
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</tr>
<tr>
<td>DF</td>
<td>83</td>
<td>84</td>
<td>-</td>
</tr>
<tr>
<td>F-Value</td>
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<td>0.53</td>
<td>-</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.896</td>
<td>0.593</td>
<td>-</td>
</tr>
</tbody>
</table>

¹ Pullets housed in a conventional rearing system (Conv) or an aviary rearing system (Avi) until 16 weeks of age Flock 1-4
² Adult hens placed into furnished cage- large (FC-L), furnished cage-small (FC-S), or conventional cages (CC) from 16-73 weeks of age Flock 1-4
³ Interaction between rearing housing system and adult housing system
Table 3.7 Quantitative Computed Tomography (QCT) bone measures of the tibia in adult laying hens at 73 wks of age

<table>
<thead>
<tr>
<th>Rearing</th>
<th>Density mg/cm³ (± SE)</th>
<th>Cross-sectional Area mm² (± SE)</th>
<th>Bone Mineral Content mg/mm (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Cortical</td>
<td>Trabecular</td>
</tr>
<tr>
<td>Conv</td>
<td>589.2 (7.68)</td>
<td>1063.2 (6.52)</td>
<td>208.19 (6.37)</td>
</tr>
<tr>
<td>Avi</td>
<td>590.6 (8.40)</td>
<td>1031.5 (7.03)</td>
<td>198.7 (6.93)</td>
</tr>
<tr>
<td>DF</td>
<td>114</td>
<td>114</td>
<td>114</td>
</tr>
<tr>
<td>F-Value</td>
<td>0.01</td>
<td>12.90</td>
<td>1.08</td>
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<tr>
<td>P-Value</td>
<td>0.905</td>
<td>&lt;0.001</td>
<td>0.301</td>
</tr>
</tbody>
</table>

Adult Housing

<table>
<thead>
<tr>
<th></th>
<th>Density mg/cm³ (± SE)</th>
<th>Cross-sectional Area mm² (± SE)</th>
<th>Bone Mineral Content mg/mm (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Cortical</td>
<td>Trabecular</td>
</tr>
<tr>
<td>FC-L</td>
<td>620.3 (8.65)</td>
<td>1061.3 (7.19)</td>
<td>213.8 (7.11)</td>
</tr>
<tr>
<td>FC-S</td>
<td>571.9 (12.42)</td>
<td>1040.0 (10.00)</td>
<td>197.6 (10.10)</td>
</tr>
<tr>
<td>CC</td>
<td>577.5 (7.92)</td>
<td>1040.7 (6.72)</td>
<td>198.9 (6.57)</td>
</tr>
<tr>
<td>DF</td>
<td>114</td>
<td>114</td>
<td>114</td>
</tr>
<tr>
<td>F-Value</td>
<td>8.34</td>
<td>3.01</td>
<td>1.51</td>
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<tr>
<td>P-Value</td>
<td>&lt;0.001</td>
<td>0.053</td>
<td>0.226</td>
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</table>

Interaction R*A

<table>
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<tr>
<th></th>
<th>Density mg/cm³ (± SE)</th>
<th>Cross-sectional Area mm² (± SE)</th>
<th>Bone Mineral Content mg/mm (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Cortical</td>
<td>Trabecular</td>
</tr>
<tr>
<td>Conv * FC-L</td>
<td>604.1 (13.00)</td>
<td>1076.8 (10.40)</td>
<td>212.6 (10.54)</td>
</tr>
<tr>
<td>Conv * FC-S</td>
<td>574.6 (15.54)</td>
<td>1053.5 (12.33)</td>
<td>210.3 (12.56)</td>
</tr>
<tr>
<td>Conv * CC</td>
<td>589.0 (10.99)</td>
<td>1056.1 (8.95)</td>
<td>201.7 (8.97)</td>
</tr>
<tr>
<td>Avi * FC-L</td>
<td>636.5 (11.40)</td>
<td>1045.7 (9.22)</td>
<td>214.9 (9.28)</td>
</tr>
<tr>
<td>Avi * FC-S</td>
<td>569.2 (19.38)</td>
<td>1023.5 (15.29)</td>
<td>185.0 (15.64)</td>
</tr>
<tr>
<td>Avi * CC</td>
<td>566.0 (11.40)</td>
<td>1025.2 (9.25)</td>
<td>196.2 (9.30)</td>
</tr>
<tr>
<td>DF</td>
<td>114</td>
<td>114</td>
<td>114</td>
</tr>
<tr>
<td>F-Value</td>
<td>2.84</td>
<td>0.00</td>
<td>0.65</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.062</td>
<td>0.995</td>
<td>0.524</td>
</tr>
</tbody>
</table>

1 Pullets housed in a conventional rearing system (Conv) or an aviary rearing system (Avi) until 16 weeks of age Flock 1-4
2 Adult hens placed into furnished cage-large (FC-L), furnished cage-small (FC-S), or conventional cages (CC) from 16-73 weeks of age Flock 1-4
3 Interaction between rearing housing system and adult housing system
### Table 3.8 Comparison of bone breaking strength in adult laying hens at 73 wks of age

<table>
<thead>
<tr>
<th>Rearing&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Humerus (± SE)</th>
<th>Radius (± SE)</th>
<th>Tibia (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conv</td>
<td>9.2 (0.45)</td>
<td>5.1 (0.13)</td>
<td>14.1 (0.75)</td>
</tr>
<tr>
<td>Avi</td>
<td>6.3 (0.44)</td>
<td>5.1 (0.12)</td>
<td>14.4 (0.75)</td>
</tr>
<tr>
<td></td>
<td>DF</td>
<td>40</td>
<td>57</td>
</tr>
<tr>
<td>F-Value</td>
<td>34.6</td>
<td>0.05</td>
<td>0.59</td>
</tr>
<tr>
<td>P-Value</td>
<td>&lt;0.001</td>
<td>0.816</td>
<td>0.445</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Adult Housing&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Humerus (± SE)</th>
<th>Radius (± SE)</th>
<th>Tibia (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC-L</td>
<td>7.7 (0.48)</td>
<td>5.2 (0.14)</td>
<td>14.8 (0.76)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FC-S</td>
<td>7.4 (0.55)</td>
<td>5.2 (0.17)</td>
<td>14.4 (0.78)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CC</td>
<td>8.1 (0.49)</td>
<td>5.1 (0.18)</td>
<td>13.5 (0.77)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>DF</td>
<td>40</td>
<td>57</td>
</tr>
<tr>
<td>F-Value</td>
<td>0.56</td>
<td>0.11</td>
<td>5.59</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.566</td>
<td>0.893</td>
<td>0.006</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Interaction R*A&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Humerus (± SE)</th>
<th>Radius (± SE)</th>
<th>Tibia (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conv * FC-L</td>
<td>9.2 (0.62)</td>
<td>5.1 (0.18)</td>
<td>14.4 (0.82)</td>
</tr>
<tr>
<td>Conv * FC-S</td>
<td>9.6 (0.74)</td>
<td>5.1 (0.19)</td>
<td>14.6 (0.85)</td>
</tr>
<tr>
<td>Conv * CC</td>
<td>8.7 (0.62)</td>
<td>5.1 (0.17)</td>
<td>13.3 (0.81)</td>
</tr>
<tr>
<td>Avi * FC-L</td>
<td>6.3 (0.62)</td>
<td>5.2 (0.17)</td>
<td>15.2 (0.81)</td>
</tr>
<tr>
<td>Avi * FC-S</td>
<td>5.3 (0.69)</td>
<td>5.2 (0.18)</td>
<td>14.2 (0.83)</td>
</tr>
<tr>
<td>Avi * CC</td>
<td>7.5 (0.65)</td>
<td>5.1 (0.18)</td>
<td>13.7 (0.82)</td>
</tr>
<tr>
<td></td>
<td>DF</td>
<td>40</td>
<td>57</td>
</tr>
<tr>
<td>F-Value</td>
<td>3.1</td>
<td>0.07</td>
<td>0.99</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.058</td>
<td>0.929</td>
<td>0.378</td>
</tr>
</tbody>
</table>

<sup>1</sup> Pullets housed in a conventional rearing system (Conv) or an avairy rearing system (Avi) until 16 weeks of age Flock 1-4

<sup>2</sup> Adult hens placed into furnished cage- large (FC-L), furnished cage-small (FC-S), or conventional cages (CC) from 16-73 weeks of age Flock 1-4

<sup>3</sup> Interaction between rearing housing system and adult housing system
Figure 3.1 Images of the Farmer Automatic Logia Pullet Portal aviary rearing system (A and B) and conventional cage rearing system (C). Image A depicts the area inside the system (System Area: 183,272 cm$^2$) equipped with feeders, waterers, perches, a suspended platform (32,371 cm$^2$) in the centre of the system (Total System Area: 215,643 cm$^2$). Image B depicts nine platforms (Platform Area: 118,887 cm$^2$) on the outer edge of the system, which are opened up at six wks of age to allow for access to the litter portion (Litter Area: 235,767 cm$^2$; Total Aviary Area: 570,297 cm$^2$) of the aviary enclosure seen in the bottom corner of the image. Image C depicts the conventional cage rearing system (Total Cage Area: 2,322 cm$^2$).
Total Density (mg/mm$^3$)

- Enrich_L
- Enrich_S
- Conv

Adult Housing System

Total Area (mm$^2$)

- FC_L
- FC_S
- CC

Adult Housing System

Total Bone Mineral Content (mg/mm)

- FC_L
- FC_S
- CC

Adult Housing System

Significance Levels:
- P < 0.001
- a
- b
- *
**Figure 3.2** Quantitative Computed Tomography (QCT) measurements comparing the effect of rearing environment on total bone measures of the radius at 16 wks (Pullet Reference Value) and 73 wks from hens housed in furnished cage-large (FC-L), furnished cage-small (FC-S), and conventional cages (CC). Significance for the effect of rearing at 16 wks is located above the pullet Reference Value. Significant effects of rearing ($P < 0.05$) in adult bones at 73 wks of age is designated by and *. Significant differences ($P < 0.05$) among adult housing systems are designated by differing letters a-c.
Figure 3.3 Quantitative Computed Tomography (QCT) measurements comparing the effect of rearing environment on cortical bone measures of the radius at 16 wks (Pullet Reference Value) and 73 wks from hens housed in furnished cage-large (FC-L), furnished cage-small (FC-S), and conventional cages (CC). Significance for the effect of rearing at 16 wks is located above the pullet Reference Value. Significant effects of rearing ($P < 0.05$) in adult bones at 73 wks of age is designated by and *. Significant differences ($P < 0.05$) among adult housing systems are designated by differing letters a-c.
### Total Density

**Adult Housing System**

<table>
<thead>
<tr>
<th>Pullet Reference Value</th>
<th>FC_L</th>
<th>FC_S</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adult Housing System</strong></td>
<td>Avi</td>
<td>Conv</td>
<td>Avi</td>
</tr>
<tr>
<td><strong>Total Density (mg/cm³)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Total Area

**Adult Housing System**

<table>
<thead>
<tr>
<th>Pullet Reference Value</th>
<th>FC_L</th>
<th>FC_S</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adult Housing System</strong></td>
<td>Avi</td>
<td>Conv</td>
<td>Avi</td>
</tr>
<tr>
<td><strong>Total Area (mm²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Total Bone Mineral Content

**Adult Housing System**

<table>
<thead>
<tr>
<th>Pullet Reference Value</th>
<th>FC_L</th>
<th>FC_S</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adult Housing System</strong></td>
<td>Avi</td>
<td>Conv</td>
<td>Avi</td>
</tr>
<tr>
<td><strong>Total Bone Mineral Content (mg/mm)</strong></td>
<td></td>
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</tbody>
</table>

*P < 0.001*
**Figure 3.4** Quantitative Computed Tomography (QCT) measurements comparing the effect of rearing environment on total bone measures of the tibia at 16 wks (Pullet Reference Value) and 73 wks from hens housed in furnished cage-large (FC-L), furnished cage-small (FC-S), and conventional cages (CC). Significance for the effect of rearing at 16 wks is located above the pullet Reference Value. Significant effects of rearing ($P < 0.05$) in adult bones at 73 wks of age is designated by and *. Significant differences ($P < 0.05$) among adult housing systems are designated by differing letters a-c.
Figure 3.5 Quantitative Computed Tomography (QCT) measurements comparing the effect of rearing environment on cortical bone measures of the tibia at 16 wks (Pullet Reference Value) and 73 wks from hens housed in furnished cage-large (FC-L), furnished cage-small (FC-S), and conventional cages (CC). Significance for the effect of rearing at 16 wks is located above the pullet Reference Value. Significant effects of rearing ($P < 0.05$) in adult bones at 73 wks of age is designated by and *. Significant differences ($P < 0.05$) among adult housing systems are designated by differing letters a-c.
The state of cortical and medullary bone (MB) growth, prior to sexual maturity at 16 wks, at initiation of 1st egg, and at 67 wks of age. Empty cortical space that was present at 16 wks of age is subsequently filled in by medullary bone as estrogen levels rise approaching egg production. By the end-of-lay at 67 wks of age, the cortical area is very thin with the bone almost entirely comprised of medullary bone.
CHAPTER 4

Rearing system affects prevalence of keel bone damage in laying hens: a longitudinal study of four consecutive flocks

4.1 ABSTRACT

High flock-level prevalence estimates of keel bone fractures and deviations in laying hens are commonly reported across a variety of housing systems; however, few longitudinal studies exist, especially for furnished and conventional cage systems. Load bearing exercise improves bone composition in laying hens and has the potential to reduce keel bone damage, especially if exercise is allowed during critical periods of bone growth throughout the pullet rearing phase. The objective of this study was to determine the prevalence of keel bone damage over time in laying hens housed in furnished and conventional cages, and assess whether opportunities for exercise during the pullet rearing phase influences the prevalence of keel bone damage throughout the laying period. Four flock replicates of 588 Lohmann Selected Leghorn-Lite pullets were reared in either standard, conventional cages (Conv) or an aviary rearing system (Avi) and placed into either conventional cages (CC), 30-bird furnished cages (FC-S) or 60-bird furnished cages (FC-L) for adult housing. Keel bone status was determined by palpation at 30, 50, and 70 wks of age. Age ($P < 0.001$) and rearing system ($P < 0.001$) had an effect on the presence of keel bone fractures. The presence of fractures increased with age, and hens raised in

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1 This manuscript is in preparation for submission to *Poultry Science* with the following authors: T.M. Casey-Trott, M.T. Guerin, V. Sandilands, S. Torrey, and T.M. Widowski
the Avi system had a lower percentage of fractures (41.6% ± 2.8 SE) compared to hens reared in the Conv system (60.3% ± 2.9 SE). Adult housing system did not have an effect on the percentage of keel fractures (P = 0.223). Age had an effect on the presence of deviations (P < 0.001), with deviations increasing with age. Rearing system (P = 0.218) and adult housing system (P = 0.539) did not affect the presence of deviations. However, keel fractures and deviations were strongly associated with each other at all ages: 30 wks: (P < 0.001); 50 wks: (P < 0.001); and 70 wks: (P < 0.001). Increased opportunities for exercise provided by an aviary rearing system reduced the prevalence of keel bone fractures through to the end-of-lay.

4.2 INTRODUCTION

Over the last two decades, numerous studies have documented fractures and deformations of the keel bone in laying hens, with prevalence estimates ranging between 5 and 97% depending on housing system and age (Fleming et al., 2004; Rodenburg et al., 2008; Wilkins et al., 2011; Petrik et al., 2015; Riber and Hinrichsen, 2016; Regmi et al., 2016a). More recent research has been dedicated to addressing the negative impact that keel damage has on the welfare of the hen in terms of pain (Nasr et al., 2012b), restricted mobility (Nasr et al., 2012a), affective state (Nasr et al., 2013a), and behavioural changes (Chapter 5). The alarmingly high prevalence combined with the concern for hen welfare has brought the issue of keel bone damage to the forefront of laying hen research.

The keel bone is an extension of the ventral surface of the sternum progressing along the midline of the sagittal plane. In avian species, the keel serves as an anchor for flight muscles and also plays a pivotal role in expanding and contracting the thoracic cavity during inhalation and exhalation (Codd et al., 2005; Claessens, 2009; Lambertx and Perry, 2015). The keel spans from
the cranial, Carina apex to the caudal tip, with the spine of the keel tapering off as it approaches the caudal portion (as described in Casey-Trott et al., 2015). The growth and ossification of the keel is a process that begins in the cranial region, progressing gradually toward the caudal tip. Ossification of the keel continues into the early stages of egg laying until approximately 28–40 wks of age (Buckner, 1949), well beyond the growth of the long bones which ceases at the onset of lay (Hurwitz, 1965; Hudson et al., 1993). Due to its slow ossification, the caudal portion of the keel is often still cartilaginous at the onset of lay (Chapter 3).

The high prevalence of keel bone damage has led to an international movement to develop prevention strategies for this problem (Harlander-Matauschek et al., 2015). Keel bone damage typically occurs in the form of fractures and deformations along the spine of the keel and at the caudal tip (Casey-Trott et al., 2015). Damage increases with age (Weitzenburger et al., 2006; Scholz et al., 2008; Kappeli et al., 2011b; Petrik et al., 2015; Stratmann et al., 2015a) across a variety of housing systems, and is most prevalent in non-cage systems due to the increased opportunity for damage from high-impact crashes and falls (Rodenburg et al., 2008; Wilkins et al., 2011; Kappeli et al., 2011a). Outfitting aviary systems with ramps (Stratmann et al., 2015a), and reducing perch obstruction and adjusting perch placement (Moinard et al., 2005) have led to positive results in non-cage systems, by reducing crashes and falls. However, keel bone damage also occurs in low-impact systems, such as furnished (Weitzenburger et al., 2006; Rodenberg et al., 2008; Scholz et al., 2008) and conventional cages (Hester et al., 2013; Petrik et al., 2015). The causes and prevalence within these low-impact systems is less understood.

As discussed by Harlander-Matauschek et al. (2015), possible causes of keel bone damage other than high-impact injuries include unequal wing-loading during wing-flapping, perch use (Pickle et al., 2011; Hester et al., 2013), compression fractures due to osteoporosis as
seen in humans (Kondo, 2008), early onset of egg production (Gebhardt-Henrich and Frohlich, 2015), nutritional inadequacies (Whitehead, 2004; Fleming et al., 2006), or genetic factors (Whitehead, 2004; Stratmann et al., 2016). Strategies to assess genetic differences in keel bone composition (Bishop et al., 2000; Hocking et al., 2003) and genetic associations with keel damage (Vits et al., 2005; Fleming et al., 2004, 2006; Stratmann et al., 2016), as well as the development of nutritional strategies such as calcium particle size (Fleming et al., 1998a) and administration of omega-3 fatty acids (Tarlton et al., 2013) have been explored.

Exercise to stimulate bone growth is another research avenue that has the potential to influence keel bone strength and composition. The beneficial effects of exercise on the long bones of adult laying hens has been demonstrated by comparing the bone characteristics of adult hens housed in a modified furnished cage system (Jendral et al., 2008), aviaries (Leyendecker et al., 2005), and free-range systems (Shipov et al., 2010) to the bone characteristics of hens housed in the relative confinement of conventional cages. Few studies have assessed the direct effects of exercise on keel bone damage (Regmi et al., 2016a); however improvement in the composition of the long bones (tibiae, humeri) has been shown to correlate with improvement in the composition of the keel bone (Hocking et al., 2003; Fleming et al., 2004; Regmi et al., 2016a). Unfortunately, the assessment of exercise on keel bone damage is often confounded by providing exercise in the form of increased space allowance, addition of furnishings, or housing in extensive systems, which increase the risk of collisions or injuries (Fleming et al., 2006; Scholz et al., 2009).

Targeting the pullet rearing phase to improve bone health has yielded positive results in terms of improving muscle growth (Hester et al., 2013; Chapter 3), bone breaking strength (Vits et al., 2005; Regmi et al., 2015; Chapter 3), and bone composition and geometry (Regmi et al.,
2015; Chapter 3) of the long bones, with the beneficial effects being sustained through the end of lay (Regmi et al., 2016b; Chapter 3). Furthermore, preliminary evidence suggests that exercise during the pullet rearing period reduces keel damage scores (Vits et al., 2005) and influences the overall growth of the keel (Chapter 3). Housing pullets in rearing systems that encourage regular, diverse forms of exercise has the potential to aid in the reduction of keel bone damage by improving motor skills within more complex systems and enhancing the strength and composition of the keel through loading exercise from wing flapping.

The objective of this study was to determine the prevalence of keel bone damage over time in laying hens housed in furnished and conventional cages, and assess whether exercise during the pullet rearing phase influences the prevalence of keel bone damage throughout the laying period. We hypothesized that the prevalence of keel bone damage would increase with age, that hens in conventional cages would have a lower prevalence of damage than hens in furnished cages, and that exercise during rearing would improve the overall keel bone status of adult hens in both adult housing systems. A secondary objective was to determine whether there was an association between the development of keel bone fractures and deviations using the Simplified Keel Assessment Protocol (SKAP; Casey-Trott et al., 2015). We hypothesized that a strong correlation would exist between both forms of keel damage in furnished and conventional cages.

4.3 METHODS

The effects of rearing system (standard cage (Conv) or rearing aviary (Avi)) and adult housing system (conventional cage (CC), 30-bird furnished cage (FC-S) or 60-bird furnished cage (FC-L)) were tested using a 2 x 3 factorial arrangement with rearing flock replicated in 4
blocks over time. Each of the 4 rearing flocks contributed 3 replicate cages to each of the adult housing systems. Animal use was approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #1947).

4.3.1 Pullet management

From 4 consecutive flocks, a group of 588 Lohmann-selected Leghorn Lite pullets per flock were conveniently selected and reared from 1 day-of-age to 16 wks of age at the University of Guelph Arkell Poultry Research Station. For each consecutive flock, half of the pullets were conveniently selected for rearing in standard conventional cages (Ford Dickinson, Mitchel, Ontario, Canada; 16 pullets/cage during wks 0-6 with a space allowance of 145 cm²/pullet followed by 8 pullets/cage during wks 6-16 with a space allowance of 290 cm²/pullet; total cage area = 2,322 cm²) and half were conveniently selected for rearing in a Farmer Automatic Logia Pullet Portal (Clark Ag Systems, Caledonia, Ontario, Canada; 756 pullets/aviary enclosure; system space allowance of 285 cm²/hen during wks 0-6; system + litter space allowance of 754 cm²/pullet during wks 6-16). The aviary system was selected to allow maximum opportunities for exercise starting at 1 day-of-age with access to the floor area of the system (183,272 cm²), perches, and a suspended platform (32,371 cm²) that was gradually raised vertically in accordance with the age of the pullet to encourage hopping and flight to access vertical space. At 6 wks of age, additional space was added by opening the sides of the system to allow for access to the litter area (235,767 cm²) and 9 elevated terraces (118,887 cm²) on the outer edge of the system, increasing the total system area to 570,297 cm² (Chapter 3: Figure 3.1).

Both the conventionally- and aviary-reared pullets from all 4 flocks were fed identical, 21% crude protein, 1.06% calcium, 0.47% available phosphorus starter diets (as crumbles) from
0-6 wks and identical 18% crude protein, 1.01% calcium, 0.45% available phosphorus grower diets (as crumbles) from 6-16 wks with the addition of granite to the Avi diet for Flock 1 and the addition of granite chick grit to both the Conv and Avi diets for Flocks 2-4. Both rearing treatments also followed identical vaccination and lighting programs. Lights were on for 16 hrs/d during wks 1 and 2, alternating 4 hrs on and 2 hrs off. Beginning at 3 wks of age, lights were on continuously for 14 hrs starting at 05:00, and subsequently reduced by 1 hr/wk until reaching a maintenance level of 8 hrs/d of light during wks 8-15. The lights were set at 40 lux at placement, and reduced by 5 lux until reaching a maintenance level of 10 lux during wks 4-16. In the aviary rearing system, water lines were located in the middle of the enclosure with the chain feeder running along the perimeter. Water lines with nipple drinkers and chain feeders were suspended from the ceiling and could be raised vertically in accordance with pullet growth. In the conventional rearing system, height-adjustable water lines with nipple drinkers were located in the middle of the cage with the chain feeders running past the front of the cage. All chicks were beak trimmed at the hatchery using infrared treatment.

Since the Conv-reared pullets from Flock 2 achieved greater body weights sooner and matured earlier than the Avi-reared pullets from the same flock, both Flock 3 and 4 were closely monitored for differences in body weight between the rearing treatments. For both Flock 3 and 4, a conveniently sampled group of 100 pullets per flock from each rearing system were weighed biweekly and compared to target weights outlined in the North American Edition of the LSL-Lite Management Guide Layers (Lohmann Tiertzucht GmbH, Germany). Pullets from within the aviary rearing system were sampled from various areas on the floor and within the system. For the standard rearing system, two pullets were selected from each cage. In an attempt to manage variation in body weights between the Conv and Avi pullets to achieve equal and recommended
target body weights at placement into the adult housing system, the room temperatures were increased slightly in the conventional room to reduce feeding behaviour when the biweekly weight of the Conv-reared pullets exceeded the Avi-reared pullets by more than 10%. Avi-reared pullets from Flock 4 remained on the starter diet for one additional wk in order to bring them up to a body weight matching that of the Conv reared pullets. All 4 flocks came into lay between 17 and 18 wks of age, and achieved 50% production at wk 19.

4.3.2 Adult laying hen management

At 16 wks of age, 294 pullets from each rearing system (Avi and Conv) from each flock (1-4) were placed into two adult rooms each holding 12 Farmer Automatic Enrichable (Furnished) Cages (Clark Ag Systems), and one adult room holding 90 standard conventional cages, of which 12 standard conventional cages (Ford Dickinson) were included in the study. In both the furnished cage rooms, and the conventional cage room, 2 flocks were housed simultaneously (Flock 1 and 2; Flock 3 and 4) with the placement into all cages equally balanced for all 4 flocks. In all rooms, a conveniently selected group of hens from a single rearing treatment (Avi or Conv) was placed into each cage, balancing both rearing treatments equally within each room. Each furnished cage room contained 6 large furnished cages (60 hens, total area = 41,296 cm², 688 cm²/hen) and 6 small furnished cages (30 hens, total area = 20,880 cm², 696 cm²/hen). Each bank of 6 cages had 3 tiers with one large and one small cage on each tier. The conventional cage room contained 12 standard conventional cages of equal size (8 hens; total area = 4,025 cm²; space allowance 503 cm²/hen), all on a single tier. The same rearing and adult rooms were used for each consecutive flock.
All flocks were fed identical, standard commercial layer crumbled pellet diets with automatic feed chains running every 3 hrs commencing at the start of a 14 hr light period from 05:00-19:00 hrs with a 15-min sunrise and sunset starting at 05:00 hr and 18:45 hr, respectively. In the furnished cage rooms, the light intensity varied among tiers, with the highest intensity recorded at the top tiers measuring 10-15 lux and the lowest intensity at the bottom tiers measuring 4-5 lux. Each furnished cage provided a curtained nest area proportional to cage size (94 cm²/hen), 10 cm high perches (15 cm²/hen) running parallel to the cage front throughout the middle area, and a smooth plastic scratch area (large: 42 cm²/hen; small: 83 cm²/hen). Nipple drinkers with cups were located above the feed auger down the middle of the cage. The feed troughs (12 cm/hen) were located on both outer sides of the cages. Conventional cages were equipped with a nipple drinker running down the middle of the cages, with the feed troughs (8 cm/hen) on the outer side of the cage. All rooms were sealed and entirely lit with artificial light (incandescent) with no natural, external light sources present.

4.3.3 Keel bone scoring by palpation

All palpation scoring was completed by the same two observers for all data collection periods for all 4 flocks. Both observers underwent training and reliability assessment as part of a previous research study (Petrik et al., 2013). Previous assessment of the lead observer (Casey-Trott) for the current study reported the accuracy, sensitivity, and specificity for detection of fractures and deviations. The accuracy of detection was 84% for fractures and 91% for deviations. The sensitivity of detection was 81% for fractures and 84% for deviations, and the specificity of detection was 87% for fractures and 97% for deviations (Casey-Trott et al., 2015). Prior to every scoring period, both observers completed consensus training by discussing scores together via palpation and inspection of excised keels. Each observer palpated exactly half of
each experimental cage to ensure balanced scoring methods. Observers were blind to rearing treatment, but not adult housing system treatment.

During placement into adult housing at 16 wks of age, all pullets were weighed and the keel bone status of each bird was scored for fractures and deviations using palpation. All subsequent weighting and keel bone scoring was completed at 30, 50, and 70 wks of age on 20% of the hens in each cage using the same palpation technique. For ease of catching, the lights were dimmed in each room and the hens were caught conveniently from multiple areas within each cage until 20% of the hens from each FC-L (N=12) and FC-S (N= 6) were caught. For CC, 20% of the hens from each cage (N=2) were scored for Flock 1; however, for Flocks 2-4, all hens in each cage (N=8) were scored to ensure a representative mean with a sample size comparable to the number scored from the furnished cages.

All keels were assessed for the presence of fractures and deviations. The hens were restrained in an inverted position by holding both legs, with the ventral surface of the keel facing away from the body of the person performing the palpation. The thumb and index finger were used to palpate the keel by running the fingers down the ventral surface of the keel. Keels were palpated from the cranial Carina apex all the way to the caudal tip. A keel was considered to be fractured (FR) if there was the presence of a sharp bend, one or more periosteal scars or calluses, or if any detached or semi-detached bone fragments were present. The presence of a FR was a binomial score denoting only the presence or absence of a keel fracture as described by SKAP (Casey-Trott et al., 2015). In addition to the SKAP scoring method, for Flocks 2-4, the location of the fracture was also recorded. The fracture was classified as a tip fracture (FR-TIP) if a fracture was detected within the last 5 cm of the caudal tip of the keel. Only the ventral surface of the keel was palpated in this study. Pushing inward into the peritoneal cavity to palpate the
dorsal surface of the caudal tip of the keel was not used. The fracture was classified as a spine fracture (FR-SPINE) if there was a fracture present anywhere on the spine of the keel from > 5cm from the caudal tip to the Carina apex.

Keel bone deviations were also scored. A keel was considered to be deviated (DEV) if it did not follow a normal, straight 180° line in the sagittal, frontal or transverse anatomical plane. The presence of a DEV was a binomial score denoting only the presence or absence of a keel deviation as described by SKAP (Casey-Trott et al., 2015). In addition to the SKAP scoring method, the severity of the deviation was also scored for all 4 flocks. A mild deviation (DEV-MILD) was described as a deviation < 1cm from the normal, 180° line of the keel in any direction (sagittal, frontal or transverse). A severe deviation (DEV-SEV) was described as any deviation > 1cm from the 180° line, typically manifesting as severe indentation in the sagittal plane, a C- or S-shaped curve in the frontal plane, or a significant folding over of the keel in the transverse plane.

4.3.4 Statistical analyses

All statistical analyses were completed using SAS statistical software version 9.4 (SAS Institute, Cary, North Carolina). The level for assessment of statistical significance of differences between means was set at \( P < 0.05 \).

4.3.4.1 Analysis of age, rearing system, and adult housing system effects on keel bone damage

To assess the effect of age (30, 50, 70 wks), rearing system (Avi, Conv), and adult housing (FC-L, FC-S, CC) on keel bone damage, general linear mixed model analyses using the PROC MIXED command were performed with age, rearing system, adult housing system, flock (1,2,3,4), and 3 interactions (age*rearing, age*adult, rearing*adult) as fixed effects. A separate
model was built for each outcome (percentage of fractures, percentage of deviations). Since cage was considered the experimental unit, the percentage of fractures or deviations present within each cage were used in the analyses. Measurements from hens in each cage were repeated at 30, 50, and 70 wks of age, and thus age was a repeated measure within the analyses. The same model building process was used for both percentage of fractures and percentage of deviations. Although keel bone status was scored by palpation at 16 wks, this measurement was meant to serve as a baseline value and was not included in any of the statistical analyses because the percentage of both fractures and deviations were zero or nearly zero at this time point. All data were tested for normality and normality of residuals using the PROC UNIVARIATE command, and the data did not require a transformation.

Assessment of the location of fractures and the severity of deviations was limited to descriptive analyses.

4.3.4.2 Analysis of relationship between body weight and keel damage

Body weight (BW) was not included in the main analyses as it was not independent from age or adult housing system (Chapter 3). However, in order to assess the relationships between body weight and keel fractures or deviations, linear regression analyses (using the PROC REG command) were used on data from individual birds at each age (30, 50, 70 wks). All data were tested for normality and normality of residuals using the PROC UNIVARIATE command, and the data did not require a transformation.
4.3.4.3 Analysis of the association between keel fractures and deviations using the SKAP method

To test the level of association between keel bone fractures and deviations, a chi-square test using the PROC FREQ command was used. An odds ratio and relative risk option was used to determine the direction of the relationship between fractures and deviations. Separate analyses were conducted for each age (30, 50, 70 wks) to monitor changes in the relationship at different time points. Although palpation scoring was also completed at 16 wks, a chi-square test could not be conducted due to the presence of zero values; fractures were absent and very few deviations were present at 16 wks. The raw means for 16 wks are reported in Figure 4.1.

4.4 RESULTS

4.4.1 Effect of age, rearing system, and adult housing system on keel bone damage

Age had an effect on the percentage of fractures ($P < 0.001$; Table 4.1 and Figure 4.1A). The mean percentage of fracture at 16 wks was $0.04\% \pm 0.002$ SE. Rearing system also had an effect on the presence of fractures ($P < 0.001$; Table 4.1 and Figure 4.1A). Hens raised in the Avi system had an overall lower percentage of fractures ($41.6\% \pm 2.8$ SE) compared to hens reared in the Conv system ($60.3\% \pm 2.9$ SE). Adult housing system did not have an effect on the percentage of keel fractures ($P = 0.223$). Flock had an effect on the overall mean percentage of keel fractures ($P = 0.014$) occurring between 30 and 70 wks of age, with Flock 4 having a lower percentage ($42.1\% \pm 3.2$ SE) than Flock 1 ($51.4\% \pm 3.1$ SE), Flock 2 ($54.6\% \pm 3.2$ SE), and Flock 3 ($55.6\% \pm 3.2$ SE). No interaction effects were significant.

Of the keel bones with a fracture(s) present, the majority of fractures were located at the caudal tip of the keel at all ages: 30 wks: $76.9\% \pm 4.1$ SE; 50 wks: $89.1\% \pm 2.9$ SE; and 70 wks:
89.5% ± 3.0 SE. Fractures occurring on the spine of the keel were less common at all ages: 30 wks: 36.8% ± 4.7 SE; 50 wks: 26.1% ± 3.4 SE; and 70 wks: 31.0% ± 3.5 SE. Some keels had both a FR-TIP and a FR-SPINE present.

Age also had an effect on the percentage of deviations (\( P < 0.001 \); Table 4.1 and Figure 4.1B). The mean percentage of deviations at 16 wks was 0.2% ± 0.09 SE. Rearing system did not have an effect on the percentage of deviations (\( P = 0.218 \); Table 4.1 and Figure 4.1B). Adult housing system did not have an effect on the percentage of deviations (\( P = 0.539 \)). Flock also had an effect on the percentage of deviations (\( P = 0.010 \)), with Flock 1 (31.0% ± 3.4 SE) differing from Flock 2 (42.1% ± 3.3 SE) and Flock 3 (47.7% ± 3.3 SE), but not Flock 4 (38.8% ± 3.4 SE). No interaction effects were significant.

Of the keel bones with a deviation(s) present, the majority of deviations were mild at all ages: 30 wks: 76.3% ± 2.4 SE; 50 wks: 60.7% ± 2.4 SE; and 70 wks: 59.9% ± 2.3 SE. However, there was a gradual increase in severe deviations with age: 30 wks: 23.7% ± 1.8 SE; 50 wks: 39.2% ± 1.8 SE; and 70 wks: 40.1% ± 1.8 SE.

Egg production and mortality were recorded daily and will be reported elsewhere (Widowski, unpublished). All mortalities were sent for post mortem analysis and there were no outbreaks of disease, feather pecking, or cannibalism throughout the duration of the study.

4.4.2 Relationship between body weight and keel bone damage

The mean body weights were 1,380.9 g ± 19.8 SE at 16 wks, 1,926.5 g ± 17.9 SE at 30 wks, 2,040.6 g ± 17.9 SE at 50 wks, and 2,136.2 g ± 17.8 SE at 70 wks. At 30 wks of age, body weight had a minor yet significant positive relationship with both the presence of fractures (\( P = 0.002 \); Adj \( R^2 = 0.1115 \)) and deviations (\( P = 0.004 \); Adj \( R^2 = 0.0997 \)). There was no relationship
between body weight and fractures or deviations at either 50 (FR: \( P = 0.119 \); DEV: \( P = 0.533 \)) or 70 (FR: \( P = 0.751 \); DEV: \( P = 0.265 \)) wks of age.

### 4.4.3 Association between keel bone fractures and deviations using the SKAP method

Keel fractures and deviations were strongly associated at all ages: 30 wks: \( P < 0.001 \); 50 wks: \( P < 0.001 \); and 70 wks: \( P < 0.001 \). The absence of both keel fractures and deviations was greatest at 30 wks of age, steadily decreasing in favour of the occurrence of both fractures and deviations at 50 and 70 wks of age (Figure 4.2).

At 30 wks of age, 55.9% of keels had no fracture or deviation present, 17.9% had only a fracture present, 9.7% had only a deviation present, and 16.5% had both a fracture and a deviation present (Figure 4.2). The odds ratio indicated that hens with a deviation were 5.3 times more likely to have a fracture than hens without a deviation (95% CI = 3.5-8.1). Based on the relative risk, the likelihood of not having a fracture was higher in hens with a non-deviated keel than in hens with a deviated keel (relative risk (RR) = 2.0; 95% CI = 1.6-2.6). Similarly, the risk of having a fracture was lower in hens with a non-deviated keel than in hens with a deviated keel (RR = 0.38; 95% CI = 0.31-0.48).

At 50 wks of age, 33.1% of keels had no fracture or deviation present, 28.0% had only a fracture present, 12.0% had only a deviation present, and 26.9% had both a fracture and a deviation present (Figure 4.2). The odds ratio indicated that hens with a deviation were 2.6 times more likely to have a fracture than hens without a deviation (95% CI = 1.8-3.8). Based on the RR, the likelihood of not having a fracture was higher in hens with a non-deviated keel than in hens with a deviated keel (RR = 1.7; 95% CI = 1.4-2.2). Similarly, the risk of having a fracture
was lower in hens with a non-deviated keel than in hens with a deviated keel (RR = 0.66; 95% CI = 0.57-0.77).

At 70 wks of age, 24.9% of keels had no fracture or deviation present, 22.9% had only a fracture present, 11.6% had only a deviation present, and 40.6% had both a fracture and a deviation present (Figure 4.2). The odds ratio indicated that hens with a deviation were 3.8 times more likely to have a fracture than hens without a deviation (95% CI = 2.6-5.5). Based on the RR, the likelihood of not having a fracture was higher in hens with a non-deviated keel than in hens with a deviated keel (RR = 2.3; 95% CI = 1.8-3.0). Similarly, the risk of having a fracture was lower in hens with a non-deviated keel than in hens with a deviated keel (RR = 0.62; 95% CI = 0.54-0.71).

4.5 DISCUSSION

4.5.1 Effect of age, rearing system, and adult housing system on keel bone damage

This is the first experimental study to demonstrate that pullet rearing systems that allow for diverse load-bearing exercise effectively alter keel bone growth in a manner that reduces keel bone fractures in adult laying hens housed in both furnished and conventional cages. It is also one of the few longitudinal studies to track the prevalence of keel bone fractures and deviations in furnished and conventional cages over the laying period of a hen. Further, it is the first study to quantifiably identify an association between keel bone fractures and deviations at different age points.

The lower prevalence of keel bone fractures in adult hens that were housed in an aviary rearing system highlights the role that diverse opportunities for loading exercise, in the form of running, jumping, wing-flapping, and flight, have in the development of keel bones that are less
susceptible to future damage. Unlike previous studies, in which increased exercise during the adult phase had a positive effect on keel bone radiographic density, yet increased the risk of keel injuries from falls and collisions (Fleming et al, 2006), this study targeted the rearing phase; taking a preventative approach by stimulating improved keel bone growth during the period of the greatest opportunity for increasing peak bone mass, before the period with greatest risk of fracture during mid to late lay. In humans, exercise prior to sexual maturity improves peak bone mass and has a protective effect on bones reducing the prevalence of osteoporotic fractures later in life (Bass, 2000). In addition to improved bone characteristics, routine, impact exercise during adolescence in humans also reduces the risk of fractures later in life by improving muscle tone, strength, and balance (Schmitt et al 2009; Body et al., 2011). Although changes specific to bone mineral density or breaking strength of the keel were not directly assessed here, the lower prevalence of keel bone fractures reported might be a result of the opportunity for exercise in the aviary pullet rearing system, encouraging improved keel bone growth and more controlled navigation of the housing systems as adults.

Although current information regarding keel bone growth in highly selected commercial lines is not readily available, preliminary evidence that the keel is not entirely ossified at 16 wks of age (Chapter 3) agrees with previous research from the 1940’s regarding the slow growth of the keel (Buckner et al., 1949). As discussed in Chapter 3, the opportunity for exercise appears to have an effect on the overall growth and ossification of the keel at 16 wks. The keel bones of aviary-reared pullets were longer, had a greater area, and larger proportion of caudal cartilage than the conventionally-reared pullets at 16 wks of age. This suggests that exercise during rearing alters the growth of the keel bone, possibly by stimulating increased overall keel bone growth, or by slowing the progression of ossification. The detailed progression of keel bone
growth using radiographic analyses throughout the life of modern lines of commercial breeds of hens is an area of research that is yet to be quantified. Understanding when the keel is completely ossified has the potential to shed light onto periods when the keel is especially susceptible to fractures, either due to weak, newly calcified bone structure, or reduction in calcium allocation to the keel due to competition for calcium supply surrounding stages of peak lay or hormonal shifts.

Our prevalence estimate for fractures, which was approximately 52.8% for furnished cages and 47.0% for conventional cages, are within the ranges of previous studies, which reported a prevalence of 62% for furnished cages (Rodenburg et al., 2008) and 25-83% for conventional cages (Hester et al., 2013; Petrik et al., 2015). The lack of adult housing system effect parallels results in human medicine where exercise in adult women does not typically increase bone strength or bone mineral density; rather continued exercise in adulthood can help preserve benefits accrued during childhood and adolescence (Kontulainen et al., 1999; Kontulainen et al., 2001).

Alternatively, the similarity between the fracture and deviation prevalence of furnished and conventional cages reported here is supportive of the notion that furnished cages during the laying period provide an intermediate improvement to overall welfare by providing the benefit of added exercise and furnishings compared to conventional cages, without a dramatic increase in the risk of injuries as a result of the collisions and falls that are often reported in non-cage systems (Lay et al., 2011). Although the increased opportunity for exercise allowed by the FC-L or FC-S compared to the CC did not manifest in a significant difference in keel damage prevalence, the beneficial effect of opportunities for exercise during rearing appears to be preserved as there is not a dramatic increase in damage with the allocation of increased space for movement and furnishings, as indicated in previous studies (Fleming et al., 2006).
Although we initially hypothesize that the prevalence of keel damage would be lower in adult hens housed in conventional cages, it is possible that the lack of a notable difference in fracture and deviation prevalence between the hens from furnished and conventional adult housing may be due to an increase in the overall bone quality of the hens housed in furnished cages compared to the hens in CC, a result reported for the same population of birds from a concurrent study with the same treatment design (Chapter 3). The greater tibiae and radii bone density of the FC-L compared to the FC-S and CC, greater bone mineral content of the FC-L and FC-S compared to the CC, and the greater breaking strength of the tibiae from the FC-L and FC-S compared to the CC indicates that adult hens in the furnished cages from the same population and treatment design had improved quality of the long bones (Chapter 3). Improvement to the bone quality of the long bones has been shown to parallel improved keel bone quality (Hocking et al., 2003; Fleming et al., 2004), suggesting the keels in the current study likely mirror the positive results report in Chapter 3. Comparable values for keel bone status between housing adult hens in conventional versus furnished cages using the same systems as the current study, were also reported by Widowski (unpublished), with the conventional hens having slightly higher keel damage scores.

This is the first study to provide prevalence estimates of keel bone deviations, as a separate measure from keel bone fractures, for hens housed in furnished (41.0%) and conventional cages (37.7%). As expected, age had an effect on deviations, with a higher prevalence at each time point; however, the lack of rearing system effect on deviations was somewhat unexpected. The slightly higher (albeit not significantly higher) prevalence of deviations in the aviary-reared hens might be related to an increased use of perches since rearing with perches typically increases perch use in adult housing systems (Roll et al., 2008; Brantsaeter
et al., 2016). Although perching differences between the rearing system treatments were not quantified in this study, a concurrent study on the same hens showed that hens with keel bone fractures present at 70 wks of age spent a greater proportion of time on the perches than hens with minimal to no keel damage present (Chapter 5). Overall nighttime perching in these flocks was low (< 10%; unreported data). Alternatively, there might be a difference in the type of deviations incurred in different housing systems during the adult period that were not detected because only the presence or absence and severity of the deviations were assessed. Perhaps if the deviations were classified by the direction of deviation in each plane, differences may arise. The lack of adult housing system effects on deviations might also be attributed to the limited benefit of exercise in adulthood as discussed above.

Several studies have reported an increase in keel bone damage with increasing age (Weitzenburger et al., 2006; Scholz et al., 2008; Tarlton et al., 2013; Petrik et al., 2015; Stratmann et al., 2015a), which is in agreement with the results reported here. This pattern is likely attributable to several factors related to the skeletal growth and body composition. Considering that the ossification of the keel is not yet complete at the onset of lay (Chapter 3), it is not possible for fractures of the tip, the most common type of fracture reported here, to form as the caudal portion of the keel is still cartilaginous. Similar to other studies (Petrik et al., 2015; Stratmann et al., 2015a), fracture prevalence steadily increased approaching 30 wks of age and generally continued to rise until 70 wks of age. The rise in fractures at or just after 30 wks of age might be a result of the caudal tip of keel no longer in a cartilaginous state, yet still not completely ossified. This weak structure might be especially susceptible to “greenstick” fractures. Greenstick fractures are commonly found in growing children and typically manifest as incomplete, bending fractures on the concave surface of a bone, with complete separation of
the cortex on the convex bone surface (Hefti et al., 2007; Verhoji et al., 2012). It is possible that these incomplete greenstick fractures are a result of minor collisions with equipment or cage-mates, muscle contractions during wing movement or even increased muscle tension applied to the keel as the keel lowers ventrally to allow for egg production (Chapman, 1943). Understanding exactly how these incomplete fractures occur needs further study and it is of utmost importance as this type of fracture is the most commonly reported. Unfortunately, even though these greenstick fractures appear to be minor (Chapter 5), they are considered unstable and increase the risk for further fracture for several wks after the initial incident (Randsborg and Sivertsen, 2009). This might explain why multiple fractures of the tip are commonly seen by the end of lay, often increasing the severity of the damage by 70 wks of age.

Increasing body weight might also play a role in greater keel damage reported late in the hen’s life. Although hens housed in non-cage systems can be especially susceptible to keel damage related to body weight increases due to a greater requirement for wing-loading (Duncker, 2000), hens in cage systems are still susceptible to fractures related to heavier body weights (Petrik et al., 2015). In the present study, the slight, yet significant association between body weight and fractures and deviations at 30 wks of age might be related to increased pressure loads on the keel while resting on perches or the cage floor. It may also be an artifact of earlier onset of sexual maturity due to increased body mass, initiating early onset of lay, which has been shown to increase susceptibility to keel damage (Gebhardt-Henrich and Frohlich, 2015).

Flock variation also had an effect on the prevalence of fractures and deviations. The variation between flocks was an anticipated result as flock variation is commonly reported in commercial barns. The flock differences were not the main interest of the research study, and
therefore are not discussed in detail. Repeating the experiment on each flock was meant to account for flock differences and increase both the internal and external validity of the results.

4.5.2 Association between keel bone fractures and deviations using the SKAP method

The relationship between keel bone fractures and deviations is not yet fully understood, although an association between the two has been previously suggested (Scholz et al., 2009; Casey-Trott et al., 2015). Especially in systems where impact injuries are less likely, such as conventional or furnished cages, the relationship between keel bone fractures and deviations is likely stronger since a fracture resulting from a single, isolated impact is less common; whereas high impact injuries in a non-cage system can lead to severe keel fractures from a single event on an otherwise straight, non-deviated keel. The greater prevalence of both deviations and fractures with age, as reported here, supports the idea that keels become more susceptible to both forms of damage over time.

The strong association between keel fractures and deviations reported here, and the increased likelihood of non-deviated keels remaining free from fractures suggests that the relationship between the two may be related to the underlying bone physiology of the bird, or the bird’s behavioural activities. In furnished cages, perching is one behaviour that is a likely cause of keel bone fractures and deviations (Chapter 5) due to the long term pressure loading on the keel (Pickel et al., 2011). Softening perch material might be beneficial across a variety of housing system considering that Scholz et al. (2014) reported a reduced risk of failed landings with soft perches, and Stratmann et al. (2015b) demonstrated that softening perch material effectively reduced both deviations and fractures; a result that may be particularly useful to reducing keel damage in a furnished cage setting where prolonged perch use is a likely cause of
keel deviations. However, it is also possible that the relationship between deviations and fractures is a result of the underlying bone physiology. Genetic differences between high bone index, low bone index, and commercial lines, have repeatedly demonstrated that genetic selection impacts the bone mineral density of the keel (Bishop et al., 2000; Fleming et al., 2006). Recent work by Stratmann et al. (2016) demonstrated that genetic selection using these same lines also influenced the presence of both keel bone fractures and deviations. Genetic differences in keel bone composition and the prevalence of keel bone deformities in commercial strains have also been reported (Regmi et al., 2016a). Perhaps if deviations and fractures are so closely related, then selection for more efficient calcium mobilization or improved bone characteristics can reduce the occurrence of both deviations and fractures with the same mechanism.

It is widely accepted that keel bone damage is multi-factorial as it manifests itself in a variety of ways throughout all housing systems. As such, a multi-faceted approach is likely required to reduce the prevalence of keel bone damage. Perhaps coupling genetic selection for improved keel bone characteristics with the allowance for exercise during rearing, the period with the greatest potential to develop peak bone mass, may have an additive effect on the underlying bone physiology, stimulating bone growth in a manner that substantially improves skeletal structure. These improvements may be further extended by improvements to housing design, namely in the form of perch placement and pliability of perch material.
**Table 4.1** Main effects of age, rearing system, and adult housing system on the percentages of keel bone fractures and deviations. Within a column, means without a common superscript differ (P < 0.05).

<table>
<thead>
<tr>
<th>Age (wks)</th>
<th>Fractures (± SE)</th>
<th>Deviations (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>35.2 (2.5)</td>
<td>28.1 (2.6)</td>
</tr>
<tr>
<td>50</td>
<td>55.2 (2.8)</td>
<td>40.0 (2.6)</td>
</tr>
<tr>
<td>70</td>
<td>62.4 (2.6)</td>
<td>51.6 (2.6)</td>
</tr>
</tbody>
</table>

DF = 136
F-Value = 22.89
P-Value < 0.001

<table>
<thead>
<tr>
<th>Rearing¹</th>
<th>Fractures (± SE)</th>
<th>Deviations (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conv</td>
<td>60.3 (2.9)</td>
<td>37.8 (2.4)</td>
</tr>
<tr>
<td>Avi</td>
<td>41.5 (2.8)</td>
<td>42.1 (2.3)</td>
</tr>
</tbody>
</table>

DF = 63
F-Value = 35.39
P-Value < 0.001

<table>
<thead>
<tr>
<th>Adult²</th>
<th>Fractures (± SE)</th>
<th>Deviations (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>47.0 (2.7)</td>
<td>37.7 (2.9)</td>
</tr>
<tr>
<td>FC-L</td>
<td>53.2 (2.7)</td>
<td>39.7 (2.7)</td>
</tr>
<tr>
<td>FC-S</td>
<td>52.5 (2.5)</td>
<td>42.4 (2.9)</td>
</tr>
</tbody>
</table>

DF = 63
F-Value = 1.54
P-Value 0.223

¹ Pullets housed in a conventional rearing system (Conv) or an aviary rearing system (Avi) until 16 weeks of age Flock 1-4
² Adult hens placed into conventional cages (CC), furnished cage- large (FC-L), or furnished cage-small (FC-S) from 16-73 weeks of age Flock 1-4.
Figure 4.1 Effect of age and rearing system on the percentage of keel bone fractures (A) and deviations (B). Age had an effect on fracture ($P < 0.001$) and deviation ($P < 0.001$) prevalence. Rearing system (aviary rearing: (Avi) vs. standard rearing: (Conv)) had an effect on fracture prevalence ($P < 0.001$) but not on the prevalence of deviations ($P = 0.218$).
Figure 4.2 Distribution of keel bone fracture (FR) and deviation (DEV) prevalence at different ages during the laying period as scored by the simplified keel assessment protocol (SKAP) method. For each age, a significant association between the presence of a fracture(s) and the presence of a deviation(s) ($P < 0.001$) is denoted by an * as determined by a chi-square test. The data from wk 16 was not analyzed as the majority of keels (99.3%) did not have a fracture or deviation.
CHAPTER 5

Behavioural differences of laying hens with fractured keel bones within furnished cages

5.1 ABSTRACT

High prevalence of keel bone fractures in laying hens is reported in all housing systems. Keel fractures have been associated with pain and restricted mobility in hens in loose housing. The objective was to determine whether keel fractures were associated with activity of hens in furnished cages. Thirty-six pairs of LSL Lite hens (72 wk) were enrolled in the study. One hen with a fractured keel and one hen without were identified by palpation in each of 36 groups of hens housed in either 30- or 60-bird cages (696 cm²/hen and 688 cm²/hen, respectively). Four observers, blind to keel status, used focal animal sampling for 10 min to record the behavioural activity of individual hens within a 2 hr period in the morning (08:30-10:30), afternoon (12:30-14:30), and evening (17:00-19:00). All hens were observed during each of the three sample periods for 3 days totaling 90 min, and individual hen data were summed for analysis. Hens were euthanized 48 hr after final observations, dissected, and classified by keel status: F0 (no fracture, n = 24); F1 (single fracture, n = 17); F2 (multiple fractures, n = 31). The percentages of time hens performed each behaviour were analyzed using in SAS 9.4 (PROC MIXED command) with fracture severity, body weight, cage size, rearing system, and tier in each model. Fracture severity affected the duration of perching ($P = 0.04$) and standing ($P = 0.001$), bout length of

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standing ($P < 0.0001$), and location (floor vs. perch) of resting behaviours ($P = 0.01$). F2 hens perched longer ($20.0\% \pm 2.9$ SE) than F0 hens ($11.6\% \pm 3.2$ SE). F2 hens spent less time standing ($15.2\% \pm 1.5$ SE) than F0 ($20.7\% \pm 1.6$ SE) and F1 hens ($21.6\% \pm 1.8$ SE). F2 hens had shorter standing bouts ($22.0$ sec $\pm 4.2$ SE) than both F0 ($33.1$ sec $\pm 4.3$ SE) and F1 ($27.4$ sec $\pm 4.4$ SE) hens. Non-fractured hens spent $80.0\% \pm 6.9$ SE of total resting time on the floor whereas F1 and F2 hens spent $56.9\% \pm 12.4$ and $51.5\% \pm 7.7$, respectively, resting on the floor. Behavioural differences reported here provide insight into possible causes of keel damage, or alternatively, indicate a coping strategy used to offset pain or restricted mobility caused by keel fractures.

5.2 INTRODUCTION

The keel bone is known to be a site of frequent fractures during the production life of laying hens with flock-level prevalence ranging from 5% to over 85% (Fleming et al., 2004; Rodenburg et al., 2008; Wilkins et al., 2011; Petrik et al., 2015; Riber and Hinrichsen, 2016). Although the flock-level prevalence is typically lowest in conventional cages (Petrik et al., 2015), birds in all types of housing systems are susceptible to keel fractures. The high occurrence of these fractures is alarming, as the welfare of the bird is potentially compromised by this reportedly painful condition (Nasr et al., 2012b).

Observing behaviour provides insight into the internal state of an animal, and behavioural measurements have been used to assess pain in animals (Zimmermann, 1986), including poultry (Duncan et al., 1991; Hocking et al., 1997; Caplen et al., 2014). Understanding the behavioural changes typically associated with pain provide an opportunity for researchers, producers, and welfare auditors to assess the current state of an animal within its given housing situation. In
birds, evidence of decreased spontaneous activity (Duncan et al., 1991), reduced mobility (Caplen et al., 2014), and reduced latency-to-lie (Weeks et al., 2002; Caplen et al., 2014) are reported in association with lameness, and standing and sitting behaviours are influenced by pain as levels of these activities were improved or restored with analgesic treatments (Hocking et al., 1997; Hocking et al., 2001).

In addition, understanding where inactive behaviours occur within the housing system also provides useful information regarding resting and coping strategies. Broiler chickens adjust their sleeping location to reduce disturbances by resting near walls when housed at high densities (Buijs et al., 2010) and similar strategies are used by laying hens by resting on perches to reduce disturbances (Olsson and Keeling, 2000) and social interactions (Cordiner and Savory, 2001). In relation to the keel bone in particular, resting location has the potential to have a significant impact. Provision of perches has been shown to increase keel deformities and fractures (Hester et al., 2013) and even the perch material itself can affect the prevalence and severity of keel damage (Pickel et al., 2011; Stratmann et al., 2015b). Where the hens rest within the cage may provide insight into the coping strategy of hens with keel damage or offer information on how the keel damage occurred in the first place.

The keel bone in particular is a critical bone to the avian skeleton as it is a structure responsible for the ability of flight in birds and plays an important role in driving the respiratory process in avian species (Duncker, 2000; Codd et al., 2005; Claessens, 2009). The keel serves as an anchor for attachment of flight muscles necessary for wing flapping and short bursts of flight required by fowl species to reach roosting sites and for escape strategies to avoid predators (Duncker, 2000). Abdominal muscles also attach to the keel allowing for ventral and dorsal oscillations of the keel to aid in the filling and emptying of the air sacs during inhalation and
exhalation in birds (Codd et al., 2005; Claessens et al., 2009). This mechanism is essential for adequate ventilation of the lungs as birds lack a muscular diaphragm and respiratory processes of birds operate with the lung volume remaining static in contrast to the dramatic lung volume changes required for mammalian respiration (Duncker, 2000).

Since the keel bone plays an important role in avian behaviours such as flight and wing flapping, as well as vital processes such as respiration, it is possible that damage to the keel bone limits daily functioning of the bird and subsequently alters behaviour and activity levels throughout the day. Nasr et al. (2012a) reported that daily activity of hens group housed in floor pens was altered by the presence of keel fractures. They (Nasr et al.) reported that hens with keel fractures spent more time sleeping on the floor and less frequently accessed perches of various heights compared to hens without keel fractures. Hens without keel fractures completed walkway tests faster and had a shorter latency to fly down from perches compared to hens with keel fractures (Nasr et al., 2012a,b). While these tests provide evidence that hens with keel fractures have altered mobility, they offer minimal insight into the behavioural differences of hens with keel damage in a furnished cage system using commercially available equipment and industry standard stocking density.

The objective of this study was to quantify general behaviour differences between hens with fractured keels and hens without fractured keels, housed in furnished cages. Determining where injured birds spend their time resting within the cage as well as which behaviours may be inhibited or amplified can provide information on how to single out hens with keel injuries and has the potential to add to the discussion of the causes, consequences, and welfare implications of how keel damage occurs in different types of housing systems. We hypothesized that behaviour would differ between hens with or without keel bone damage, especially in regards to
inactivity, namely sitting, standing, and sleeping, and that hens with keel damage would likely spend more time perching.

5.3 METHODS

5.3.1 Animal Housing

Animal use was approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #1947). Three consecutive flocks of 588 Lohmann-selected Leghorn-Lite (LSL-Lite) laying hens were reared from 1 day-of-age to 73 wks of age at the University of Guelph Arkell Poultry Research Station. As part of a concurrent experiment, half of the hens were reared in conventional cages (N=294) and half were reared in an aviary system (N=294). Rearing details described in Chapters 3 and 4. At 16 wks of age, hens from both rearing systems were placed into two rooms holding 12 Farmer Automatic Enrichable (Furnished) Cages (Clark Ag Systems, Ontario, Canada) each, with one rearing treatment placed into each cage. Each room contained six large (41,296 cm²; 60 hens) and six small (20,880 cm²; 30 hens) cages, providing a space allowance of 696 cm²/hen and 688 cm²/hen, respectively. Placement of rearing treatments was balanced for cage size. Each bank of six cages had three tiers with one large and one small cage on each tier. The same rearing and adult rooms were used for each consecutive flock.

Hens were fed a commercial layer crumbled pellet diet with automatic feed chains running every three hours commencing at the start of a 14 hr light period from 05:00-19:00 with a 15-min sunrise and sunset starting at 05:00 and 18:45, respectively. The light intensity varied among tiers, with the highest intensity recorded on the top tiers measuring 10-15 lux and the lowest intensity at the bottom tiers measuring 4-5 lux. Each furnished cage provided a curtained
nest area proportional to cage size (94 cm$^2$/hen), 10 cm high perches (15 cm$^2$/hen) running parallel to the cage front throughout the middle area, and a smooth plastic scratch area (large: 42 cm$^2$/hen; small: 83 cm$^2$/hen). Nipple drinkers with cups were located above the feed auger down the middle of the cage. The feed troughs (12 cm/hen) were located on both outer sides of the cages. All rooms were sealed and entirely lit with artificial light with no natural, external light sources present. Beak trimming was performed at the hatchery at 1 day-of-age using infrared treatment.

5.3.2 Treatment Selection

A set of twelve pairs of LSL-Lite hens were selected for inclusion in this study at 72 wks of age from each of three consecutive flocks for a total of 72 hens. Selection for enrollment in the study was based on the keel status of each individual bird determined by palpation by the trained, lead observer (Casey-Trott; training details described in Chapter 4). Lights were dimmed for ease of handling and hens were caught conveniently from various locations within each furnished cage until one hen with a normal, non-fractured keel and one hen with a fractured keel was found. A keel was considered non-fractured if it followed a normal, straight 180° line without the presence of any sharp bends or periosteal scars or callus indicative of a healing fracture. A keel was classified as fractured if there was the presence of a sharp bend or deviation from the 180° line accompanied by one or more than one periosteal scar or callus (Casey-Trott et al., 2015). Three days prior to the observation period, each fractured and non-fractured hen was weighed, marked with a colored, numbered livestock ID tag (Allflex, Québec, Canada), with one tag placed in each wing web with an ATag One Piece Applicator (Allflex, Québec, Canada) to allow for visual identification within the cage. Only hens free from moderate to severe foot damage were included in the treatment selection process.
5.3.3 Behavioural Observations

Four trained observers, blinded to keel status, recorded behavioural activity of individual hens following a specified ethogram (Table 5.1) with the number of observations for each observer balanced between rooms, cages, and keel status category. Reliability among all four observers was determined by using Kendall’s Coefficient of Concordance (W) to rank the behaviours recorded by each observer. Based on the initial palpation score, observers were balanced across the keel status category (fractured vs non-fractured), and following dissection at the end of the trial, the balance of observers across keel status category was confirmed. Agreement among observers was acceptable ($W = 0.725; \chi^2 = 11.6; P = 0.0206$). According to Martin and Bateson (2007), inter-observer reliability scores above 0.70 are considered to be acceptable, especially when multiple observers are used. The percentage of total observations for each observer of hens in the F0 category was 34.5-36.7%, the F1 category was 25.1-30.3%, and the F2 category was 32.9-40.2% as confirmed at dissection.

Each hen was continuously recorded using a handheld machine (Psion Workabout Pro3, Motorola Solutions, Illinois, USA) to collect data using Pocket Observer software (Observer XT, Noldus Information Technology, Wageningen, The Netherlands) for a focal animal sampling period of 10 min within a sample period of 2 hr in the morning (08:30-10:30), afternoon (12:30-14:30), and evening (17:00-19:00) repeated for 3 days. Nighttime resting location could not be recorded since the majority of hens huddled in a group on the floor of the cage and the individual hen ID tags were not visible. To reduce the confounding effect of behaviours related to nesting, observations began after the completion of the peak morning laying period for each flock as determined by research previously completed on site (Hunniford et al., 2014).
The time of observation for each cage was shifted everyday by 40 min within the 2 hr period to ensure observation of each hen occurred at a different time point within the 2 hr period on all three days. The duration of general activities (forage, eat, drink, preen, sit, stand, walk, sleep, dust bathe, perch) described by the ethogram were recorded and the data for each hen over all three days was summed for analysis. All behaviours, except perching, were recorded as mutually exclusive state behaviours in that initiation of one behaviour terminated the recording of the previous behaviour. Location (nest box, cage middle, scratch area, cage front) was recorded simultaneously for all behaviours. Occurrence of perching was recorded in conjunction with any behaviours occurring while the hen was located on the perch allowing for a description of specific activities occurring on the perches.

5.3.4 Keel bone status classification by dissection

Within 48 hours following the final observations, hens were euthanized by cervical dislocation, dissected, and re-classified by keel bone status at dissection by the lead researcher (Casey-Trott): F0 (no fracture and no deviation from 180°, n = 24); F1 (single, “greenstick” fracture at the caudal tip of the keel without any deviation from 180°, n = 17); F2 (multiple fractures (including at least one complete fracture) with deviation from 180°, n = 31).

Egg production and mortality were recorded daily and will be reported elsewhere (Widowski, unpublished). All mortalities were sent for post mortem analysis and there were no outbreaks of disease, feather pecking, or cannibalism throughout the duration of the study.
5.3.5 Statistical Analyses

All statistical analyses were completed using SAS statistical software version 9.4 (SAS Institute, Cary, North Carolina). The level for assessment of statistical significance of differences between means was set at $P < 0.05$.

For all statistical procedures, only the true damage status of the keel, as determined by dissection, was used in the analyses. Although the sensitivity and specificity of the identification of keel status by palpation is moderate to high (Wilkins et al., 2011; Petrik et al., 2013; Casey-Trott et al., 2015) reclassification of keel status at dissection is commonly used in keel bone research (Nasr et al., 2012a,b; 2013b) to ensure accurate treatment allocation.

5.3.5.1 Analysis of general activity

The total duration of each behaviour within the ethogram for each individual hen was summed and used to create a proportion of total time out of the 90 min that each hen was observed. Each behaviour was assessed in a separate general linear mixed model analysis (PROC MIXED command) with fracture severity (F0, F1, F2), body weight, rearing system (conventional cage, aviary), cage size (large, small), and tier (top, middle, bottom) in the model as fixed effects. Flock number and room were included as random effects to account for any flock and room variation. Interactions of fracture severity and all other variables were initially included in the model, but were subsequently removed due to lack of significance. All data were tested for normality and normality of residuals and the proportion of sleeping behaviour required arc sin square-root transformation.

The mean bout lengths and bout frequencies for sitting, standing, and sleeping were also analyzed. A general linear mixed model analysis (PROC MIXED command) was used for each
outcome with fracture severity (F0, F1, F2), body weight, rearing system (conventional cage, aviary), cage size (large, small), and tier (top, middle, bottom) in the model as fixed effects. Flock number and room were included as random effects to account for any flock or room variation. Interactions were initially included in the model, but were subsequently removed due to lack of significance. Square-root transformation was used to achieve normality for the bout length and bout frequency for sleep.

5.3.5.2 Analysis of resting behaviour location

With the intention of assessing differences in resting location between hens with or without keel damage, only hens that exhibited resting behaviour could be assigned a resting location and subsequently be included in the analysis of resting location. A category of resting behaviour was created by combining the duration of all sitting and sleeping behaviour for an individual hen. In an effort to include hens expressing prolonged resting behaviour, and not bias resting location with hens initiating another behaviour (i.e., dustbathing), only hens that rested for at least 10% of their total observation period, with resting bouts occurring within a minimum of three of the nine 10-min observation periods were included in this data set. A total of 48 hens met this criteria (N= F0:16, F1:9, F2:23). The proportion of time resting on either the perch or the floor of the cage was then assessed in a general linear mixed model analysis (PROC MIXED command) with fracture severity (F0, F1, F2), body weight, rearing system (conventional cage, aviary), cage size (large, small), and tier (top, middle, bottom) in the model as fixed effects. Flock number and room were included as random effects to account for any flock or room variation. Interactions between fracture severity and all other variables were initially included in the model, but were subsequently removed due to lack of significance. The proportion of time
resting on the perch and the proportion of time resting on the floor were transformed using arc
sin square-root.

5.4 RESULTS

5.4.1 General activity

Keel fracture severity had an effect on the percentage of time perching ($F_{2,61}=3.30$, $P = 0.0436$; Table 5.2). Hens with a keel status of F2 perched for a greater percentage of time (20.0% ± 2.9 SE) than both F0 and F1 hens, 11.6% ± 3.2SE and 13.9% ± 3.6 SE, respectively. Rearing system ($F_{1,61}=4.26$, $P = 0.0432$) and cage size ($F_{1,61}=5.34$ $P = 0.0243$) also affected perching. Aviary reared hens spent more time on the perches (18.2% ± 2.8 SE) than conventionally reared hens (12.7% ± 2.8 SE). Hens in small cages spent more time perching (18.6% ± 2.8 SE) compared to hens in large cages (11.7% ± 2.9 SE). There was no effect of body weight ($F_{1,61}=0.43$, $P = 0.5130$), or tier ($F_{2,61}=1.14$, $P = 0.3256$) on the percentage of time perching.

Keel fracture severity also had an effect on the percentage of time standing ($F_{2,61}=7.65$, $P = 0.0011$; Table 5.2). Hens with an F2 keel spent less time standing (15.2% ± 1.5 SE) than both F0 and F1 hens, 20.7% ± 1.6 SE and 21.6% ± 1.8 SE, respectively. There was no effect of body weight ($F_{1,61}=1.45$, $P = 0.2329$), rearing system ($F_{1,61}=2.46$, $P = 0.1217$), cage size ($F_{1,61}=1.93$, $P = 0.1700$), or tier ($F_{2,61}=0.43$, $P = 0.6524$) on the percentage of time standing. The effect of keel fracture severity on all other behaviours can be found in Table 5.2.

Keel fracture severity had an effect on the mean bout length of standing ($F_{2,61}=11.88$, $P < 0.0001$; Figure 5.1) with F2 hens standing for shorter bouts (22.0 sec ± 4.2 SE) than both F1 (27.4 sec ± 4.4 SE) and F0 (33.1 sec ± 4.3 SE) hens. Cage size also affected standing bout ($F_{1,61}=8.09$, $P = 0.0060$) with hens in small cages exhibiting a longer bout length (30.4 sec ± 4.2
SE) compared to hens in large cages (24.7 sec ± 4.2 SE). Keel fracture severity did not affect bout length for sitting ($F_{2,61}=0.46, P = 0.6347$) or sleeping ($F_{2,55}=1.23, P = 0.3012$).

The effect of fracture severity on the frequency of sitting bouts approached significance ($F_{2,61}=2.84, P = 0.0659$) with F2 hens having a mean bout frequency of 10.5 sitting bouts ± 1.2 SE compared to F1 (8.2 sitting bouts ± 1.5 SE) and F0 (7.4 sitting bouts ± 1.3 SE). The number of bouts for standing and sleeping were not significant, $F_{2,61}=2.27, P = 0.1121$ and $F_{2,55}=2.23, P = 0.1165$, respectively.

Body weight had an inverse relationship with the percentage of time foraging ($F_{1,61}=5.89, P = 0.0182$) and walking ($F_{1,61}=10.76, P =0.0017$); however, body weight accounted for only a small degree of variation based on the low $R^2$ values (0.0729 and 0.1619, respectively). Tier also had an effect on the percentage of time foraging ($F_{2,61}=3.51, P = 0.0359$) with hens on the top tier spending more time foraging (10.6% ± 2.7 SE) compared to the bottom tier (6.4% ± 2.6 SE). The middle tier did not differ from either the top or bottom tier in terms of time foraging (7.2% ± 2.6 SE).

5.4.2 Resting behaviour location

Fracture severity and cage size had an effect on the location of resting behaviour. The percentage of time resting on the floor of the cage was significantly greater for F0 hens (80.0% ± 6.9 SE) compared to F1 (56.9% ± 12.4 SE) and F2 hens (51.5% ± 7.7 SE; Table 5.3. $F_{2,37}=4.63$, $P = 0.0161$) and subsequently F1 and F2 hens spent a greater percentage of time resting on perches compared to F0 hens (Table 5.3). A larger percentage of hens in large cages rested on the floor of the cage (79.5% ± 8.7 SE) compared to hens in small cages (47.5% ± 8.4 SE;
and consequently small cages had a larger percentage of hens resting on the perches than hens in large cages.

5.5 DISCUSSION

The primary objective of this study was to assess whether hens with keel bone damage behaved differently or rested in different locations than hens without keel bone damage, and it is the first to consider this relationship within a commercially available, furnished cage setting. Although we hypothesized that differences in all inactive behaviours would be associated with keel status, only standing behaviour was significantly different between fractured and non-fractured hens. Differences in sitting and sleeping behaviours were not identified. Our prediction that hens with keel fractures would spend more time perching and resting on the perches than hens without keel damage was supported by our data. This prediction was based on several studies that suggest the perches may cause keel bone damage (Abrahamsson and Tauson, 1993; Tauson and Abrahamsson, 1994; Abrahamsson et al., 1996; Wilkins et al., 2004; Wilkins et al., 2011; Hester et al., 2013).

In contrast to Nasr et al. (2012a) who reported that hens with keel fractures spent more time sleeping on the floor and less time up on the perches, these results indicate the opposite. This is likely due to the difference in housing design (floor pen vs furnished cage) which allows for dramatically different perch heights. Unlike the perches used by Nasr et al. (2012a,b) which ranged from 50 cm to 150 cm off the ground, the furnished cage perch is only 10 cm off the cage floor and requires no flight or jumping for access. As it is suggested that keel fractures are painful and restrict mobility, especially in regards to flight, the theory that pain or restricted flight mobility deters the hen from perching applies to Nasr et al. (2012a,b) and likely other non-
cage, perch systems; however, it is not applicable to the furnished cage. The differences in results reported here in comparison to Nasr et al. (2012a, b) highlight the importance of considering how housing environments can alter the expression of pain behaviour.

The current study details behavioural differences specific to hens housed in furnished cage systems. The significant differences in perch use and standing behaviour among hens with varying degrees of keel bone damage provides evidence of a potential causal link between certain behaviours that may leave the hen more susceptible to keel damage, or alternatively offers insight into pain- or physiologic function-related changes that result from damage to the keel bone.

Several authors have suggested that although perches satisfy a motivated behaviour, they may actually cause more keel bone damage (Abrahamsson and Tauson, 1993; Wilkins et al., 2004; Sandilands et al., 2009; Wilkins et al., 2011; Hester et al., 2013), most often through crashes or falls. However, even in the relatively low-impact environment provided by furnished cages, there is still a relationship between perches and keel damage. Although impact injuries are most often discussed regarding perches and keel damage, the type of perch material, shape, peak force, and contact area between the keel and the perch surface have also been shown to influence keel bone deformations (Pickel et al., 2011). Keel damage in other low-impact systems, namely conventional cages equipped with perches, also reported increased keel damage with the perch provision (Hester et al., 2013). The greater amount of perch use in fractured hens reported in this study likely parallels this causal hypothesis that increased exposure to continuous loading pressure on the keel causes damage. This is further supported by the choice of hens with intact keels to rest on the floor of the cage more often than on the perch while fractured hens spent significantly more time resting on perches, with their keel in contact with the perch surface, than
their intact counterparts. The long term pressure of the perch on the keel during sitting or sleeping behaviour may be causing the deformation and fractures. Although this theory of perch use causing fractures in furnished cages correlates with previous research, within this current study it is impossible to definitively support this causal hypothesis since hen selection criteria was based on palpation for keel status and perch use behaviour prior to keel damage is unknown.

Alternatively, it is possible that this difference in perching behaviour could be a coping strategy to use the perch as a support structure either to relieve strain on the keel by relaxing the attached abdominal muscles or to reduce the involvement of the keel in respiration (Codd et al., 2005). This concept also applies to standing behaviour, which requires substantial oscillatory movement of the keel during respiration in a standing position (Codd et al., 2005) and increases the strain and pressure on the keel due to the added gravitational weight of the visceral organs.

In domestic fowl, Hocking et al. (1997; 2001) reported that sitting and standing behaviours are influenced by both naturally-induced pain as well as pharmacologically-induced pain models and the behaviours are subsequently restored with administration of analgesics and anti-inflammatory steroids. These behavioural changes are suggested to be an attempt to reduce weight bearing on the injured limb or relieve pressure on the spine. Not only this, but standing is costly, requiring a 40-45% increase in metabolic effort (Deighton and Hutchinson, 1940) and causing 20-40% greater heat loss (DeShazer et al., 1970) compared to sitting. Although the keel bone is not a load bearing bone for standing, it is a bone that is subjected to the gravitational weight of the internal organs. When the keel is resting on a supportive surface, the degree of involvement of the attached abdominal muscles to support the visceral weight is decreased. Therefore, it is possible that in a strategy similar to reducing load bearing on painful limbs, the reduction of standing behaviour may be a mechanism of pain relief for hens with fractured keels.
by limiting the time spent in a metabolically costly position where the visceral weight adds to the strain on the keel induced by the activation of the external oblique. This hypothesis requires further research involving the administration of analgesics.

In a similar manner, this increase in perching and reduction in standing behaviour may be an attempt to alter physiologic function in relation to respiration. The keel plays a vital role in driving the respiratory process; however, its involvement and displacement during inhalation and exhalation changes when the hen is in a seated versus standing position. When the hen is seated in a resting position with the keel flush against a surface such as the floor or perch, the oscillatory movement of the keel is restricted and subsequently the involvement of the ribs becomes more substantial (Codd et al., 2005; Claessens et al., 2009). When the keel is restricted, the appendicocostalis drives the expansion of the thoracic cavity by flaring the ribs laterally, whereas when standing, the keel is allowed full oscillatory movement with the external oblique primarily responsible for pulling the keel dorsally during the exhalation (Codd et al., 2005). When the keel is fractured, the hen could be subjected to reduced flexibility or mobility of the keel and its muscle attachments forcing the involvement of alternative respiratory strategies, or the hen could be intentionally reducing the involvement of the keel in respiration by reducing standing behaviour and increasing the time spent supporting the keel on the perch. Since there was no significant difference in sitting duration in regard to fracture severity, it cannot be assumed that the hens are directly substituting standing behaviour with sitting; however, the reduction in stationary standing suggests that aspects of this position are possibly uncomfortable or less efficient. This hypothesis is supported by the shorter bout duration for standing and the increased number of sitting bouts expressed by hens with keel fractures. Perhaps the desire to rest is present, but discomfort or metabolic inefficiency induces a more restless expression of
behaviour. This hypothesis is supported by the results reported in Chapter 6, where the hens with keel bone fractures had a decreased level of stationary, inactivity compared to hens without keel bone fractures as determined by an accelerometer.

The proportion of the behaviours reported here are similar to previous descriptions of general activities including dustbathing (Klein et al., 2000; Scholz et al., 2011), preening (Dawkins, 1989; Webster and Hurnik, 1990; Klein et al., 2000), sitting (Dawkins, 1989), walking, resting (Klein et al., 2000), sleeping, eating, and drinking (Webster and Hurnik, 1990). Foraging was noticeably lower in the current study compared to previous reports in red jungle fowl (Dawkins, 1989) and LSL hens (Klein et al., 2000); however, this is likely due to genetic differences between red jungle fowl and modern lines in terms of the degree of time budget devotion to energetically costly activities (Schutz and Jensen, 1998; Schutz et al., 2001) as well as the fact that the hens in the current study were housed in cages where litter substrate was not provided. It is also possible that the classification of eating and foraging in the ethogram used here led to mixing of the two behaviours since LSL hens are known to spend more time feeding and foraging in the feed trough rather than scratching at the floor (Klein et al., 2000). The differences in the percentage of time standing reported here is likely a consequence of housing. Caged hens spend a large portion of their time standing, approximately 70-75% of their daily time budget (Webster and Hurnik, 1990), whereas hens in natural environments and floor pens spent less the time standing too, as low as 5% in red jungle fowl (Dawkins, 1989) and 10-12% in LSL hens (Klein et al., 2000). Furnished cages likely lie in between conventional cages and floor pens in terms of the range of expression of behaviours as they offer opportunities for a greater diversity of activities than conventional cages, yet, the complexity of the environment and overall space allowance is typically lower in furnished cages than in natural or floor pens.
Although not of primary concern, the effects of several covariates on behaviour add to the discussion and provide evidence that the focal sampling technique used here adequately captured behavioural patterns. The effect of rearing system and cage size on perch use reported here are likely related to different learned behaviours and use of space. Chicks and pullets offered perches early in life have been shown to maintain different spatial usage later in life compared to pullets without early perch access (Gunnarsson et al., 2000; Brantsaeter et al., 2016; Nicol, 2015). In addition, even though the individual space allowance in both the large and small cages were equal, the overall increased space to navigate and larger nest area in large cages has the potential to alter the movement and flow within the cage design. The inverse relationship between body weight and the proportion of both foraging and walking behaviour is not surprising, and neither is the increased foraging on the top tier which was noticeably brighter due to its closer proximity to the light source compared to the middle and lower tiers.

One final question to address is why hens with minor fractures more closely resemble the behavioural description of hens without keel fractures than hens with severe keel damage. The lack of behavioural difference between F0 and F1 hens with fractures at the caudal tip begins to address the question of whether or not this minor damage to the keel is negatively impacting the hen. From the results presented here, it appears that minor caudal tip fractures do not significantly impact the daily activity of the hen in the same way that keels with large deviations and multiple fractures sites do. This may be in large part due to the type of fracture occurring in each case.

Unlike the complete, displaced fractures seen in severely fractured keels, classified by complete separation of the bone and displacement from its original position, the minor caudal tip fractures appear to more closely resemble a “greenstick” fracture. Greenstick fractures are
characterized by an incomplete fracture on the concave side of a bone and a complete separation on the cortex on the convex region of the bone (Hefti et al., 2007; Vernooji et al., 2012). The minor caudal tip fractures reported in the current study followed this description with a small callus indicative of a healing fracture on the convex tip of the keel and evidence of bending on the concave portion of the caudal tip. In a previous report using an LSL-Lite flock raised at the same research station as the current study, this type of fracture was reported to be responsible for 64% of the total fractures present (Casey-Trott et al., 2015). It is possible that this minor fracture, although still potentially painful, does not disrupt the periosteal nociceptors enough to dramatically alter behaviour.

One concern with greenstick fractures is that they are considered unstable and are at risk of further displacement for several wks after the initial injury (Randsborg and Sivertsen, 2009). Even though the F1 hens spent a lower total proportion of time on the perches than F2 hens, almost half of their time on the perches was for resting, closely resembling the F2 group. While this mixed result is possibly related to a small sample size and larger standard deviation for the F1 group in terms of resting location, it could also be relating back to a causal relationship between perch use and fractures suggesting hens with greenstick fractures are at risk for more extensive, fracture displacement injuries in the future as a result of their time spent resting on the perches.

Since the primary goal of this study was to assess behavioural expression and resting location in relation to keel bone damage in furnished cages, it is now possible for further studies to be designed to quantify the behavioural differences reported here in more detail and potentially assess changes to these behaviours with administration of analgesics.
CONCLUSIONS AND ANIMAL WELFARE IMPLICATIONS

Keel bone damage is a welfare concern in all housing systems. Understanding how keel bone fractures are caused by certain behaviours or how they subsequently alter behaviour can provide valuable information for appropriate housing design, genetic selection traits, and identification of affected hens in commercial settings. In addition to further research into pain related to keel bone damage, identifying physiologic and metabolic effects of severe keel bone damage will add to the scope of understanding of this prevalent welfare concern.
**Table 5.1 Ethogram used for behaviour observations**

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forage</td>
<td>Pecking or scratching at the floor of the cage with head below rump (adapted from Klein et al., 2000).</td>
</tr>
<tr>
<td>Eat</td>
<td>Head in the feed trough or completely through the cage over the feeder. Can include standing breaks of ( \leq 5 ) sec followed by resumption of behaviour.</td>
</tr>
<tr>
<td>Drink</td>
<td>Repeated pecks at nipple drinker followed by swallowing. Can include standing breaks of ( \leq 5 ) sec, with beak still within the plane of the drinker, followed by resumption of drinking behaviour.</td>
</tr>
<tr>
<td>Preen</td>
<td>A hen uses her beak to clean wing and body feathers. Related behaviours include head scratching, wing stretching, feather ruffling and/or feather erection.</td>
</tr>
<tr>
<td>Walk</td>
<td>Moving more than 3 paces in one direction, head erect.</td>
</tr>
<tr>
<td>Stand</td>
<td>Hen standing on feet, legs extended, no movement of the body but with eyes open (adapted from Webster and Hurnik, 1990). Head in either erect or relaxed posture.</td>
</tr>
<tr>
<td>Sit</td>
<td>Hen’s body is flush with the bottom of the cage, wings tucked, and head either erect or in relaxed posture. Eyes are open.</td>
</tr>
<tr>
<td>Sleep</td>
<td>Hen in a relaxed posture, either sitting or standing, with eyes closed. Head may be tucked. (adapted from Blokhuis, 1984)</td>
</tr>
<tr>
<td>Dustbathe</td>
<td>A hen performs vertical wing shakes on the wire, bill raking, circular foot motions. Includes sham dustbathing. Hen may pull feed from feeder to use as substrate. Can be social or individual (adapted from Scholz et al., 2011).</td>
</tr>
<tr>
<td>Perch</td>
<td>A hen has 2 feet on a perch (or feed auger) for more than 3 sec (i.e. not stepping over the perch).</td>
</tr>
</tbody>
</table>
Table 5.2 Description of each behaviour as a percentage of the total 90 min observation period. All behaviours were mutually exclusive, except for perching which was recorded in conjunction with any behaviour occurring while the hen was on the perch. Within a row, means without a common superscript differ (P < 0.05).

<table>
<thead>
<tr>
<th>Keel Bone Status</th>
<th>F0</th>
<th>F1</th>
<th>F2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>24</td>
<td>17</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forage</td>
<td>7.2 ± 2.7</td>
<td>7.7 ± 2.8</td>
<td>9.3 ± 2.6</td>
<td>0.4034</td>
</tr>
<tr>
<td>Eat</td>
<td>23.7 ± 3.1</td>
<td>26.2 ± 3.5</td>
<td>24.2 ± 2.9</td>
<td>0.7973</td>
</tr>
<tr>
<td>Sit</td>
<td>10.9 ± 2.0</td>
<td>12.8 ± 2.3</td>
<td>13.8 ± 1.8</td>
<td>0.4744</td>
</tr>
<tr>
<td>Stand</td>
<td>20.7 ± 1.6a</td>
<td>21.6 ± 1.8a</td>
<td>15.2 ± 1.5b</td>
<td>0.0011</td>
</tr>
<tr>
<td>Walk</td>
<td>7.4 ± 0.8</td>
<td>8.4 ± 0.9</td>
<td>8.4 ± 0.7</td>
<td>0.1536</td>
</tr>
<tr>
<td>Drink</td>
<td>6.9 ± 0.7</td>
<td>7.4 ± 0.8</td>
<td>6.7 ± 0.6</td>
<td>0.7766</td>
</tr>
<tr>
<td>Preen</td>
<td>7.5 ± 1.1</td>
<td>10.4 ± 1.4</td>
<td>9.8 ± 1.0</td>
<td>0.2116</td>
</tr>
<tr>
<td>Sleep</td>
<td>9.1 ± 2.1</td>
<td>4.4 ± 0.9</td>
<td>9.9 ± 1.8</td>
<td>0.2423</td>
</tr>
<tr>
<td>Dustbathe</td>
<td>1.6 ± 0.05</td>
<td>1.1 ± 0.04</td>
<td>1.1 ± 0.03</td>
<td>0.9559</td>
</tr>
<tr>
<td>Other</td>
<td>5.0 ± 0.6</td>
<td>0</td>
<td>1.6 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td>Perch</td>
<td>11.6 ± 3.2a</td>
<td>13.9 ± 3.6a</td>
<td>20.0 ± 2.9b</td>
<td>0.0436</td>
</tr>
</tbody>
</table>

1 F0: no fracture and no deviation from 180°, (n = 24); F1: single, “greenstick” fracture at the caudal tip of the keel without any deviation from 180°, (n = 17); F2: multiple fractures (including at least one complete fracture) with deviation from 180°, (n = 31).
Table 5.3 Relationship between fracture severity and the percentage of time resting on the floor vs perch. Within a row, means without a common superscript differ (P < 0.05).

<table>
<thead>
<tr>
<th>Keel Bone Status</th>
<th>F0</th>
<th>F1</th>
<th>F2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0: no fracture and no deviation from 180°, (n = 24); F1: single, “greenstick” fracture at the caudal tip of the keel without any deviation from 180°, (n = 17); F2: multiple fractures (including at least one complete fracture) with deviation from 180°, (n = 31).</td>
<td>n = 16</td>
<td>n = 9</td>
<td>n = 23</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest on Perch</td>
<td>20.2 ± 6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.5 ± 12.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.1 ± 7.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0114</td>
</tr>
<tr>
<td>Rest on Floor</td>
<td>80.0 ± 6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.9 ± 12.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.5 ± 7.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0161</td>
</tr>
</tbody>
</table>

<sup>a</sup> F0: no fracture and no deviation from 180°, (n = 24); F1: single, “greenstick” fracture at the caudal tip of the keel without any deviation from 180°, (n = 17); F2: multiple fractures (including at least one complete fracture) with deviation from 180°, (n = 31).
Figure 5.1 Mean bout length of sitting, standing, and sleeping behaviours for hens with varied keel status. Fracture severity is described as follows: F0 (no fracture and no deviation from 180°, n = 24); F1 (single, “greenstick” fracture at the caudal tip of the keel without any deviation from 180°, n = 17); F2 (multiple fractures (including at least one complete fracture) with deviation from 180°, n = 31).
CHAPTER 6

Validation of an accelerometer to quantify inactivity in laying hens with or without keel bone fractures

6.1 ABSTRACT

Accelerometers are used in a variety of species to remotely monitor active and inactive phases for extended periods of time in studies involving pain quantification or mitigation, expression of behavioural patterns, and individual differences in activity. Although commercially available accelerometers are not yet validated for use with laying hens, behaviours with the potential to be recorded by accelerometers in poultry, e.g. periods of stationary, inactive behaviours (sitting, standing, and sleeping), are typically altered by pain, sickness, or injury. Our objectives were to validate a commercially available accelerometer (Actical) for quantifying inactivity in laying hens, and compare inactivity levels between hens with severely fractured keel bones and hens with minimal to no keel damage. For validation, seven LSL-Lite hens wore accelerometers around their necks, and were simultaneously observed for stationary, inactive behaviour (SI) inside their (furnished) home cages for a one hour period on two consecutive days for a total of 14 hours of Actical inactivity (AI) counts which were paired with focal behaviour observations of SI. Actical inactivity was quantified by summing the total number of 15 sec intervals with a zero recorded (no acceleration) for the activity count during the observation

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1 This manuscript is in preparation for submission to Animal Welfare with the following authors: T.M Casey-Trott, M. T. Guerin, S. Torrey, V. Sandilands, and T. M. Widowski
period. Pearson’s correlation was used to assess the relationship between AI and SI. Correlation between the AI and SI was high with an \( R^2 \) value of 0.8592 \( (P < 0.0001) \) with < 7% of the differences > ± 2 SDs from the mean difference. Therefore, the Actical can be used to accurately quantify the amount of time hens spend inactive. Following validation, 61 LSL-Lite hens (71-72 wks of age) were equipped with Actical accelerometers to measure inactivity level within their home cages (60 hens/ furnished cage; 688 cm\(^2\)/hen) for a period of seven days. Hens were selected for inclusion in the study based on palpated keel status, selecting only hens with severely fractured keels \( (n = 20) \) and hens with minimal to no keel damage \( (n = 41) \). A mixed model analysis of variance assessed the effect of keel status on AI. Severely fractured hens spent less time motionless, (1280 zero counts ± 202 SE), than hens with minimal to no keel damage (1461 zero counts ± 196 SE; \( P = 0.0358 \)). Further investigation into inactivity differences related to keel status before and after acquisition of keel fractures or administration of analgesics is warranted.

6.2 INTRODUCTION

Quantifying physical activity with accelerometers has been used in a variety of animal species as a method to detect behavioural changes related to sickness (Marais et al., 2013; Smith et al., 2014), estrus (Hunnell et al., 2007; Madureira et al., 2015), seasonal or temporal organization (Mann et al., 2010; Ware et al., 2012), lameness (Conte et al., 2015; Solano et al., 2016; Dalton et al., 2016), and chronic pain caused by osteoarthritis (Lascelles et al., 2008; Brown et al., 2010; Riallanc et al., 2013). Accelerometers have been used since the early 1900s to detect vibrations associated with movement, and technological advances in the 1980s allowed the accelerometer to be used for objective quantification of activity in various species (John and Freedson, 2012). Understanding the activity patterns of an animal provides insight into daily
rhythms, individual differences in behaviour, and changes to an individual’s activity before and after a specified drug treatment or procedure. Technological advances in commercially-available accelerometers now offer the ability to cost-effectively and minimally-invasively monitor long-term activity outputs in a quantifiable manner.

Acute and chronic pain has been shown to alter physical activity and mobility in several species, such as cats (Lascelles et al., 2008), dogs (Brown et al., 2010), cattle (Newby et al., 2013), and pigs (Conte et al., 2015). Veterinarians and physicians frequently use the measurement of physical activity as an outcome measure to quantify the recovery process and the response to treatment in companion animals (Hansen et al., 2007; Wernham et al., 2011) and humans (Inoue et al., 2003; van Hemert et al., 2009; Collins et al., 2012). In poultry, changes in activity level (Duncan et al., 1991) and resting behaviours such as sitting and standing (Hocking et al., 1997), are reported as indicators of pain. Although changes in activity level are believed to be affected by pain stimulated by locomotion (Duncan et al., 1991; Hocking et al., 1997), a method for direct quantification of activity in relation to pain or injury has yet to be explored in poultry.

The use of remote sensing equipment, such as RFID tags and accelerometers, to monitor activity in poultry has been applied to assessing resource and range use in non-cage systems (Quwaider et al., 2010; Daigle et al., 2012; Richards et al., 2012; Gebhardt-Henrich et al., 2014), detecting hyperactivity in high feather pecking genetic lines (Kjaer et al., 2009), monitoring sickness behaviour (Marais et al., 2013), and quantifying convulsive activity during euthanasia (Dawson et al., 2007; Rankin et al., 2013); however, use of remote sensing for assessment of behavioural changes related to pain in poultry has been largely unexplored.
In laying hens, the keel bone is a site of frequent fractures during their production life (Fleming et al., 2004; Rodenburg et al., 2008; Wilkins et al., 2011; Petrik et al., 2015; Chapter 4). Damage to the keel bone alters mobility and flight behaviours (Nasr et al., 2012a), standing and perching behaviours (Chapter 5), and hens with keel damage respond positively to treatment with analgesics, suggesting keel damage is painful and negatively affects daily activities (Nasr et al., 2012b). Using an accelerometer to quantify activity in hens with keel damage has the potential to determine if this type of injury impacts daily activity level or behaviour patterns. If a difference exists, further investigation into how analgesics affect the activity of fractured hens can be used in conjunction with accelerometers to offer detailed analyses of changes in activity.

Previous use of accelerometers in poultry showed promising results in the measurement of steps and activity; however, the duration of recording was limited to a maximum of one hour for HOBO® Pendant® data loggers (Dalton et al., 2016) and 60 hours for a wireless sensor device (Quwaider et al., 2010). Although observation time was limited, the use of accelerometers in pullets and hens to describe the behavioural repertoire and variety in activity levels has the potential to be an extremely valuable tool in housing design and management strategies (Kozak et al., 2016).

The light-weight and durable Actical (Philips Respironics, Bend, Oregon, USA) accelerometer offers the ability to continuously monitor activity for more than 250 days and it has been used in a variety of species to quantify activity. The Actical is an omnidirectional accelerometer designed to detect vibrations from movement in all directions, and is quantified by a unit-less, arbitrary activity count that is based on the duration and magnitude of the acceleration. When the device is stationary, with no acceleration detected in any direction, a zero is recorded for the designated period (Lascelles et al., 2008; John and Freedson, 2012). To date,
Actical accelerometers have not been validated for use in poultry. In order to quantify activity levels of hens with keel damage, validation of the accelerometer for use in laying hens is essential.

Previous validation studies using cats and dogs demonstrate a strong correlation between the activity count of the Actical accelerometer and the distance traveled or time spent mobile; however, discrepancies repeatedly arose during non-locomotor, active behaviours, such as grooming (Lascelles et al., 2008; Andrews et al., 2015), shaking, scratching (Andrews et al., 2015), and tail wagging (Hansen et al., 2007). Likewise, birds perform comparable non-locomotor, active behaviours that also have the potential to influence the accelerometer output. Long durations or frequent bouts of preening pose a risk of registering high activity counts during periods of relatively low locomotor behaviour, as do other avian behaviours, such as dustbathing, wing flapping and body shaking (Dalton et al., 2016), which although short in duration and frequency, have the potential to skew the activity count results due to the relatively high acceleration associated with each of these behaviours.

Previous studies validating the Actical in other species focused on active behaviours; however, frequently their results showed that the strongest overlap between the Actical and specific behaviours occurred during periods of stationary, inactive behaviour classified as resting (Hansen et al., 2007) or immobile (Lascelles et al., 2008) behaviour. Behavioural changes in relation to pain or injury in poultry affect levels of inactivity, such as lying time (Duncan et al., 1991), rather than cause dramatic changes in the intensity or duration of active behaviour. The purpose of this study was to quantify the amount of time hens spend inactive so as to capitalize on the significant overlap between zero activity (no acceleration detected) as measured by the Actical and the observation of stationary, inactive (SI) behaviours: sitting, standing, and
sleeping. Following validation, the Actical was used to quantify the level of inactivity of hens with keel bone fractures and hens without keel bone fractures as a secondary objective to determine if keel damage affected daily inactivity level.

6.3 METHODS

6.3.1 Animal Housing

Animal use was approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #1947). Two consecutive flocks of 588 Lohmann-selected Leghorn-Lite (LSL-Lite) laying hens were housed from one day-of-age to 73 wks of age at the University of Guelph Arkell Poultry Research Station.

As part of a concurrent experiment, half of the hens were reared in conventional cages (Ford Dickinsen, Ontario, Canada) and half were reared in an aviary system (Farmer Automatic Pullet Portal: Clark Ag Systems). Rearing details can be found in Chapters 3 and 4.

At 16 wks of age, pullets from both rearing systems were placed into two rooms holding 12 furnished cages (Farmer Automatic Enrichable (Furnished) Cages; Clark Ag Systems) each, with rearing treatment segregated by cage. Each room contained six large (41,296 cm$^2$; 60 hens; 688 cm$^2$/hen) and six small (20,880 cm$^2$; 30 hens; 696 cm$^2$/hen) furnished cages. Only hens from large cages were used in this experiment. Each bank of six furnished cages had three tier levels. The same rearing and adult rooms were used for each consecutive flock.

Hens were fed a commercial layer crumbled pellet diet with automatic feed chains running every three hours commencing at the start of a 14 hr light period from 07:00-21:00 hrs with a 15-min sunrise and sunset. Each furnished cage provided a curtained nest area (94
cm²/hen), 10 cm high perches (15 cm²/hen) running parallel to the cage front throughout the middle area, and a smooth plastic scratch area (42 cm²/hen). Nipple drinkers with cups were located above the feed auger down the middle of the cage. The feed troughs (12 cm/hen), were located on both outer sides of the cage.

6.3.2 Accelerometer

The Actical accelerometer was 28 x 27 x 10 mm, and weighed 17.5 g. The Actical is an omnidirectional accelerometer constructed of a rectangular piezoelectric bimorph plate and seismic mass designed to detect movement in all directions; however, the device is most sensitive in a position parallel to the longest direction of the case (John and Freedson, 2012). To account for this positioning on a laying hen, necklace harnesses were created to hold the Actical in a vertical plane with the hen. Necklaces were comprised of a #10 brass nickel-coated beaded chain, with a key ring used to attach the accelerometer. The device was protected by white duct tape, for a total weight of < 30 g (Figure 6.1). Necklaces were designed to breakaway if caught within the cage to prevent strangulation.

As determined by small pilot studies (data not reported), to achieve maximum sensitivity, all Actical accelerometers were programmed to record 1 sec epochs and set for subjects with the following settings: height 10.0 cm, weight 0.5 kg, gender female, and age 2. These settings were adjusted based on subject size and weight to appropriately calibrate the Actical. A full description of the Actical accelerometer mechanism of operation can be found in Lascelles et al. (2008) and John and Freedson (2012).
6.3.3 Part 1: Actical Validation: Evaluation of Actical accelerometer as a measure of inactivity

Seven LSL-Lite laying hens each housed in a separate group in a furnished cage (60 hens/cage) were conveniently selected from various locations throughout the cage, marked with livestock paint, and fitted with Actical accelerometer necklaces at 70 wks of age.

After acclimatization (> 24 hrs), hens with Actical necklaces were focally observed for 1 hr on each of two consecutive days. Observation periods were equally allocated throughout the hours of 0900-1600 to allow for one morning and one afternoon observation per hen. Trained observers followed a detailed ethogram (Table 6.1) to record behaviour. Reliability among all four observers was acceptable ($W = 0.733; \chi^2 = 8.8; P = 0.0303$). All behaviours were recorded using a Noldus handheld Pocket Observer 3.1 software (Noldus Information Technology, Wageningen, The Netherlands). All behaviours, except perching, were recorded as mutually exclusive state behaviours in that initiation of one behaviour, terminated the recording of the previously occurring behaviour. Occurrence of perching was recorded in conjunction with any behaviours observed while the hen was located on the perch, allowing for a description of specific activities that occurred on the perches.

Following two days of observations, the Actical necklaces were removed. All data were uploaded in 15 sec epochs and the total number of zero counts from each hour that the hen was concurrently focally observed was summed to create the variable Actical Inactivity (AI). A “zero count” was any 15 sec epoch interval that recorded a “0” for acceleration, meaning that no movement in any direction was detected by the Actical over the entire 15 sec period. The total duration of AI was then converted to total seconds of inactivity for each individual hen on each of the two days.
A variable of total duration of inactivity was also created by summing the total duration of stationary, inactive behaviours (sit, sleep, and stand) for each hen during each hour of focal behaviour observation. This variable was subsequently termed Stationary Inactivity (SI). A variable of total duration of activity was also created for each hen by summing the total duration of all active behaviours (walk, preen, eat, drink, forage, and dustbathe).

6.3.3.1 Statistical analysis

The data were analyzed in SAS statistical software version 9.4 (SAS Institute Inc, Cary, North Carolina, USA) using a Pearson’s correlation analysis (PROC CORR command). The duration of AI and SI for each hen on each day were plotted against each other to determine an adjusted Pearson correlation ($R^2$ value). The Pearson correlation coefficient for the duration of SI, active behaviours, perching, and Actical-directed behaviour, and AI for each individual were assessed.

The level for assessment of statistical significance of differences between means was set at $P < 0.05$.

6.3.4 Part 2: Evaluation of daily inactivity of hens with varied keel damage using Actical

6.3.4.1 Treatment Selection

Two hens with fractured keels and two hens without fractured keels were selected from 24 large furnished cages when the hens were 71-72 wks of age ($N = 96$). Selection for the study was based on the keel bone status of each individual bird determined by palpation, with a focus on selection for hens with severe keel damage and hens with minimal to no keel damage. Lights were dimmed for ease of handling and hens were caught from various locations from within each
cage until two hens with a non-fractured keel and two hens with a severely fractured keel were found. A keel was considered non-fractured if it followed a normal, straight, 180° line without the presence of any sharp bends or periosteal scars or calluses indicative of a healing fracture. A keel was classified as severely fractured if there was the presence of a sharp bend or deviation from the 180° line accompanied by one or more periosteal scars or calluses (Casey-Trott et al., 2015). All palpation scoring was completed by the lead investigator (Casey-Trott) who was trained in palpation as described in Chapter 4. Immediately following palpation, selected hens were fitted with an Actical necklace and were marked with livestock paint on their back to allow for monitoring and retrieval of the devices.

Actical necklaces were placed on the hens for a total of seven days, with the first day of data collected from the Actical excluded from analyses to allow for acclimatization of the hen to the device. Since the necklaces were designed to detach from the hen if caught within the cage, data were only included in the analysis if the necklace remained on the hen for ≥ 4 complete days of data collection. Necklaces were not reattached if they fell off to ensure that birds were not disturbed beyond daily management routines throughout the entire data collection period.

After seven days of data collection for each group of 24 hens, hens were caught and the remaining necklaces were removed. All data were uploaded in 15 sec epochs and the total number of zero counts from the lights- on period, 07:00 to 21:00, was summed to determine the total time inactive for each hen during daylight hours. The daily duration of daytime inactivity was then averaged to create a mean duration of AI (sec) for each individual hen.

Following the final data collection, hens were euthanized by cervical dislocation, dissected, and re-classified by keel status at dissection: F0: No fracture or only minimal damage
(single, green-stick fracture at the caudal tip of the keel, potentially accompanied by $< 5^\circ$ deviation from $180^\circ$, $n = 41$); F1: Severe keel damage (multiple complete fractures, potentially accompanied by $> 5^\circ$ deviation from $180^\circ$, $n = 20$). The decision to compare hens with severely damaged keel bones to a combined category of hens with minimal to no keel bone damage was based on our previous finding that the behaviour of hens with minimal keel bone damage closely resembled the behaviour of hens with no keel bone damage, whereas hens with severe keel bone damage showed behavioural differences from both groups (Chapter 5). The discrepancy between sample sizes was a result of the loss of necklaces due to their intentional break-away design, as well as the re-classification of keels possessing a deviation without the presence of a fracture. Although an attempt was made during palpation to find keels with the greatest extremes, in some cases, severely damaged keels could not be found.

6.3.4.2 Statistical Analysis

For all statistical analyses, only the true damage status of the keel, as determined by dissection, was used. The level for assessment of statistical significance of differences between means was set at $P < 0.05$.

The mean daily duration of daytime AI zero count for each keel status category was assessed using SAS 9.4. The mean AI zero count was analyzed using a PROC MIXED command with keel status (F0 or F1), body weight, room, rearing system, and tier as fixed effects. Flock number was included as a random effect to control for variation between flocks.
6.4 RESULTS

6.4.1 Part 1: Actical validation

From periodic visual observation over the first 24 hours, the Actical accelerometer was well tolerated by the hens, with the most disturbance of behaviour occurring within two hours of application. Actical-directed behaviours, such as pecking at or shaking the device, were most frequently observed and most vigorous during this time. Walking backwards, a behaviour not commonly expressed by hens, was observed in a few birds within the first 30 minutes of necklace attachment, but was not seen thereafter. On the day following necklace attachment, a full range of behaviours (sit, stand, walk, forage, eat, drink, perch, dustbathe, preen, wing-flap, and sleep) were exhibited by each hen involved without impedance by the Actical necklace.

A total of 14 hours of simultaneous focal, live bird observation and Actical accelerometer data was collected. One observation was considered an outlier, as it was greater than ± 2 standard deviations from the group mean difference, and was subsequently removed. The corresponding behaviour that occurred during the Actical data collection of this outlier was frequent bouts of preening and continuous standing for the entire 1 hour observation period.

There was a strong, positive correlation between periods of SI and AI, with an $R^2$ value of 0.8592 ($P < 0.0001$: Figure 6.2). Likewise, the relationship between periods of active behavioural states and AI had an inverse relationship with an $R^2$ value of 0.5904 ($P = 0.0022$). The total duration of perching had a positive relationship with AI ($R^2 = 0.4932; P = 0.0236$). The correlation between AI and the total duration of Actical-directed behaviours was not significant ($R^2 = 0.2926; P = 0.0652$) although it did show an inverse trend with AI.
The average duration of SI as recorded by focal behaviour observations was 37 min ± 2.5 SE, and 32 min ± 2.7 SE for AI, per hour long observation.

A graphical representation of the one hour focal behaviour observation output compared to the concurrent Actical Actogram activity output is described in Figure 6.3.

6.4.2 Part 2: Effect of keel status on Actical Inactivity

Keel bone status had a significant effect on AI; hens with severely damaged keel bones (F1) spent less time inactive (1280 zero counts/day ± 202 SE) than hens with minimal or no keel bone damage (1461 zero counts/day ± 196 SE; \( P = 0.0358 \)). There was no effect of body weight \( (P = 0.3945) \), room \( (P = 0.5431) \), rearing system \( (P = 0.9303) \), or tier \( (P = 0.5954) \) on AI. The distribution of individual F0 and F1 hens by their mean AI zero count can be found in Figure 6.4.

With AI converted to a unit of time, the raw mean total duration of inactivity for F0 was 316 min ± 20 SE and for F1 was 243 min ± 12 SE. An example of an Actical Actogram activity output for an individual hen over a period of nine consecutive days can be found in Figure 6.5.

Egg production and mortality were recorded daily and will be reported elsewhere (Widowski, unpublished). All mortalities were sent for post mortem analysis and there were no outbreaks of disease, feather pecking, or cannibalism throughout the duration of the study.

6.5 DISCUSSION

6.5.1 Part 1: Actical validation

The Actical accelerometer output correlates strongly with periods of stationary, inactivity corresponding with sitting, sleeping, and standing behaviours in laying hens. To our knowledge, this is the first study to assess the validity of the Actical accelerometer for use as a measure of
inactivity in poultry. Previous studies validating the Actical as a tool for monitoring activity, reported that the relationship between Actical activity counts and distance traveled produced $R^2$ values within the range of 0.80-0.90 for cats (Lascelles et al., 2008) and 0.78 for dogs (Hansen et al., 2007). The $R^2$ value reported here falls within that range and demonstrates the validity of using the Actical to strictly quantify periods of SI behaviours that correspond with a motionless Actical with no acceleration detected.

Since the Actical accelerometer calculates the arbitrary activity count by accounting for both the duration and intensity of a given acceleration, activities such as dustbathing, wing-flapping, or body shaking produce a relatively high activity count with virtually no locomotor movement. In dogs, periods of vigorous tail wagging, ground snifffing, and toy chewing produced exaggerated activity counts in relatively sedentary subjects (Hansen et al., 2007); the researchers also noted that the strongest overlap in observed behaviours and Actical activity counts occurred during periods of quiet rest. By focusing only on periods of inactivity, the effects of behaviours that skew the Actical activity count can be reduced. For future studies, if the intention is to quantify behavioural patterns or the frequency of occurrence of specific behaviours, the approach of classifying activities by threshold, as described by Kozak et al. (2016), should be used.

For laying hens in the relatively confined environment of a furnished cage, correlating Actical activity with distance traveled might not be biologically relevant, as the degree of locomotion and behavioural expression varies within different housing systems (Hansen, 1994). Laying hens spend approximately 90% of their active time performing feeding behaviour (Dawkins, 1989) and have reduced the frequency of behaviours that are energetically costly as a result of genetic selection for feed efficiency (Schutz and Jensen, 2001). Especially in an enclosed environment, such as a furnished cage, many active behaviours such as preening,
eating, dustbathing, and spot pecking take place in a single location with virtually no distance locomotor movement. Even foraging, arguably one of the more active locomotor behaviours in hens, does not involve a large distance traveled as it does in mammals; rather, foraging behaviour in hens is focused on repeated ground scratching movements and ground pecking in a relative small area (Lindqvist, 2008) often in close association with the feed trough in caged birds (Mench, 2009). Focusing only on periods of stationary inactivity, acknowledges the diversity of active behaviours expressed in laying hens and offers a conservative approach to quantifying true periods of inactivity.

The decision to include standing, in addition to sitting and sleeping postures, in the definition of stationary, inactive behaviours was based on previous focal behaviour observations by the researcher where dozing behaviour was frequently observed. Dozing is thoroughly described by Blokhuis (1984) as a stationary behaviour that is part of the normal rest repertoire of poultry. It can take place in either the sitting or standing position, and while it is believed to offer a form of rest in and of itself, it was also believed to transition into more explicit sleeping positions in which the head is tucked beneath the wing. Blokhuis (1984) also noted that the commercial hybrid strain spent a significantly larger proportion of their resting time in a standing position, 20.4% compared to only 4.9% in Red Jungle Fowl. Since we observed a similar, high percentage of standing behaviour in a previous study (Chapter 5), we decided that stationary standing behaviour should be included in the description of stationary, inactive behaviours, as it represents a substantial portion of resting strategy of commercial hens.

Similar to previous accelerometer validation studies, in which 4-6% of observations were considered to be outliers and excluded from the analysis, < 7% of the observations in our study showed a discrepancy between AI and SI greater than two standard deviations from the mean.
The one observation that was considered to be an outlier and was subsequently excluded resulted from a hen that spent 40 minutes standing in the middle of the cage, with more than 10 minutes spent preening. The large discrepancy between the low value of recorded AI and the high value of SI recorded by visual observation is likely a combination of two factors. First, the standing position was located in the middle of the cage, in a relatively high traffic area located in a narrow pathway between the nest box curtains and end of the perches. It is possible that even though the hen was standing in a stationary position, the loosely attached Actical necklace may have been stimulated by contact with passing cage mates. The placement of the Actical on a loosely attached necklace was intentional and allowed for detection of even slight movement as well as a break-away mechanism if the equipment became caught within the cage. While this is an advantage for detecting motion in a relatively small, light-weight species such as a laying hen and has been used in marmosets (Mann et al., 2010), the disadvantage of a necklace attachment is that the Actical likely underestimated the amount of inactivity due to some extraneous movement of the Actical caused by contact with nearby chickens and the ability of the hen or cage mates to peck or shake the device. Although there was a trend highlighting an inverse relationship between Actical-directed behaviours (pecking or shaking the device) and inactivity, the lack of significance suggests that Actical-directed behaviours did not dramatically influence inactivity output. Perhaps a more secure location of Actical attachment may reduce this extraneous stimulation; however, this problem was only a concern for one observation out of 14.

The second likely reason for the low AI and high SI discrepancy of the outlier hen was the high level of preening expressed by this hen, which was greater than any other hen by more than one minute and greater than the average preening time of the group by six and a half minutes. Lascelles et al. (2008) and Andrews et al. (2015) reported a similar problem with cats.
that spent a large amount of time grooming. Although we initially hoped that the skewed activity count triggered by immobile grooming behaviours would be reduced by focusing on only the AI periods, it appeared that this was not the case. Frequent, short bouts of preening triggered enough movement of the Actical to record a value other than zero acceleration. Since the strategy of this study was to take a conservative approach and include only complete periods of 15 sec of entirely zero acceleration as recorded by the Actical, slight preening motions might have triggered very brief, low threshold bouts of activity reducing the overall total zero count for this particular hen. Again, this only occurred in one hen, which is consistent with other studies (Lascelles et al., 2008; Andrews et al., 2015). Although this may be initially perceived as a problem, the ability of the Actical to detect such subtle movements as preening does provide the opportunity to study individual differences in levels of excessive preening or hyperactivity, which has been shown to be related to feather pecking and genetic selection (Kjaer, 2009).

6.5.2 Part 2. Effect of keel status on Actical Inactivity

Across a variety of species, sickness behaviour manifests as several non-specific behavioural characteristics such as malaise, anorexia, lethargy, and withdrawal from social activities (Dantzer and Kelley, 2007). More specifically, chickens express sickness behaviour by increasing the time spent sitting while reducing other behaviours such as eating, drinking, standing and moving (Cheng et al., 2004). Likewise, acute or chronic pain and inflammation in turkeys with untreated hip disorders (Duncan et al., 1991), laying hens in response to a sodium urate injection (Hocking et al., 1997), and broilers with untreated articular inflammation (Hocking et al., 2001) indicate painful stimuli induce higher levels of inactivity. On the other hand, visceral pain in dairy cattle has been shown to increase overall activity, inducing a state of restlessness (Rialland et al., 2014). All of the conditions listed above use changes in inactivity as
an outcome measure of assessment, a value that appears to be easily quantifiable by measuring AI as demonstrated by this validation study.

The results of the second experiment demonstrate an interesting contradiction with current literature in that although keel fractures have been demonstrated to be painful (Nasr et al., 2012b), hens with fractures spent less time stationary and inactive than hens without keel fractures; however, this lower level of stationary inactivity may be related to lower levels of standing behaviour seen in hens with keel fractures, as described in Chapter 5. While these results may lead to the premature assumption that keel fractures are not painful since they do not increase levels of inactivity, it is important to realize that if that were the case, the keel fractured hens would show no differences in inactivity level from the non-fractured hens. In order to truly understand the dynamics of pain related to keel damage, a traditional cross-over design pain study with subjects as their own controls and monitoring Actical Inactivity before and after treatment with analgesics needs to be carried out.

The difference in Actical Inactivity between severely fractured hens and hens with minimal to no keel damage does indicate that hens with keel damage are behaviourally different; however, it is important to note that this difference could manifest differently depending on the housing situation (cage vs non-cage) of the bird. One possibility suggests a causal relationship, in which hens that spend less time stationary are subsequently exposed to greater risk of injury to the keel. Alternatively, perhaps the coping strategy of hens with keel damage does not follow the traditional description of coping with pain related to lower limb injuries which describes the majority of pain research in poultry. Keel bone damage may be more closely related to the visceral pain. Rialland et al. (2014) reported that cattle with higher levels of visceral pain exhibited higher levels of activity due to a state of restlessness. Our previous research indicated
that hens with keel bone fractures reduced their time spent standing compared to hens with no keel bone damage (Chapter 5). Since stationary standing was one component within our definition of SI, and SI correlated strongly with AI, it is possible that the lower AI exhibited by hens with keel fractures compared to hens without keel damage is related to a decrease in standing behaviour.

Although the keel bone serves as the anchor for flight muscles and is subjected to the gravitational weight of the visceral cavity, it is not a load bearing bone in the traditional sense of standing and walking activity. Discomfort related to a keel bone injury may not be alleviated by increasing the duration of sitting behaviour, as it is with pain in the lower limbs. Instead, hens with severe keel damage may be spending less time inactive because it is uncomfortable to remain in a sitting or standing position with pressure or strain on the keel for long periods of time resulting in more frequent shifting of position or changes in behavioural state. Restless behaviour and disturbed sleep patterns are commonly reported in human patients experiencing musculoskeletal pain (Moldofsky, 2001; Smith et al., 2004), and restlessness is used as an outcome measure for pain assessment in non-verbal infants (Patel et al., 2001). Similarly, we found shorter bout length for standing behaviour and a trend for more frequent sitting bouts in hens with keel fractures compared to hens with no keel damage (Chapter 5). Although it is impossible to definitively state the cause of the decreased AI exhibited by the hens with fractured keel bones, the difference in inactivity level compared to hens with minimal to no keel bone damage indicates that severe keel damage alters the behaviour of the hen. Further, understanding the level of inactivity expressed by individual laying hens may permit us to quantify changes in inactivity level due to administration of analgesics in pain trials, age, injury, environmental or husbandry conditions, or even disease status.
Table 6.1 Ethogram used for focal behaviour observations

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forage</td>
<td>Pecking or scratching at the floor of the cage with head below rump (adapted from Klein et al., 2000).</td>
</tr>
<tr>
<td>Eat</td>
<td>Head in the feed trough or completely through the cage over the feeder. Can include standing breaks of ≤ 5 sec followed by resumption of behaviour.</td>
</tr>
<tr>
<td>Drink</td>
<td>Repeated pecks at nipple drinker followed by swallowing. Can include standing breaks of ≤ 5 sec, with beak still within the plane of the drinker, followed by resumption of drinking behaviour.</td>
</tr>
<tr>
<td>Preen</td>
<td>A hen uses her beak to clean wing and body feathers. Related behaviours include head scratching, wing stretching, feather ruffling and/or feather erection.</td>
</tr>
<tr>
<td>Walk</td>
<td>Moving more than 3 paces in one direction, head erect.</td>
</tr>
<tr>
<td>Stand</td>
<td>Hen standing on feet, legs extended, no movement of the body but with eyes open (adapted from Webster and Hurnik, 1990). Head in either erect or relaxed posture.</td>
</tr>
<tr>
<td>Sit</td>
<td>Hen’s body is flush with the bottom of the cage, wings tucked, and head either erect or in relaxed posture. Eyes are open.</td>
</tr>
<tr>
<td>Sleep</td>
<td>Hen in a relaxed posture, either sitting or standing, with eyes closed. Head may be tucked. (adapted from Blokhuis, 1984)</td>
</tr>
<tr>
<td>Dustbathe</td>
<td>A hen performs vertical wing shakes on the wire, bill raking, circular foot motions. Includes sham dustbathing. Hen may pull feed from feeder to use as substrate. Can be social or individual (adapted from Scholz et al., 2011).</td>
</tr>
<tr>
<td>Perch</td>
<td>A hen has 2 feet on a perch (or feed auger) for more than 3 sec (i.e. not stepping over the perch).</td>
</tr>
<tr>
<td>Actical-directed</td>
<td>Pecking, pulling, or shaking of the Actical device by the individual wearing the Actical device or by a cage-mate</td>
</tr>
</tbody>
</table>
Figure 6.1 Actical necklace attached to hen (A) and image of Actical accelerometer attached to beaded chain necklace with a size comparison for reference (B).
Figure 6.2 Correlation between Stationary Inactivity (SI), as determined by focal, live bird observation, and Actical Inactivity (AI) zero count (adjusted Pearson’s correlation coefficient: $R^2 = 0.859; P < 0.001; n = 13$).
Figure 6.3 Comparison of the Actical Actogram graph output (A) and focal behaviour observation output (B) of a single bird over a concurrent one hour period.
Figure 6.4 Distribution of mean Actical Inactivity (AI) zero count/day by keel bone status for each individual hen. Solid bars represent hens with severe keel damage (F1; n = 20) and patterned bars represent hens with minimal to no keel damage (F0; n = 41).
Figure 6.5 Example of Actical Actogram activity graph for a single hen over 9 days. Lights on at 07:00 hr and off at 21:00 hr.
CHAPTER 7

DISCUSSION

7.1 General Discussion of Main Findings

The results reported in Chapters 3 and 4 demonstrate a clear, beneficial effect of providing the opportunity for exercise during the pullet rearing phase on both overall bone quality and keel bone status of adult laying hens, supporting the hypothesis of the first main objective. The increased bone area, density and mineral content reported in aviary-reared pullets at 16 wks of age indicates that increased opportunities for exercise during rearing positively affected the skeletal growth of the aviary-reared pullets, supporting the initial prediction of the first objective. The evidence of a sustained effect of rearing system seen at the end of lay in adult hens suggests that rearing in a pullet aviary with ample room for diverse exercise from one day-of-age gave the aviary-reared pullets a skeletal advantage from the onset of lay onwards. Perhaps the increased bone area of aviary-reared pullets allowed for greater deposition of mineral content and medullary bone resources, providing a larger calcium-sink from which to draw during egg production. It is possible that this improvement to bone structure could be combined with advances in genetic selection for improved skeletal integrity or improved metabolic function to produce an additive effect for a dramatically stronger, more substantial skeletal frame.

Rearing system affected the structure of the keel bone at 16 wks of age and had lasting positive effects that resulted in a reduction in the prevalence of fractures during the laying phase. This also supports the hypothesis and predictions of the first objective. The differences in keel bone characteristics at 16 wks of age suggests that rearing system either slowed the growth or
ossification of the keel or stimulated a slight increase in overall keel size. Further research, similar to the work completed by Buckner in 1949 mapping the progression of keel bone ossification over time, needs to be carried out on modern commercial lines to assess the rate of ossification and growth of the keel bone during the pullet and adult laying phase. Understanding the growth and ossification process of the keel may provide insight into periods where the keel bone is particularly vulnerable to damage either due to a weak, newly ossified structure, or periods of reduced calcium availability due to physiological changes in hormones or calcium allocation at peak lay.

The second main objective is also addressed in Chapters 3 and 4 by providing evidence that a greater allowance of exercise through provision of increased space allowance, area, and furnishings in adult housing positively impacted the quality of the wing and leg long bones in adult hens, although keel bone status was unaffected. While the greater bone density and mineral content values of the furnished cage hens support the initial hypothesis and predictions of the second objective, the lack of significant differences between adult housing systems was surprising. Since it is well documented that the provision of furnishings, perches in particular, increase the risk of keel bone damage, it was initially predicted that housing in furnished cages would increase the prevalence of keel bone damage in comparison to hens housed in adult conventional cage housing. While the lack of significant difference may be indicative of a positive effect of sustained exercise in adulthood on the overall strength of the keel bone, it is also possible that the lack of difference in keel bone damage is related to the low utilization of the perches in the furnished cages seen in the hens used here. Although differences in daytime use of perches were present in hens with or without keel bone fractures as discussed in Chapter 5, the overall utilization of perches at night was extremely low (< 10%; data not reported)
compared to previous reports (85%; Appleby et al., 1992). In order to determine whether the lack of difference in keel bone damage between furnished or conventional cages was due to improved keel characteristics or low perch utilization, detailed analysis of the keel bone composition and thorough assessment of day and night perch use is required.

The lack of adult housing effects on the prevalence of keel bone damage reported here contradicts previous literature as discussed in the Sandilands et al. (2009) review; however, perhaps the lack of adult housing differences also highlights the importance of making improvements to skeletal health in pullets, rather than waiting until the adult phase to provide opportunities for exercise. The prevalence of keel bone damage in the current study is still confounded by the presence of furnishings potentially causing the damage, as seen in other studies. As the bone quality characteristics of the keel bone were not quantified in this study, it cannot be conclusively determined if the keel bones from any given rearing or adult housing system were of better bone quality or strength at end of lay. All that can be determined here is that hens reared in an aviary system as pullets exhibited a lower prevalence of keel bone fractures at all ages, regardless of adult housing system. This information, along with the prevalence data of keel bone fractures and deviations over time, and the clear evidence of an association between keel bone deviations and fractures, addresses the hypothesis and predictions of the third objective.

Another interesting finding was related to differences in bone composition between the hens housed in FC-L and FC-S. The finding of improved bone quality in hens allowed a greater opportunity for exercise in furnished cages compared to conventional cages in adulthood parallels the work of numerous studies addressing this question as discussed in Chapter 3; however, it is interesting to note that the increase in cage area alone between the FC-L and FC-S
was enough to positively influence the bone composition in adult hens. The FC-L and FC-S were nearly identical in terms of the furnishings provided, and provided the same space allowance per hen. Since the consistent opportunity for walking is considered a sufficient method of creating loading-strain on bones stimulating bone growth (Shipov et al., 2010), it is likely that the additional area for locomotion provided within the FC-L stimulated additional deposition of bone mineral content. The greater bone mineral content in the radius of hens housed in FC-L also suggests that this additional area allowed for a greater amount of wing-loading exercises, perhaps in the form of wing-flapping, which is supported by research reporting that furnished cages holding > 60 hens provided sufficient space to support wing-flapping (Mench and Blatchford (2014).

The final objective moved away from the use of physiological and health related measures of quantifying bone health and keel bone status, and shifted towards a behavioural approach to assess the effects of keel bone damage on the behaviour of laying hens. Unlike physiological indices which take indirect measurements of welfare, behavioural indices are the only indicators that can directly measure the animal’s perception of the stimulus. Behaviour is what the animal uses to change and control their environment to both maintain and return to homeostasis, and it provides insight into preferences, needs, and internal states. Understanding the behaviours associated with physiological or health related changes in an animal provides a more robust understanding of the state of welfare as perceived by the animal.

The methods described in Chapters 5 and 6 cannot conclusively determine whether differences in behaviour and inactivity levels between hens with or without keel bone fractures occurred before or after the development of the keel damage; however, the lower proportion of standing, greater proportion of perching, and decreased level of inactivity seen in hens with keel
bone fractures compared to hens without keel bone fractures provides evidence that behavioural differences exist between hens with or without keel bone fractures. This supports the hypothesis for the fourth objective; however the initial predictions were less accurate in regards to inactive behaviours and overall inactivity.

As predicted, hens with keel bone fractures exhibited a greater proportion of time perching. This is supported by previous research describing an association between perch use and keel bone damage (Tauson and Abrahamsson, 1994; Abrahamsson et al., 1996), and likely parallels the previous claims of a causal relationship between perch use and keel bone damage (Sandilands et al., 2009).

The decreased proportion of standing behaviour in hens with keel fractures potentially indicates a pain-related behavioural adjustment, but this finding requires further research. The decreased proportion of standing behaviour coupled with the shorter standing bouts of the hens with fractured keels, potentially indicates an agitated state, a finding supported by the unexpected result of lower levels of inactivity in the hens with fractures as detected by the Actical accelerometer described in Chapter 6. Restless behaviour and disturbed sleep patterns are commonly reported in human patients experiencing musculoskeletal pain (Moldofsky, 2001; Smith et al., 2004) and restlessness is used as an outcome measure for pain assessment in non-verbal infants (Patel et al., 2001). Perhaps the lower inactivity level as detected by the Actical and the frequent shifts in standing behaviour of the hens with keel bone fractures, provides insight into how keel bone damage affects behavioural rhythms and resting behaviour.

Using this information and the availability of newly validated technology (Actical accelerometer; Chapter 6), the behaviour of hens with or without keel bone damage can be
Further quantified. Studies assessing the behaviour and inactivity levels of hens prior to keel bone fractures, and continued recording of behaviours and inactivity level after fractures occur, would allow for the assessment of changes in behaviour and inactivity level directly related to the occurrence of keel bone damage, using the individual hen as a baseline. Use of radiographic technology such as QCT or a portable x-ray to determine initial keel status and track keel status in live hens will be especially useful in longitudinal assessment of behaviour and inactivity changes in laying hens. This is one area of research that I was lucky enough to begin exploring at the end of my PhD research, and is an opportunity that I hope to explore further in the future. These techniques could also be used in conjunction with administration of analgesics or self-medication studies to take the assessment of behavioural and inactivity changes a step further and attempt to quantify if hens with fractured keels are in a state of pain. This is one area of research regarding keel bone damage that is in great need of further investigation.

7.2 Future Directions for Improving Skeletal Health in Laying Hens

While advances have been made to mitigate severe cases of osteoporosis, it still remains a pandemic problem. Even if the mortalities can be reduced and fracture incidence decreased, is it ethical to produce an animal that is at a high risk of injury with any sort of handling or disturbance? If improving animal welfare standards and achieving sustainability in the production of food truly is a priority for society and is the goal for the future of the agricultural and food industry, using the information gained over the past half century to generate a more interdisciplinary, collaborative approach to reducing osteoporosis may be required.

In my opinion, further research exploring the importance of various aspects of the pullet rearing phase has the greatest potential to offer immediate improvements to the welfare of adult
laying hens. As discussed previously in more detail, providing complex rearing systems that encourage physical and cognitive development will better prepare the hens for lasting egg production and aid in the transition into alternative adult systems. Addressing practical questions related to training pullets for placement into various adult housing systems, and designing rearing systems that encourage maximal musculoskeletal growth without inducing unnecessary risks are two areas of applied research that could offer immediate benefits to producers attempting to embrace the implementation of alternative systems. Integrating a combination of housing, nutritional, and genetic strategies during the pullet phase should also be explored as the combinations may produce additive beneficial effects.

Further understanding the role of the other systems involved in the process of calcium utilization, such as the overall health of the kidney (Beck and Hansen, 2004), activity of the parathyroid gland (Miller et al., 1984), or the role of a fatty liver on vitamin D metabolism, may offer opportunities to improve calcium uptake and mobilization to maximize any benefit provided by selection advances (Etches, 1987). Striving to achieve the full functionality of digestive organs, such as the gizzard, by including a diversity of nutrient sources as well as feed structure in the form of fiber, whole grains, or varied particle size distribution improves gut motility and stimulates the secretion of pancreatic enzymes and gastroduodenal refluxes (Amerah et al., 2007). The slower passage rate of digesta results in more exposure of nutrients to digestive enzymes and improved digestibility of feed. Even additives such as prebiotics and organic acids have proven useful in improving mineral availability (Swiatkiewicz and Arczewska-Włosek, 2012). Not only does this diversity of feed provide better nutrient availability with the potential to increase the utilization of key skeletal metabolites (calcium, phosphorus, Vitamin D), but it also can help to satisfy the strong motivation of poultry to seek
feed structure. Utilizing the specific appetite of poultry (Wilkinson et al., 2011) to help treat fresh fractures or prevent fractures from occurring may also be a nutritional research avenue worth pursuing in relation to keel bone damage.

The same interdisciplinary approach can also aid our understanding of keel bone damage. In addition to addressing the question of whether or not keel bone fractures are painful, future research should attempt to quantify the effects of keel bone damage on overall physiological processes such as metabolism and respiration. The integral involvement of the keel in respiratory function as discussed in Chapter 4 addresses the potential that damage to the keel bone may be affecting the hen in a more profound manner than initially suspected. Assessing the effect of keel bone damage on respiratory and metabolic efficiency using controlled metabolic chambers is a research question that should be addressed to more holistically quantify keel bone damage on health and daily function.

Finally, genetic solutions likely provide the greatest potential to improve the bone health of laying hens. Over the last half century, genetic selection has proven to be an incredibly useful tool in accomplishing the task of improving efficiency and productivity in laying hens. Now at a point of transition between valuing sustainability over efficiency, genetic selection again has the chance to play a pivotal role in the progression of the agricultural industry towards improving animal well-being. Several researchers have suggested that it is not the large number of eggs over the lifetime of the hen that depletes the skeleton, but rather the prolonged periods of laying without any pauses that is to blame (Whitehead, 2002; Hocking et al., 2003). It has also been suggested that by selecting against the natural broody period in hens, we may have eliminated genes or mechanisms for sensing calcium depletion (Beck and Hansen, 2004) and subsequently produced hens that continue to lay large amounts of eggs without any sort of physiological
warning system to prevent skeletal exhaustion. Perhaps selection for hens that produce fewer eggs/year, with periodic pauses in production, would aid in the replenishment of the structural bone allowing the hen to better maintain a healthy skeleton for a period longer than the typical 52+ weeks of production. Although it may seem counterintuitive to take a few steps back in terms of genetic selection for eggs/hen/year, it is possible that a shift towards a more sustainable hen in combination with advances in nutritional and housing strategies can offset any reduction in productivity while accomplishing the goals of producing food with improved animal welfare as a priority. However, as public pressure continues to rapidly drive welfare related changes across the majority of animal production industries, a perfectly equal trade-off between ethics and economics is unlikely. During this period of transition, it is imperative that science-based recommendations help guide the industry changes, without dismissing the ethical concerns of the public.

7.3 Defining Animal Welfare

In the study of animal welfare science, there are many philosophical viewpoints and variations of the definition of animal welfare; however, when looking at the history of animal welfare, it can be suggested that regardless of the specific details, all definitions of animal welfare focus on the overall consideration of the animal’s quality of life. How one defines or determines the details of ‘quality of life’ are where the discrepancies lie, for this requires the involvement of judgements based on personal values, morals, or circumstances (Fraser et al., 1997; Fraser, 2008).

Traditionally, there are three main approaches to understanding animal welfare as discussed by Duncan, Appleby, and Fraser (Fraser, 2008). These three approaches are basic
health and functioning, natural living, and affective state with each classification focusing
primarily on one aspect of the individual animal, namely body, nature, and mind, respectively.
Affective state focuses solely on the feelings of the animal and how it perceives or experiences
the environment and interactions of its daily life, and is the viewpoint where I find myself most
often in regard to my own definition of good animal welfare.

In my opinion, animal welfare in its truest form should ultimately focus on the relative
state of the quality of life as experienced from the animal’s point of view, independent of human
values. The current pursuit of this illusive insight continues to drive the refinement of a wide
variety of methods in an attempt to quantify the animal’s well-being.

The different viewpoints described in the diagram of animal welfare perspectives put
forth by Fraser (2008) are essential to a thorough discussion of animal welfare and encourage the
critical assessment of animal well-being, a topic undeniably intertwined with ethical
considerations. Efforts to understand the complexity of welfare related interventions by
considering societal, economic, and circumstantial pressures may aid in the successful
implementation and facilitation of changes related to animal welfare (Whay, 2007).

As an animal welfare scientist, my ideal definition of welfare relies predominantly on
addressing the question of what the animal feels or perceives to determine whether the welfare of
the animal is good or bad. With that in mind, in my opinion, it would be expected that animal
welfare is improved when an animal is allowed to make choices within its environment and
perform a variety of motivated behaviours, while being free from distress and chronic or
unnecessary pain and fear. For me, offering choice to animals is one of the most likely ways to
offer them the opportunity for good welfare. Not only does this provide them with a sense of
control over their environment, but also their individual choices most closely reflect the preferences and motivations that they seek at any given time. This helps to eliminate individual differences and reduces the focus on what humans find valuable.

With all the current policy changes and public pressure to move towards alternative housing systems for laying hens, it has been a very exciting time to work on completing a PhD evaluating the use of furnished cages as an alternative to conventional cage housing. Furnished cages attempt to improve hen welfare by offering hens a degree of choice within their environment, additional space for exercise, and a range of furnishings to aid in carrying out motivated behaviours. Although there is pressure to move towards entirely non-cage systems, it is important to remember that although non-cage systems offer a higher degree of choice and opportunities for a larger range of motivated behaviours, there is also a trade-off of additional risk of injuries in the majority of the currently available housing designs, especially when combined with the modern genetic lines of laying hens.

The majority of this thesis addresses the desire to improve bone health and reduce the prevalence of keel bone fractures in laying hens in order to accomplish the latter part of the above definition of providing animals with a life free from distress, and chronic or unnecessary pain. This is one area of welfare research that I think we have great potential to improve by reducing the prevalence of osteoporotic fractures and keel bone damage. As we move forward towards transitioning into alternative housing systems, it is our responsibility to ensure that the alternative housing systems truly improve hen welfare without inducing unnecessary injuries due to insufficient housing design or inadequate skeletal or cognitive capacities of the hens that we house in them.
Instead of improving welfare for the sake of profit, or because legislation demands it, I still hope that welfare improvements will at least in part be made because it is our moral responsibility to provide the best life we can for the animals that we use. I am utilitarian in my beliefs not simply because I see great value in the end products that animals provide us with, but also I find our interactions and relationships with animals both rewarding and somewhat mystifying. Their existence in many forms and specializations of different metabolisms, physiological capacities, and successful survival in a vast diversity of biological niches serve as a reminder that the human form is just one of many successful organisms in existence, and highlights the fact that we do not fully understand the depth of the world around us. Since it is unlikely that we ever will, I support the notion that it is better to err on the side of caution and believe that ‘beyond a reasonable doubt’ animals are capable of both mental and physical suffering, and therefore their state of welfare deserves our thorough consideration.
LITERATURE CITED


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