The Effects of Light Intensity During Rearing on Brown- and White-Feathered Egg Strain Pullets' Use of Space, Behaviour, and Health

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Overall Abstract

This study aimed to determine the effects of light intensity (L) and strain (S) on growth, behaviour, frequency and success of jumping between structures, bone health, and welfare of pullets reared in a perchery. Three L (10, 30, or 50 lux, provided by white LED lights) and two Lohmann S (Brown-Lite (LB) and LSL-Lite (LW)) were tested. Pullets were floor reared in pens within light tight rooms from 0 to 16 weeks of age (wk). Each pen contained a system of four parallel perches, a ramp, drinker line, and two tube feeders. Data collected included body weight (BW), behaviour, jumping frequency and success, fear and stress response, keel bone damage (KBD), breast muscle weight, tibia bone characteristics and strength, and mortality. L did not affect BW, aggression, jumping success, fear, stress, KBD, breast muscle weight, tibia bone characteristics and strength, or mortality. At 13 and 16 wk, pullets reared in 50 lux spent more time preening than 10 lux pullets. At four wk, pullets reared in 30 and 50 lux had higher jumping frequency than 10 lux pullets, however jumping success did not differ. LB pullets had a higher BW than LW pullets at eight and 16 wk. Throughout the experiment, LB pullets spent more time pecking at litter and walls than LW pullets, while LW pullets spent more time resting and preening. LW pullets performed more jumps than LB pullets and were equally as successful in navigational jumps. S did not affect aggression, however LB pullets had higher fear and heterophil/lymphocyte ratio, suggesting S characteristic differences. S did not affect KBD. LB pullets had heavier breast muscle and tibia, however LW pullets had a proportionally higher breast muscle yield and thicker and stronger tibia. Mortality was higher in LW pullets than LB pullets in the first wk. The results suggest that pullets could navigate their environment safely under the Canadian industry standard of 10 lux. Higher L at 30 or 50 lux may have a minor improvement on welfare by increasing bird activity. Conclusively, these light intensities can prepare pullets for navigating complex environments during the laying phase.

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List of Abbreviations

°C	degree Celsius
BW	Body weight
cm	centimeter
g	gram
H/L	Heterophil/lymphocyte ratio
HPA	Hypothalamic-pituitary-adrenal
k	kelvins
kg	kilogram
L	Light intensity
LB	Lohmann Brown-Lite
LED	Light-emitting diode
LW	Lohmann Selected Leghorn-Lite
m	meter
mL	milliliter
mm	millimeter
nm	nanometer
S	Strain
s	second
SEM	Standard error of mean
UV	Ultra-violet
vs	versus
wk	Weeks of age

1.0 Chapter 1. Literature review: The impact of light intensity during pullet rearing on use of space, behaviour, and health

1.1 Introduction / Historical Background

The use of conventional cages in Canada has been steadily decreasing. In 2016, 82% of the layer industry used conventional housing (Egg Farmers of Canada, 2019). Currently (as of 2019), this percentage has been reduced to 66% (Egg Farmers of Canada, 2019). Conventional cages are unfurnished enclosures with wire mesh and sloping floors, feed troughs and a drinker line. Typically housing four to eight hens per cage, conventional cages provide a controllable environment and protect hens from a range of health and injury problems. However, due to limited space, hens are restricted in their movement and are unable to perform many natural behaviours (Lay et al., 2011; Widowski et al., 2013). As a result, conventional cages are beginning to be phased out in Canada, and by 2036, the Canadian National Farm Animal Care Council Codes of Practice for Pullets and Laying Hens has mandated that all laying hens will be housed in alternative housing systems (NFACC, 2017).

This transition away from conventional cages in Canada has already been long established in some other nations worldwide. Conventional cages were banned in Switzerland in 1992 and Sweden in 1999 (Häne et al., 2000). The European Commission (1999) also prohibited the use of conventional cages within the EU by 2012. Instead, only furnished cages or alternative systems can be used (European Commission, 1999). With growing pressure from retailers, animal rights organizations, and consumers (Parrot, 2004; Spain et al., 2018), producers in other countries have also begun transitioning away from conventional cage systems and adopting the use of alternative housing and more specifically non-cage systems instead.

Alternative housing systems include furnished (enriched) cages and non-cage systems, both of which provide more space for the birds to move around and express more natural behaviours. Increases in exercise and load-bearing activities such as wing flapping and running can strengthen bones (Regmi et al., 2015). Resources such as next boxes, perches, and foraging and dustbathing areas are also often provided in alternative housing to satisfy behavioural needs (Olsson and Keeling, 2000; Sandilands et al., 2009). These additional spaces and environmental enrichments improve flock health and welfare (Sandilands et al., 2009; Campbell et al., 2019). However, there is a trade-off. The increase in freedom of movement comes with the cost of an increased risk of injury, especially keel bone damages if hens crash or collide into their surroundings (Whitehead, 2004; Sandilands et al., 2009).

One factor that directly plays a role in navigational ability, and indirectly on skeletal development of the birds, is light intensity (L). Currently, the Canadian Code of Practice for Pullets and Laying Hens (2017) requires a minimum L of 10 lux for pullets reared in alternative housing systems. However, it is recommended that L for newly placed chicks should be higher than 10 lux during the first week to allow chicks to easily locate food and water (Kristensen, 2008; NFACC, 2017). This same logic can be applied to navigation; higher L can improve visual acuity, and is supported by a few studies (Taylor et al., 2003; Moinard et al., 2004b). However, some studies discourage high L as it can increase fear and incidences of negative behaviours such as feather pecking and cannibalism, negatively affecting the well-being of the flock (Kjaer and Vestergaard, 1999). Among studies, the level of L treatments and type of poultry varies (many studies were on broilers). Therefore, it is difficult to accurately summarize what L level is best for laying hens. As such, there is a gap in literature on what L can help bird navigation without negatively affecting health and welfare.

In addition, while many studies have evaluated the effects of alternative housing systems on the health of adult laying hens, few studies have been conducted on pullets. Information in this area is needed because wing feathers grow and short distance flights begin to take place in pullets during the rearing phase (Regmi et al., 2015). Jumping, flying, and any form of exercise positively affects musculoskeletal development with lifelong effects on the health and production of laying hens (Casey-Trott and Widowski, 2016). Additionally, pullets exposed to perches at a young age tend to perform better than those exposed to perches at a later stage in life (Gunnarsson et al., 2000; Regmi et al., 2015). Therefore, ensuring pullets have strong cognitive and navigational ability is crucial in preventing them from crash landings into objects in the environment. Lighting can play an important role since L can act as an assistant for visual acuity for navigation. Therefore, it is important for industry to know what level of L is bright enough to aid pullets in moving around the environment without provoking negative behaviours in the flock.

This chapter will explore different housing systems, how L can impact the environment in which birds are raised, bird vision, and different measurable factors to record or observe when understanding the effect of L on pullets reared in alternative housing. This review aims to better understand the visual, behavioural, navigational ability, and musculoskeletal development of a pullet to navigate a complex environment.

1.2 Alternative Housing Systems

The main features of alternative housing systems are added space and environmental resources. There are various types of alternative housing for laying hens, which will be discussed in the next few sections.

1.2.1 Equipment

The modern laying hen has long been domesticated and selected from its ancestor the Red jungle fowl (*Gallus gallus)*. However, basic biology and behaviour remain similar. The four main behavioural needs of poultry are nesting, perching, foraging, and dustbathing (Weeks and Nicol, 2006; Campbell et al., 2019). As such, there are four main types of equipment that can be found in an alternative housing system as opposed to a conventional cage system. They are nests, perches, and litter for foraging and dustbathing (or dustbaths).

Laying hens are highly motivated to access confined nest sites (Cooper and Albentosa, 2003; Weeks and Nicol, 2006; Engel et al., 2019). Conventional cages do not contain nesting areas, whereas alternative housing systems provide artificial nest boxes (Villanueva et al., 2017). Artificial nest boxes provide enclosed spaces with softer flooring and have been found to elicit nesting behaviour in hens (Appleby and Smith, 1991). Ringgenberg et al. (2014) reported that hens prefer smaller, individual nest boxes as opposed to larger, communal types of nest boxes. It is also recommended for nest boxes to have curtains to ensure proper privacy and shading during laying (Hunniford and Widowski, 2018).

Perching is a natural behaviour in birds. In Red jungle fowl, tree branches are used as perches to allow escape and prevent detection from natural predators (Newberry et al., 2001). This behaviour is still performed by poultry today; perching is a natural behaviour for sleeping and resting (Duncan, 1998). Laying hens are highly motivated to perch and will do so to roost at night or escape from other birds (Duncan, 1998; Newberry et al., 2001). In fact, Olsson and Keeling (2000) reported that lack of access to perches can cause frustration and reduced welfare. Perches offer vertical space and can increase bird activity as it requires pullets to move in more than two dimensions (Newberry, 1995). Understanding a bird’s ability to navigate and utilize perches or raised platforms is important as some alternative systems contain food, water, nest boxes and other equipment on different tiers or levels within the housing environment.

In addition to perches increasing bone strength, they can also contribute to fractures and deformed keel bones (Newman and Leeson, 1998; Whitehead, 2004; Sandilands et al., 2009). The shape, material, and cleanliness of perches can impact foot health and influence the rate at which bone fractures occur (Taylor et al., 2003). For instance, some perches may be easier to see than others, especially in different light intensities. Brown or natural wood coloured perches blend into the background easily and can cause an error in judgement during landing, resulting in crashes and injury (Taylor et al., 2003). In contrast, black and white perches are more easily distinguishable (Taylor et al., 2003). Chicks can perch as early as seven to ten days of age and should be introduced to perches at an early age to allow adaptation (Workman and Andrew, 1989; Gunnarsson et al., 2000). Workman and Andrew (1989) also reported that the amount of time spent perching in pullets will steadily increase over time. Ramps, ladders, or elevated platforms should be included to aid chicks with accessing perches (Stratmann et al., 2015a).

Foraging consists of pecking and scratching at the ground and is related to looking for and eating food. Johnsen et al. (1998) reported that the development of feather pecking is affected during the first four weeks of life by the type of environmental condition the pullets are raised in. Therefore, providing litter or other appropriate substrates for foraging such as straw bales, hay bales, oat hulls, or insoluble grit can encourage foraging behaviour, redirect the attention of the pullets, and reduce the development and incidence of feather pecking within the flock (Kjaer and Vestergaard, 1999; ADSA et al., 2010). In some types of housing systems, a foraging area can be provided in the form of a sandbox or scratching mat (Glatz, 2004).

Dustbathing is observed when a bird squats into the ground and performs a vertical wing shake and rubs its body against the ground (Ericsson et al., 2014). The purpose of dustbathing is to maintain plumage condition and remove feather lipids (van Liere and Bokma, 1987; Lindberg and Nicol, 1997). Some studies disagree that dustbathes are necessary for poultry and instead, sham-dustbathing (in the absence of dustbathing material) is an adequate substitute (Petherick et al., 1991; Lindberg and Nicol, 1997). On the other hand, sham-dustbathing damages the feathers of the birds, affecting their ability to thermoregulate (Hughes and Duncan, 1988). Other studies argued that the presence of a vacuum activity such as sham-dustbathing is enough indication that welfare is reduced, thereby making dustbathing a behavioural need (Van Putten and Dammers, 1976; Hughes and Duncan, 1988). Nonetheless, dustbaths can be provided along with foraging material or a sandbox for a contained area.

1.2.2 Types of Alternative Housing Systems

At the beginning of this review, the properties of conventional cages and the need for alternative housing were discussed. The transition away from conventional cages for laying hens has resulted in the development and improvement of alternative housing systems. The major categories for alternative housing systems include furnished cages, aviaries, free-run, and free-range.

Compared to conventional cages, furnished cages provide opportunities for hens to express more natural movements and behaviours through the provision of additional resources such as perches, nest boxes, forage and dustbathing areas, and scratching mats (Appleby, 1998; Appleby et al., 2002). Furnished cages are larger than conventional cages and may house up to a hundred hens in each cage (Rodenburg et al., 2005). Furnished cages work well to limit the risks of diseases and injuries and allow birds to nest, perch, and forage, thereby improving bone strength and bird welfare compared to birds in conventional cages (Rodenburg et al., 2005; Widowski et al., 2013).

Comparing furnished cages and non-cage systems, major differences exist. These are group size, freedom of movement, and environment complexity, and each system has its own advantages and disadvantages (Rodenburg et al., 2005). Two examples of differences among housing systems include space allowance and group size. Although the space and bird allowance in furnished cages are higher than conventional cages, they are smaller than non-cage systems (Rodenburg et al., 2005). Smaller group size in furnished cages (and conventional cages) can reduce the risk of feather pecking and cannibalism which results in mortality being lower compared to non-cage systems (Lay et al., 2011). In non-cage systems with much higher group size and space allowance, studies have reported higher mortality due to cannibalism, increased risk of disease or parasites, and reduced air quality (dust, ammonia) levels (Tauson, 2005; Fossum et al., 2009). In contrast, non-cage systems contain larger horizontal and vertical space that furnished cages do not have. Although furnished cages can also contain scratch pads and dustbathing systems, a non-cage environment contains access to area with litter and is important because it allows birds to walk, run, and fly which improves welfare and encourages exercise to strengthen bones (Olsson and Keeling, 2000; Widowski et al., 2013). However, there is also increased risk of keel bone fractures from collisions with objects in the environment. Therefore, while both furnished cages and non-cage systems have their advantages, it is important to consider these trade-offs. Nonetheless, both systems provide more behavioural freedom and welfare advantages than conventional cages (Tauson, 2005).

Non-cage systems include free-run, free-range, and organic housing. Free-run and free-range systems typically house groups of hens in larger numbers than conventional and furnished cages. Free-range systems provide access to outdoors, while free-runs do not. Similar to furnished cages, free-run and free-range systems provide birds freedom of movement and expression of natural behaviours such as nesting, perching, and dustbathing, as well as with the added benefit of litter pecking (Widowski et al., 2013). Indoor non-cage systems include single-tier systems or multi-tier aviaries and protect birds from natural predators and weather conditions outdoors. In aviaries, birds have access to different tiers which allow birds more freedom to utilize vertical spaces and improve flight activity and musculoskeletal development of the birds (Newman and Leeson, 1998; Rodenburg et al., 2005). Outdoor non-cage systems such as free-range systems provide hens with access to an outdoor area. As opposed to indoor non-cage systems, birds reared in outdoor systems are at greater risk of contracting disease from wild birds, parasites, natural predators, and health risks from harsh weather conditions (Lay et al., 2011). Organic housing also contains access to an outdoor area with natural light (Berg, 2001). Indoors, organic systems apply loose housing on littered floor with slatted floors, tiers, and nest box spaces, and have slightly larger space allowance for birds (Berg, 2001).

The shift to alternative housing brings about improved welfare for laying hens. There is a general agreement that alternative housing systems such as furnished cages and non-cage systems provide greater freedom of movement and more space to express natural behaviours, benefitting the overall well-being and welfare of the birds. However, while non-cage system environments allow birds more freedom to jump, run, and fly, they can also increase the incidence of bone fractures through crashes and collisions with environmental elements (Sandilands et al., 2009; Lay et al., 2011; Harlander-Matauschek et al., 2015). Studies alluded that the alternative housing environment may not be suited for modern hens lack of flying abilities (Wilkins et al., 2011; Campbell et al., 2019). However, this flaw can be prevented by exposing the birds to these systems during the rearing phase and allowing them to adapt to the housing environment (Whitehead, 2004; Janczak and Riber, 2015; Regmi et al., 2015). To further support the basis of this study, adjusting L may serve as a valuable abettor in helping pullets navigate their environment.

1.3 Light

To understand L, it is important first to comprehend the role light settings play in the rearing of birds. Understanding how light can impact the biological rhythms of a bird is important when managing birds. Light can also affect physiological aspects, which in turn may affect growth, behaviour, and reproduction (Lewis and Morris, 2006). Important characteristics of light are photoperiod (or duration) of lighting, wavelength (or colour), and intensity (or brightness). This review will focus on L.

1.3.1 Light Intensity

Light intensity is the level of brightness projected from a source and can influence bird behaviour and welfare (Boshouwers and Nicaise, 1992; Rubene, 2009). As a result, L can be used to manage birds. Currently, the Canadian Code of Practice for Pullets and Laying Hens (2017) requires a minimum of five lux for laying hens in cages, and ten lux for laying hens kept in non-cage multi-tier systems. Current intensities of five to ten lux keep birds calm and reduce overall aggression within the flock (Widowski et al., 2013). However, it is unknown whether the lack of activity is due to a calming effect or a lack of perception.

Light intensity is typically high during chick placement to stimulate chicks to be more active, eat, and drink (Deaton et al., 1981; Siopes et al., 1983). Many studies on L have been conducted on broilers, and studies on the varying effects of L on laying hens are limited. Further, most of the research has been completed using adult laying hens (Boshouwers and Nicaise, 1987; Taylor et al., 2003; Moinard et al., 2004b; Bleak, 2008; O’Connor et al., 2011), whereas studies on growing pullets are fewer in number (Hughes and Black, 1974; Davis et al., 1999; Kjaer and Sørensen, 2002). Research on L’s impact on pullets is crucial to know because this information can be helpful as producers transition to alternative housing systems. Compared to adult laying hens, pullets are smaller in size and are still growing, developing their skeletal and muscular strength. The effect of activity levels is critical since exercise can affect bone formation due to load bearing (Tauson and Abrahamsson, 1994, 1996; Hester et al., 2013). While low light intensities might discourage successful navigation (Taylor and Scott, 2002; Taylor et al., 2003), high light intensities can increase fear, aggression, and its related welfare issues (Hughes and Duncan, 1972; Kjaer and Vestergaard, 1999; Rubene, 2009). The outcomes of aggression and severe feather pecking have negative consequences on bird health and welfare, which in turn would decrease production value and lead to economic losses for producers (Rubene, 2009). Therefore, it is important to understand and find an intermediate balance between the two that will not compromise the behaviour, health, and welfare of these birds.

1.3.2 Source of Light

A number of light sources are currently available, namely incandescent, fluorescent, and light-emitting diode (LED) lights, each one varying from another in characteristics. Incandescent lights emit high amounts of red light, which can appear red-saturated to chickens, and have been shown to increase aggression and severe feather pecking activity (Prescott and Wathes, 1999; Rubene, 2009). Lewis and Morris (1998) also reported delayed sexual maturity in broiler breeders kept under incandescent lights. Fluorescent lights, on the other hand, emit very little red light and some ultra-violet light and were preferred by broiler chickens in a study by Kristensen et al. (2007). Widowski et al. (1992) reported preference towards fluorescent than incandescent lighting (both at 12 lux) in 24-week-old laying hens. The birds preened more frequently in fluorescent lighting, which the authors indicated may be due to the different appearances of plumage under different light sources (Widowski et al., 1992). The presence of ultra-violet (UV) light can act as visual enrichment and plays an important role in social interactions and mating behaviour in chickens (Prescott and Wathes, 1999; Maddocks et al., 2001). In fact, Maddocks et al. (2001) suggested that a lack of UV light in poultry housing could increase stress levels and alter behaviour in birds. However, Fitzsimmons and Newcombe (1990) reported that despite differing light intensities in fluorescent or incandescent lighting, similar agonistic behaviour in laying hens was observed. Meanwhile, LED lights are becoming more common in the poultry industry due to their many advantages over other light sources, including high energy efficiency, long operating life, and low cost (Parvin et al., 2014). Huth and Archer (2015) reported a preference for LED lighting over incandescent lighting in broilers. LED lights also provide the ability to alter wavelength (colour), which some studies have reported may be advantageous in influencing specific behaviour, growth, and reproductive function in birds (Parvin et al., 2014). In contrast, Lewis and Morris (2006) reported that there is no evidence that one type of light source is better than another in performance.

1.4 Vision in Birds

Now that the basic characteristics of alternative housing and how L may play a role in pullet navigation have been discussed, it is important to understand how vision in birds works. This section covers the mechanism for how birds perceive light, how birds perceive brightness (rods and cones), and the influence of L on bird vision.

1.4.1 Light Perception

Although birds perceive light through their retina like humans, their large, highly sensitive eyes allow them to have a higher relative sensitivity to particular light wavelengths than humans (Figure 1.1; Hassan et al., 2013). As a result, birds perceive certain colours as brighter than humans (see next section, Hassan et al., 2013). The second way chickens respond to light is through extra-retinal light receptors present in the pineal gland and hypothalamus (Lewis and Morris, 2006). For the stimulation of these receptors, light needs to penetrate through the top of the skull and tissues. Stimulation of the pineal gland maintains circadian rhythmic balance controlling the secretion of melatonin, which influences night body temperature, regulates sleep, stress, and immunity levels (Lewis and Morris, 2006). The hypothalamus regulates the pituitary gland, which secretes growth hormone and reproductive hormones such as follicle-stimulating hormone, luteinizing hormone, and 17-estradiol (Mitchell, 1970; Lewis and Morris, 2006). Lewis and Morris (2006) reported that for extra-retinal light stimulation, the L must be at least 1.1-3.3 lux to penetrate the skull and cranial tissues to reach the pineal gland and hypothalamus.

1.4.2 Ocular Anatomy

Relative to body size, birds have the largest eyes among vertebrates, indicating the dependency on vision (McFadden, 1993). The anatomy of the avian eye is similar to that of most other vertebrates; light enters anteriorly and is refracted by the cornea and lens, and an image is projected onto the retina (Figure 1.2; Hughes, 1977). The large size of the avian eye correlates to the number of photoreceptors, allowing high amounts of light to be reflected and thus producing good quality of images to the bird (Hughes, 1977; Hall and Ross, 2007).

There are several similarities and differences between the avian and the human eye. While humans have a small pit located in the retina called the fovea that functions to enlarge images, birds have two foveas, for near and distant vision (Figure 1.2; Jones et al., 2007). Birds also have a nictitating membrane that acts as a third eyelid (Jones et al., 2007). Acting independently from the other two eyelids, the nictitating membrane possesses its own lubricating duct and serves to protect, clean, and moisten the eye (Jones et al., 2007).

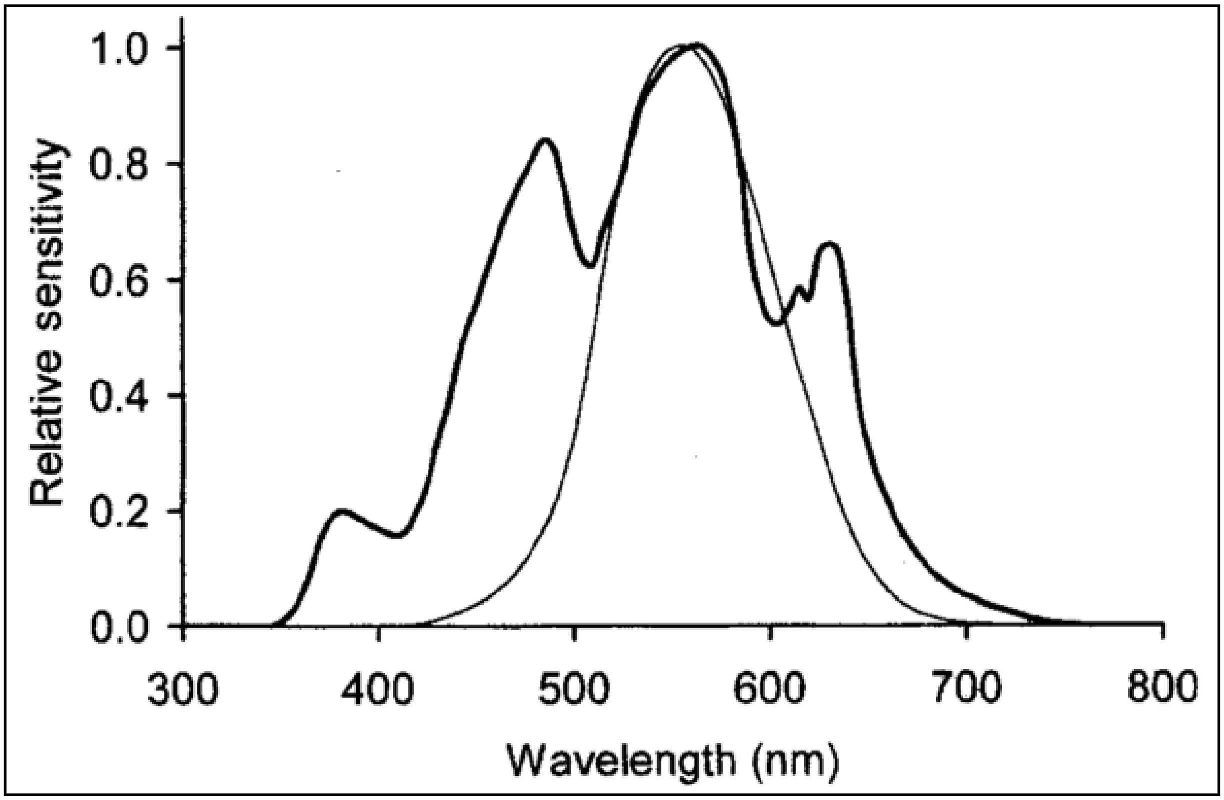

Figure 1.1 Spectral intensities of domestic fowl (bolded) and humans (Lewis and Morris, 2006). Domestic fowls have higher relative sensitivity to certain wavelengths than humans.

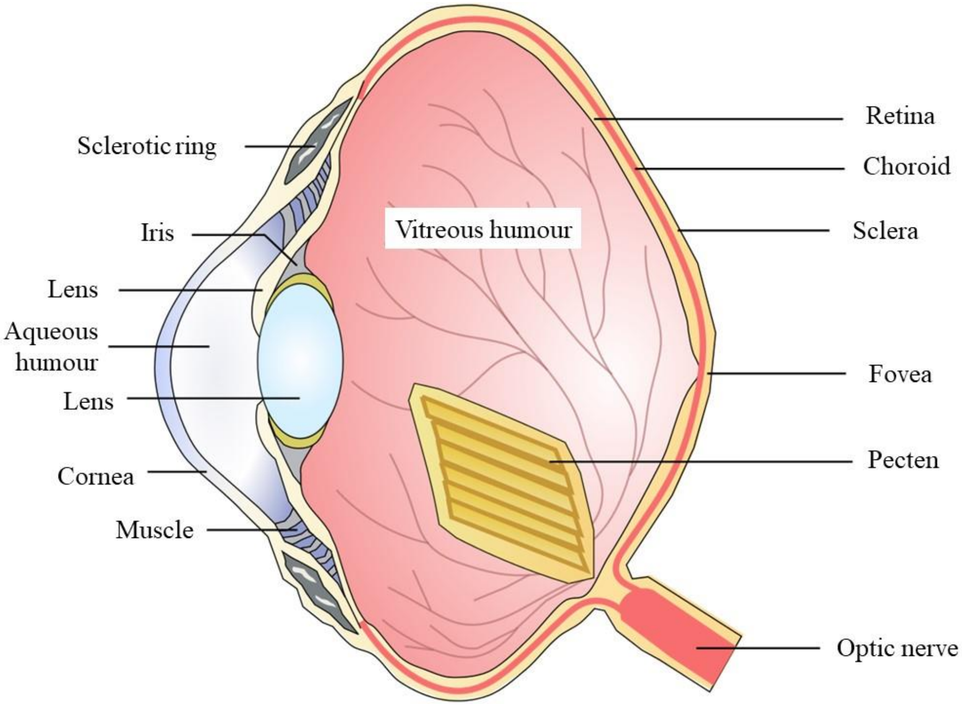

Figure 1.2 Anatomy of a bird's eye (adapted from Bleak, 2008).

Additionally, the ratio between the axial (vertical and horizontal) lengths of the eye differs between birds and humans and plays a role in visual acuity (Green et al., 1980; Hall and Ross, 2007). The larger the axial length, the larger the image projected onto the retina, and the better the resolution of the image (Green et al., 1980).

Avian eyes are more oval than humans and have a shorter horizontal axial length, however birds have a larger retina that contains more photoreceptor cells, thus allowing them to receive a heightened sense of visual acuity (Green et al., 1980; Hall and Ross, 2007). Additionally, diurnal animals such as chickens are visually dependent, therefore the size of the cornea is larger, maximizing the chances for light to enter the eye (Hall and Ross, 2007).

1.4.3 Rods versus Cones

In the retina, there are two types of photoreceptor cells: rods and cones. Rods are highly sensitive photoreceptors, which allow vision in poor light or scotopic conditions (Lewis and Morris, 2006). Several rods are linked to one nerve fibre, resulting in images lacking in definition and colour (Lewis and Morris, 2006), however, birds have better night vision than humans since rods in birds are more sensitive to light than humans (Purves et al., 2004; Lewis and Morris, 2006).

The second type of photoreceptor, cones, allows vision in bright light or photopic conditions and produces high definition images in colour as a result of each cone having its own nerve fibre (Lewis and Morris, 2006). Humans have a trichromatic colour vision (Lewis and Morris, 2006), meaning that the human eye possesses three types of cones that allow specific wavelengths of light to penetrate through, namely violet/blue- (419 nm), green- (531 nm), and red- (559 nm) sensitive cones (Figure 1.1; Purves et al., 2004). Birds have an additional type of cone, labelling birds as tetrachromatic organisms (Lewis and Morris, 2006). Each cone of a bird’s eye contains a coloured oil droplet which sharpens their vision with a peak vision sensitivity including at 415 nm, falling in the ultraviolet- or violet-sensitive zone (Figure 1.1,Govardovskii and Zueva, 1977; Vorobyev et al., 1998). This spectral sensitivity is greater than that of humans, resulting in poultry perceiving most wavelengths of light as brighter than humans. The term “lux”, which is a measure of the illuminance of light as perceived by the human eye does not reflect L as perceived by chickens and the term “clux” or “gallilux” is used instead for poultry (Lewis and Morris, 2006). Another unit of measure “foot candles” is also used as a measure of illuminance of light. In this review, the unit “lux” will be used.

1.4.4 Light Intensity and Vision

With regards to the effect of L and vision, several studies were found. Harrison et al. (1968) reported enlarged eyes, an abnormality which may lead to eye lesions and reduced visual ability (Harrison et al., 1968), in White Leghorns housed in approximately six lux (versus 269 lux), and Siopes et al. (1983) also observed a similar result in turkey poults reared in 1.1 lux (versus 11, 110, or 220 lux). In another study, Olanrewaju et al. (2007) observed more eye lesions in broilers reared in 0.2 lux versus 20 lux. Deep et al. (2010) reported larger and heavier eyes in broilers reared in one lux (versus 10, 20, or 40 lux). Rault et al. (2017) also reported 5% heavier eyes in broilers reared in five lux than those in 20 lux. Another study by Blatchford et al. (2009) reported that the eyes of broilers reared in five lux were heavier than those reared in 50 and 200 lux, which the authors claimed is due to inflammation of the choroid and degeneration of the retina. However, the authors also stated that the effect of these conditions on bird vision was not evaluated (Blatchford et al., 2009). Kristensen et al. (2002) also reported decreased visual acuity in broiler chicks in five lux versus 100 lux. Based on these studies, it appears that L of less than five lux can negatively affect the eye and to some extent compromise visual acuity, leading to sensory deprivation and eye abnormalities (Manser, 1996).

1.5 Brown- versus White-Feathered Strains

Genetic differences can result in character differences in terms of behaviour and reaction. For instance, Troilo et al. (1995) stated that different strains (S) could vary in eye size which can result in different sensitivity to L. This review will focus on overarching differences between brown-feathered and white-feathered S of laying hens.

One major difference is growth morphology. Brown-feathered hens are heavier and have larger bone sizes than white-feathered hens (Knowles et al., 1993; Bishop et al., 2000; Riczu et al., 2004), whereas, white-feathered hens are smaller and can generate energy for flights more easily than brown-feathered hens (Marden, 1994; Moinard et al., 2004b; Tobalske and Dial, 2007). This may be reflected in studies which observed higher levels of activity in white-feathered hens than brown-feathered hens (Fraisse and Cockrem, 2006; Kozak et al., 2016; Pusch et al., 2018).

For vision, between brown- and white-feathered S, Karlsson et al. (2009) and Lisney et al. (2012) reported no difference in the intensity to flicker fusion frequency curves (a measure of visual function and sensitivity) between the two S. Despite this, chickens can display differences in behaviour and reactivity, and therefore it is important that both brown- and white-feathered S are tested in studies (Lindqvist and Jensen, 2009; Lisney et al., 2011).

Another difference between the two S is their coping mechanisms. Pusch et al. (2018) described two main coping styles in animals that correlate with the strength of stress responses: proactive and reactive. These can further be assigned to whether they are behavioural or physiological (Peixoto et al., 2020). Proactive responses tend to be fast through lower physiological response and a faster behavioural response (Peixoto et al., 2020). On the other hand, reactive animals produce a higher physiological and slower behavioural response (Cockrem, 2007; Peixoto et al., 2020). White-feathered S tend to produce stronger physiological and faster behavioural responses to acute stressors, whereas brown-feathered S exhibit less dramatic fear responses and have a lower physiological response (Cockrem, 2007; Pusch et al., 2018; Peixoto et al., 2020). Understanding the nature of these characteristics can provide insight into the reactions, behaviours, and physiological responses of these S, especially when exposed to higher L.

1.6 Assessing Welfare

The previous sections have established the foundations for the rest of the literature review. Firstly, the differences between conventional cages and alternative housing were discussed, along with the benefits and need for alternative housing for laying hens. Secondly, bird perception and image processing were reviewed to understand vision in birds and its importance to navigate complex environments such as in alternative housing. Light was acknowledged as an important factor in managing birds and L was introduced to play a key role, especially with navigation. The last background section provided insight into the different characteristics of brown- and white-feathered S and their responses to environmental cues. Combined, these sections increased the understanding of how L affects egg-strain chickens reared in different types of housing. All these fundamental details were described with the main intention of discovering ways to improve the welfare of egg-strain birds.

Welfare is defined and classified into three main categories: biological function, affective states, and ‘natural’ living (Fraser, 2008). Biological function refers to the basic aspects of health of an animal, including growth, diseases, injuries, and productivity, whereby jeopardization of any of these parameters indicates a reduced welfare state (Moberg, 1985). Affective states promote the concept of an animal’s experience, citing the importance of incorporating animal feelings to determine welfare (Dawkins, 2012). Natural living involves many elements that simply put, enables an animal to perform various normal and natural behaviours (Fraser, 2008). In combination, assessing these categories can provide insight into the well-being and welfare state of an animal. These three categories are commonly used in the scientific measure of animal welfare and will be applied to this study as well.

Understanding bird perception and responses to different housing environments and L – as shown in the previous sections – will provide a clearer view on the types of measures required to assess welfare properly. In this case, there is interest in discovering the effect of L on egg-strain pullets reared in alternative housing containing enriching devices such as perches. As such, possible welfare measures include behavioural assessments, physiological response, health, and growth of pullets. The next sections will explain each welfare measure, as well as reports from current scientific literature.

1.7 Behaviour

One of the first ways producers can tell about the health, comfort, and well-being of a bird is by observing its behaviour. In many scientific studies, behavioural performance is also used to assess welfare. Animal behaviours are often reflective of their affective state, therefore, understanding and observing different animal behaviours can help determine the animal’s current state of welfare (Duncan, 1998). The different behaviours performed by pullets can be classified into different categories. In this study, they are active, inactive, comfort, nutritive, exploratory, severe feather pecking, and aggressive behaviours. Behaviours that occur during the laying phase of a hen’s life, such as nesting, are not measured in this study, and therefore are not covered.

1.7.1 Active Behaviour

Active behaviour includes standing, walking, and wing assisted jumping and flying. These active behaviours are important in a bird’s life and may be an indicator of positive welfare (Dawkins, 2003). On the surface, these behaviours help birds move from one place to another and promote exercise. Internally, these movements strengthen bones and improve muscle quality (Le Van et al., 2000; Bessei, 2006). If a bird is balancing while perching or using wing assisted jumping or flying, wing and breast muscles are also used, promoting muscle development (Newberry and Hall, 1990; Le Van et al., 2000). In fact, early development in such areas can increase the bird's strength and help to reduce future risks of fractures and bone breakages (Casey-Trott and

Widowski, 2016). This behaviour can be encouraged through provision of perches and ramps (LeBlanc et al., 2018).

According to published scientific literature, laying hens increase active behaviours with increasing L. In broilers, Newberry et al. (1988) reported increased walking and standing in broilers reared in 180 versus six lux. Kristensen et al. (2007) also observed more standing in broilers in 100 lux than five lux. In layers, several papers have also reported an increase in activity with L, such as a study by Kjaer and Vestergaard (1999) looking at ten-week-old ISA Browns reared in floor pens in 30 vs three lux, a study by Hughes and Black (1974) with 14-week-old White Leghorns reared in cages in 55 to 80 lux versus 17 to 22 lux, a study by O'Connor et al. (2011) looking at 16-week-old Hy-line Browns reared in floor pens with enrichment devices in 150 versus five lux, and a study by Boshouwers and Nicaise (1987) with 35-week-old White Leghorns reared in battery cages with L that ranged from 0.5 to 120 lux.

1.7.1.1 Jumping Frequency and Accuracy

Red jungle fowl, the ancestor of modern chickens, frequently jumps and flies between locations to roost in trees at night (Moinard et al., 2004b). These behaviours require accurate manoeuvring and visual accuracy especially during low light levels during dusk and may be well adapted in poultry today (Moinard et al., 2004b). In fact, Taylor and Scott (2002) reported that visual acuity decreases when light in the environment falls below five lux. Mentioned previously, active behaviours such as jumping increase bird exercise which can improve bone health and strength. Identifying whether increasing L can increase not only jumping behaviour but also navigational success of pullets can improve both the health and welfare of the flock.

Several studies have reported the effects of L on jumping behaviour of pullets. Taylor and Scott (2002) and Taylor et al. (2003) reported that 59-week-old Hyline Plus hens had a higher latency to jump from one perch to another (distances of 0.5 m and 1.0 m) at lower light intensities (1.5 lux and 0.8 lux) than higher light intensities (six lux and 40 lux). On the other hand, Moinard et al. (2004b) reported no difference in jumping behaviour between 25-week-old Lohmann Brown hens reared in five, ten, or 20 lux. The difference in results between the two studies could be due to the L or age used. Indeed, Kozak et al. (2016) reported that 10-16 week-old pullets performed more aerial ascents than the 17-24 and 25-37 week-old hens. It is possible that jumping behaviour decreases with age and would explain the disagreement between results from different authors.

1.7.2 Inactive Behaviour

Inactive behaviour includes resting which promotes better energy utilization (Alvino et al., 2009b). Rest is a prolonged period of inactivity allowing energy conservation, tissue restoration, and growth (Blokhuis, 1984; Malleau et al., 2007). Rest is also an important contributor to welfare, as proposed by Blokhuis (1984) that rest helps the animal cope with stress and adaption to its environment. Resting is identified behaviourally in two ways. When dozing, the head is retracted and when sleeping, the head is tucked under the wing (Blokhuis, 1984; Yngvesson et al., 2017). However, excessive levels of resting can suggest reduced welfare (O'Connor et al., 2011). In a study by O'Connor et al. (2011), laying hens exposed to continuous high levels of noise spent more time resting than those not, which the authors suggested may be due to disrupted deep sleep, requiring an increased rest time. Therefore, although resting is a contributor to welfare through energy regeneration, excessive resting behaviour may suggest lethargic birds which could have negative health and welfare impacts (Jones et al., 2010).

With L, in broilers, Alvino et al. (2009a) observed an increase in resting behaviour in broilers reared in five lux than 50 or 200 lux, and Deep et al. (2012) reported increased resting in broilers in one lux (versus 10, 20, or 40 lux). In both studies by (Alvino et al., 2009a) and Deep et al. (2012), increased resting in broilers referred to excessive resting which had negative impacts on bird health. In layers, Boshouwers and Nicaise (1987) reported that resting is independent of L ranging between 0.5 and 120 lux in laying hens. However, Davis et al. (1999) reported that six-week-old ISA Brown pullets preferred L at six lux than 200 lux to perform inactive behaviours.

1.7.3 Comfort Behaviour

Comfort behaviour in laying hens typically involves feather and body maintenance behaviours (Nicol, 1990). These include preening, which is a self-directed grooming activity to maintain healthy plumage (Delius, 1988; Appleby et al., 2004), wing or leg stretching, tail wagging, head shaking, self-scratching, feather ruffling, and wing flapping which are bodily movements (Albentosa and Cooper, 2004; Nicol et al., 2011). Wing or leg stretching, tail wagging, and wing flapping are more commonly found in birds reared in spacious environments (Albentosa and Cooper, 2004). Dustbathing is also a comfort behaviour. It is a natural behaviour to maintain plumage condition by balancing lipid levels in feathers (Olsson and Keeling, 2005; Shields and Greger, 2013). It is observed in the presence of litter or dustbathes, however in the absence of dustbathing material, sham-dustbathing can also occur as a vacuum activity, which may be associated with stress (Lindberg and Nicol, 1997). Additionally, preening can be associated with stress, known as displacement preening. This usually occurs when a bird is expressing frustration or stress (Duncan and Wood-Gush, 1972).

Due to the time budget, in laying hens, behavioural requirements are prioritized towards nesting, perching, and foraging, resulting in lower frequencies and observations of comfort behaviours (Cooper and Albentosa, 2003; Albentosa and Cooper, 2004). Similarly, disruptions of comfort behaviours can indicate feelings of discomfort and frustration. This is reflected in the effects of L on comfort behaviours. Nonetheless, these behaviours are still important as they reflect the comfort level and welfare status of the bird (Nicol, 1990; Albentosa and Cooper, 2004). In broilers, Alvino et al. (2009a) reported that broilers spent more time preening when reared in 200 lux compared to five lux. The authors suggested that higher light intensities (such as the 200 lux intensity) may improve visual acuity and encourage the birds to maintain good plumage condition than those housed in a dimmer environment (Alvino et al., 2009a), possibly because in high L the birds are able to see things on the plumage that attracts them and stimulates the behaviour (Widowski et al., 1992). This is supported by Deep et al. (2012) who observed that broilers reared in one lux preened less than the other intensities in their study (10, 20, or 40 lux). In layers, Vandenberg and Widowski (2000) observed that Leghorn hens preferred high intensity high-pressure sodium lighting than low intensity incandescent lighting via increased preening and nesting.

1.7.4 Nutritive Behaviour/Growth

All creatures need food and water to maintain health and well-being. Observing the feeding and drinking behaviours in birds can serve as a good indicator of a bird's basic health and functioning, which suggests their current state of welfare. For instance, laying hens require an increase in feed intake for the onset of lay (Ward and McKague, 2007). They also need specific nutrients, such as calcium, to maintain egg production (Hurwitz, 1965). Over half of the water intake in poultry is related to feed consumption (Ward and McKague, 2007). Water consumption rates are affected by environmental temperature and humidity, water temperature, salinity, and impurities (Ward and McKague, 2007).

In relation to L, dim conditions (less than one lux) can impede birds from seeing clearly and navigating their way around clearly, which may result in starvation, dehydration, and death (Prescott and Wathes, 2002; Deep et al., 2012). On the other hand, a study by Newberry et al. (1988) comparing six and 180 lux observed that despite increase in broiler activity in higher L, no difference in feed conversion was found. The authors suggested that the increased activity does not affect the energy requirements of the birds (Newberry et al., 1988). This was supported by Deaton et al. (1981) who found no difference in body weight between broilers reared in 75 and five lux. These findings could be due to the difference in energy distribution in broilers since broilers have been selected for their meat and rapid body weight gain, resulting in high feed intake. In laying hens, Davis et al. (1999) reported increasing feed and water consumption with increasing L (six, 20, 60, or 200 lux) in ISA pullets at two and six weeks of age (wks). Prescott and Wathes (2002) also observed that ISA brown hens preferred feeding in high intensity (200 lux) than low intensity (less than one lux) lighting and were willing to work harder to gain access to feed in high L. However, Kjaer and Vestergaard (1999) reported that feeding behaviour was not affected by L (three or 30 lux).

1.7.5 Exploratory Behaviour

The purpose of exploratory behaviour is to gather information by interacting with the environment and its resources (Newberry, 1999). As such, lack of stimuli in the environment can result in boredom and frustration which compromise welfare (Newberry, 1999). Birds use their beaks to explore the environment (Rogers, 1995). Exploratory behaviours can be directed towards the ground, including litter pecking (also known as foraging), ground scratching, and head sweeping. This behaviour can also be directed towards objects in the environment, such as pecking at walls, perches, feeder bins (without consumption), or drinker lines (without consumption). Finally, gentle feather pecking at other birds in the environment is a form of social exploration (Riedstra and Groothuis, 2002). Gentle feather pecking is observed when a bird runs another bird's feathers through its beak without harming the recipient (Kjaer and Vestergaard, 1999). Feather pecking can develop into a behavioural disorder that compromises the health and welfare of a bird. Injurious or damaging feather pecking has been studied by many scientists, however, to avoid confusion in the present study, this type of feather pecking is referred to as ‘severe feather pecking’. Severe feather pecking is covered in the next section.

Regarding the effect of L, when reporting on feather pecking, many authors focused on severe feather pecking and not gentle pecking. One paper was found on gentle pecking in layers. Kjaer and Vestergaard (1999) observed that gentle pecking was expressed more frequently in ten-week-old ISA Brown hens reared in three lux than 30 lux, which the authors attributed to the reduced ability to identify environmental cues.

Other forms of exploratory behaviour were reported more frequently. In broilers, Kristensen et al. (2007) observed more foraging (litter pecking) in broilers reared in five lux than 100 lux. This is in contrast with Alvino et al. (2009a) who found that broilers reared in five lux foraged less than those in 50 or 200 lux. A study by Deep et al. (2012) reported that broilers in one lux foraged less in comparison to their other L treatments (ten, 20, 40 lux). In layers, Kjaer and Vestergaard (1999) observed that ten-week-old ISA Brown pullets tended to forage more in 30 lux than three lux.

1.7.6 Severe Feather Pecking

Severe feather pecking occurs whereby feathers are plucked resulting in feather damage, skin damage, and blood loss (Hughes and Duncan, 1972; Huber-Eicher and Audigé, 1999). Severe feather pecking may arise from pecking during dustbathing (Vestergaard et al., 1993) or as redirected ground pecking behaviour (Blokhuis, 1986) and can lead to toe pecking and cannibalism, which places the health of the entire flock at risk (Kjaer and Vestergaard, 1999; Rodenburg et al., 2013).

Light intensity may affect severe feather pecking as increased L may amplify the birds' perception of colours and attraction towards other birds' plumage (Nicol et al., 2013). A few studies have reported increased severe feather pecking with increasing L. Kjaer and Vestergaard (1999) observed increased feather damage and decreased feather plumage at high L (30 lux versus three lux) in 28-week-old ISA Brown pullets. However, the authors also noted that severe pecking was reduced at 45 wks, indicating the possibility that L effects are slowly reduced after the start of the laying period (Kjaer and Vestergaard, 1999). Hughes and Duncan (1972) also reported that from three to 21 wks, egg-strain birds (Thornber 808, 909, and Shaver 288) reared in high L received more pecking than those in low L. In their experiment, all the birds were housed in a floor pen with the two high light intensities (six lux to 44 lux) placed at the end of each side of the pen, and the gradual decline in L towards the center was used as the low intensity treatment (one to 11 lux).

On the other hand, many authors reported that L does not affect severe feather pecking. In a study by Kjaer and Sørensen (2002), L (three lux versus ten lux) did not affect the plumage condition of ISA and LSL hens at 35 wks. The authors suggested that the L treatment differences may have been too little to have significant effects. However, Hartini et al. (2002) who used a bigger difference between intensity treatments also reported that the incidence of cannibalism was not influenced by high L (60-80 lux) vs low (five lux) during the housing of ISA brown hens. Similarly, Hughes and Black (1974) found no consistent effect of L (17-22 lux vs 55-80 lux) on feather pecking in 14-week-old white leghorns. Huber-Eicher and Audigé (1999) conducted interviews on table egg farms in Switzerland and reported that L did not affect feather pecking (low intensity was less than six lux, average high intensity not stated).

1.7.7 Aggressive Behaviours

Aggression consists of agonistic behaviours and in poultry, stems from fights for social dominance, competition for food and other resources, living spaces, and survival conditions (Pagel and Dawkins, 1997). Aggressive behaviour include bird-to-bird pecking which are forceful pecks directed at the head or neck of the recipient (Rodenburg et al., 2013). Aggressive behaviours are accompanied by changes in the physiological state of the animal and are reflected likewise (Candland et al., 1969). As such, measuring physiological responses such as the stress response can be used as a welfare indicator and is addressed in the next few sections (see Stress Response).

The effect of L on aggressive behaviour is reported in a study by Shinmura et al. (2006). White Leghorns at 17 wks were housed in battery cages, furnished cages, or aviaries, with natural light through windows or fluorescent lights, both at an equivalent of 680 lux at the feed trough (Shinmura et al., 2006). Due to increased aggression, L was decreased to 70 lux for all housing types and birds in the furnished cages had their beaks re-trimmed (Shinmura et al., 2006). Behavioural observations reported decreased aggression in the flock that received a beak re-trim, but not in the others, indicating that decreasing L did not reduce aggression (Shinmura et al., 2006). The authors suggested that 70 lux may not have been dim enough to control aggressive pecking within the flock (Shinmura et al., 2006). Another study by Wechsler and Schmid (1998) on male Japanese Quails reported that only when L was reduced to one to five lux (versus 39 to 370 lux, seven to 36 lux, or 15 to 170 lux, from the corners to the center of the pen) aggressive pecking rates were reduced.

1.8 Stress Response

Change in physiological systems is another tool to assess bird welfare. In response to a stressor, the central nervous system activates the sympathomedullary and hypothalamic-pituitary-adrenal (HPA) axis. The sympathomedullary pathway is also known as the ‘fight or flight’ response, whereby adrenaline is released to give the body quick bursts of energy. On the other hand, the HPA axis prepares the body for sustained exertion especially when a bird is stressed for a longer duration. Through a series of releasing hormones and signals along the HPA pathway, the adrenal cortex releases glucocorticoids such as corticosterone in birds. Another consequence of a long-term stressor is the body undergoing several physiological changes where energy expenditures are directed toward coping or dealing with the source of stress and away from maintenance and production status (Gross and Siegel, 1983). This results in changes in immune system and function such as heterophil/lymphocyte (H/L) ratio, which is a reliable measure for the stress response in chickens (Maxwell, 1993; Maxwell and Robertson, 1998).

Heterophils are the avian equivalent of neutrophils in mammals; they work as the first line of defence in the immune system in activating several anti-microbial mechanisms, such as phagocytosis (Genovese et al., 2013). Lymphocytes are white blood cells that recognize non-self-antigens and initiate the body's immune response through the production of cytokines and antibodies (Lilliehöök et al., 2004). In chickens, the H/L ratio has been found to increase in response to different types of stressors, such as road transportation, poor air quality, cold stress, fear, and acute noise (Maxwell and Robertson, 1998). However, a decrease in H/L ratio can be observed during life-threatening situations, such as severe feed restriction (or multiple fasting events) and extreme heat exposure of over 40 degrees Celsius (Maxwell, 1993; Maxwell and Robertson, 1998). These strong stressors cause heteropenia, whereby mature heterophil numbers are exhausted and immature cells are released, resulting in a decreased H/L ratio (Maxwell, 1993). Since different stressors cause differential increase or decrease in H/L ratio, it is important not to base the interpretation of a study solely on one measurement. Rather, it is important to incorporate other measurements, such as behavioural observations and health-related indicators to appropriately assess whether the birds are stressed in their environment (Lentfer et al., 2015).

There are a few reports on the effect of L on H/L ratios. Lien et al. (2007) reported no effect of L on H/L ratio in broilers reared in one versus ten lux. Using blood corticosterone levels, Olanrewaju et al. (2011) reported no difference in stress levels in broilers reared in 0.2, 2.5, 5.0, 10.0, and 25.0 lux. Similarly, Rault et al. (2017) reported no difference in corticosterone levels in broilers at five versus 20 lux. In a study by Fidan et al. (2015), broilers reared in 50 lux were exposed to 50, 100, or 200 lux of light for 30 seconds every two hours. Similar to previous reports, the authors reported no effect of L on H/L ratios (Fidan et al., 2015). In laying hens, O'Connor et al. (2011) observed no physiological differences or H/L ratio and corticosterone change in 16-24 week-old laying hens housed in five versus 150 lux, however other differences were noted; hens housed in five lux had lower egg production than those in 150 lux.

1.9 Fear Response

Another method to assess the welfare of birds is by analyzing fear responses. Fear is characterized by various behavioural, emotional, and physiological reactions leading to different responses and strategies (Jones, 1996; Campler et al., 2009). In poultry, behavioural and physiological fear responses may vary depending on the type of stimuli and genetic differences (Forkman et al., 2007). Several methods can be used to determine the level of fearfulness in animals. A typical fear test involves measuring the reaction of an animal when exposed to a situation or stimulus that can be unpleasant. These tests include placing the animal in a novel environment, also known as the "open field test", or placing a novel object into the test arena, known as the "novel object test", or even exposing the animal to a simulated predator attack, known as the "predator test" (Forkman et al., 2007). The reactions of the animals are recorded up to a set maximum time and these fear responses can be correlated physiologically and behaviourally (Jones, 1996; Campler et al., 2009).

In poultry, a common reaction to stimulus is avoidance or immobility (Jones, 1996). If unthreatened, this cautionary behaviour will be reduced and fear responses lowered while the bird begins to move and investigate (Jones, 1996). As such, a fear test such as the novel object test measures the latency for a bird or a flock to peck at a novel object introduced to the environment. Studies have reported that the longer the time taken for the birds to peck at the novel object (or the longer the duration of avoidance), the more fearful the flock is (Hughes and Black, 1974). Fear responses may also be S dependent and was mentioned in the previous section (see Brown- versus White-Feathered Strains).

Light intensity appears to affect the level of fear in chickens. Hughes and Black (1974) reported that hens were more fearful in 17-22 lux than in 55-80 lux. In contrast, Perkins (2001) observed broilers to be less fearful in five lux than 20 lux. Olanrewaju et al. (2007) also reported no difference in tonic immobility, another indicator of fear, in broilers housed in 0.2, 2.5, 5, 10, and 25 lux. Fidan et al. (2015) also performed tonic immobility test on broilers housed in 50 lux which were exposed to 50, 100, or 200 lux of light for 30 seconds every two hours. The authors reported no effect of L on the fear response on broilers.

Fear is also associated with abnormal behaviours in poultry such as feather pecking (Jones, 1996), which was discussed in the previous section (Severe Feather Pecking). It is important to note that behaviour, stress indicators, and fear responses should not be used alone as the sole indicator of bird welfare. Rather, a combination of these assessments is encouraged.

1.10 Bone Health

Previously, jumping behaviour was discussed as a common activity performed by laying hens which can increase muscle mass and bone strength. As such, especially in this study with navigation during the rearing phase being the focus, understanding bone structure and formation and the types of damages are crucial. The following sections will focus on bone formation and the evaluation of bone strength, as well as keel bone health.

1.10.1 Bone Formation

There are three forms of bone in birds: cortical, trabecular, and medullary. The cortical and trabecular bone supports bone structure; the cortical bone forms the dense outer surface while the trabecular bone consists of bone marrow and blood flow for bone remodelling and repair (Mueller et al., 1964; Whitehead, 2004). The medullary bone is unique to birds and crocodilians and serves as a labile source of calcium for eggshell formation (Mueller et al., 1964).

The formation of bones in laying hens has been reviewed extensively by Whitehead (2004). Two types of bone development occur during the rearing phase of a laying hen. The first is longitudinal growth through a process called endochondral ossification (Whitehead, 2004). Located in the germinal layer of the epiphyseal growth plate (area of growing tissue), chondrocytes (cells found in the cartilage tissue) that contain fibroblastic properties grow and proliferate to form columns of cells packed within an extracellular matrix (Whitehead, 2004). These cells separate within their columns and begin to differentiate and enlarge or undergo hypertrophy (Whitehead, 2004). Meanwhile, various compounds (type II collagen, type X collagen, proteoglycans, growth factors) are secreted and help regulate chondrocyte development (Whitehead, 2004).

Later, chondroclasts (cartilage absorbing cells) absorb the extracellular matrix while bone mineral develops (Whitehead, 2004). The chondrocytes are replaced by osteoblasts (bone-forming cells) which begin to form the matrix of a bone (Whitehead, 2004). During this period, osteoclasts (bone-resorbing cells) are also activated to help with bone remodelling. The coupling of these two cells at work eventually forms the inner trabecular bone, which is the irregular structure of collagen fibrils (Whitehead, 2004). The bone continues to elongate and begins to widen through a process called intramembranous ossification (Whitehead, 2004). Osteoblasts secrete layers of lamellar bone which form and strengthen the cortical bone layer, and osteoclasts resorb the inner surface of the bone, thus widening while forming the outer surface of the bone (Whitehead, 2004).

At the onset of sexual maturity in a laying hen, many changes take place in the bone. Initially forming lamellar cortical bone, osteoblasts change their function to produce medullary bone which is an irregular arrangement of collagen fibrils (Whitehead, 2004). The medullary bones are built up rapidly within the structural bones especially of leg bones initially during the laying period and is then used over the laying period to supply calcium for eggshell formation (Whitehead and Fleming, 2000). Meanwhile, osteoclasts still function to resorb structural bone, resulting in a gradual decline of structural bone content (Whitehead, 2004). This progressive loss during the laying period results in a net reduction of structural bone, a weakened skeleton, and increased risks for fractures (Whitehead, 2004). Despite this, the medullary bone is accumulated within the structural bone, allowing total bone content to remain constant over the laying period (Whitehead and Fleming, 2000). However, due to its composition, the medullary bone is weaker than the structural bone and reduces the overall strength of the hen's skeleton (Whitehead, 2004). This issue is typically resolved when the hen goes out of lay. In the wild, hens do not lay an egg every day. Instead, they go out of lay and into incubation, allowing the structural bone to reform on top of the medullary layer, maintaining good bone quality over time (Whitehead, 2004). However, the domesticated hen has been selected to maintain long periods of egg-laying, rendering it unable to regenerate structural bone (Whitehead and Fleming, 2000; Whitehead, 2004). This progressive loss of cortical bone results in loss of structure and contributes to osteoporosis (deterioration of bone tissue) and making them prone to painful fractures (Whitehead and Fleming, 2000). Because of this, it is important to ensure that bone health and strength is well-established during the pullet rearing phase. Building a better and stronger frame of structural bone in young pullets can positively affect bone attributes for the birds lifetime (Gunnarsson et al., 2000; Regmi et al., 2015). Increase in exercise can increase muscle development and improve muscle quality, which increases bone strength (Le Van et al., 2000; Janczak and Riber, 2015; Casey-Trott et al., 2017).

1.10.2 Bone Strength

Many variables contribute to bone strength, such as bird activity, nutrition, genetics, and others. In the case of this study, the effect of L on exercise in pullets is of interest. Exercise can stimulate bone growth and improve bone strength, particularly during the rearing phase (Whitehead, 2004). Especially with the use of perches in non-cage systems, there is evidence to suggest that these load-bearing exercises improve overall bone composition of pullets by increasing bone structure and improving mineral composition (Regmi et al., 2015). Load-bearing exercises include wing-flapping, walking, running, jumping, or flying (Regmi et al., 2015).

One way to measure bone strength is by conducting a 3-point bending test. In a bending test, the bone is elevated and supported at each end while a force is applied midspan (middle of the bone). The bending test involves two types of forces: compressive and tensile (Crenshaw et al., 1981). A compressive force pushes an object together and is exerted on the top fibres of the bone during the test, whereas a tensile force pulls or lengthens an object and is applied on the bottom fibres of the bone (Crenshaw et al., 1981). During a bending test, both compressive and tensile forces act together, creating a moment of force and together with the distance over the force applied, the breaking strength of the bone is determined in units of force and distance (Crenshaw et al., 1981).

The effect of L on bone strength is not well studied, especially in laying hens. In broilers, Newberry et al. (1986) observed no difference in leg disorders in broilers housed in L ranging 0.1 to 100 lux (0.1, 0.5, 1, 10, 20, 30, and 100 lux). Rault et al. (2017) reported that despite higher activity in 20 lux than five lux, there was no difference in leg strength in broilers (reflected by latency to lie).

1.11 Keel Bone

The keel bone is a pronounced bone extending from the ventral surface of the sternum and runs axially along the midline. The keel grows from the cranial border, also known as the *Carina apex*, through the spine and ends at the caudal border, also known as the caudal tip. Unlike the growth of long bones mentioned previously, keel bone growth and ossification are slow processes that continue into the egg production phase (Buckner et al., 1949; Casey-Trott, 2016). The keel bone plays several important roles in avian species. It anchors flight muscles used for wing motion and is used during inhalation and exhalation (Duncker, 2000).

1.11.1 Keel Bone Damage

Due to increased freedom of mobility, the prevalence of keel bone damage occurs most commonly in non-cage systems (Wilkins et al., 2004; Sandilands et al., 2009; Sherwin et al., 2010). This could be due to poor placement of perches or L settings resulting in increased crashes in the environment (Wilkins et al., 2011; Campbell et al., 2016). Regardless of the cause, keel bone damage is a major welfare issue in the laying hen industry and can result in decreased egg production and increased mortality (McCoy et al., 1996; Riber et al., 2018).

Keel bone damage can be in the form of a deviation or a fracture. A keel bone deviation can be easy to misinterpret during palpation, however it is considered deviated when the ventral surface is departed from a straight line, resulting in a bent, S-shaped, or twisted curvature in the keel bone (Casey-Trott et al., 2015). These deviations are assumed to form when long-term pressure is exerted on the keel bone, such as from perching (Scholz, 2007; Sandilands et al., 2009; Pickel et al., 2011). In fact, several studies reported that soft perches can reduce keel bone problems (Tauson and Abrahamsson, 1994; Pickel et al., 2011; Stratmann et al., 2015b). The consequences of keel bone deviations possibly include unequal bone loading during wing-flapping, impaired motion, and increased risk of fractures, all of which reduce bird welfare (Harlander-Matauschek et al., 2015).

A keel bone fracture is a sharply bent, sheared, or fragmented section, commonly in the tip of the keel bone (Casey-Trott et al., 2015). Although the main cause of keel bone fractures is unknown, possible causes include crashes, collisions with objects in the environment, failed landings, or present keel bone deviations which can further exacerbate fractures (Moinard et al., 2004a; b; Sandilands et al., 2009; Harlander-Matauschek et al., 2015). Environmental collisions occur more frequently in non-cage systems, during the beginning of the dark period, and if perches are higher or farther apart in distance (Taylor et al., 2003; Moinard et al., 2005; Stratmann et al., 2015a). To prevent this, Stratmann et al. (2015a) suggested that ramps be placed to facilitate movement between landing platforms or tiers, especially in aviary systems.

To identify keel bone damage, palpation serves as a reliable and inexpensive method. Provided that proper training is given, palpation can provide an accuracy of more than 70% in detecting old and new bone breakages and deviations, and where on the bird's body the damage is (Wilkins et al., 2004). Another method is to dissect the keel bone post-mortem and visually inspect for damages. Other techniques of keel bone damage detection include radiography, ultrasonography, and peripheral quantitative computed tomography (Casey-Trott et al., 2015).

Light intensity can affect keel bone damage. To date, only few studies have evaluated the effect of L on keel bone damage in pullets or laying hens. Gregory et al. (1993) reported that laying hens housed in battery cages in 0.5 or two lux had fewer incidences of healed breaks than when housed in 15 lux, which is likely due to the fact that birds do not move much in the dark. In pullets, the keel bone is still not yet fully ossified and finding a fracture in the keel bone of a pullet is uncommon (Buckner et al., 1949; Rufener and Makagon, 2020). Nonetheless, many studies state that in more complex environments, if the L is too dim (less than one lux), especially during the beginning of dark periods, visual acuity can be compromised and affect navigation or landing success, negatively affecting the bone health of the bird overall (Prescott and Wathes, 2002; Taylor et al., 2003).

1.11.2 Keel Bone Integrity

One important part to maintaining keel bone quality is the development of keel bone muscles, which are the breast muscles, *Pectoralis major* and *Pectoralis minor*. The pectoral muscles anchor the keel bone and are what makes flight possible in birds, especially the breast muscle mass to body weight ratio (Duncker, 2000; Fleming et al., 2004; Casey-Trott, 2016). Duncker (2000) reported breast muscles should be at approximately 20% of the body weight for controlled lifts in flights. Since the domestication of the Red jungle fowl and genetic selection for increased egg production, the breast muscle of the modern laying hen has decreased and overall body mass increased (Jackson and Diamond, 1996; Fleming et al., 2004). The result is a more exposed keel bone and less efficient flights, further jeopardizing breast muscle quality and flight success (Harlander-Matauschek et al., 2015). Additionally, Fleming et al. (2004) reported that a low breast muscle mass could result in more vulnerable keels and increased risk of deviations and fractures.

Similar to keel bone damage, the effect of L on breast muscle weight of pullets or laying hens is not well studied. One study by McKee et al. (2009) reported that broilers housed in one lux had longer and heavier filets than broilers housed in 150 lux. Another study in turkey toms by Yahav et al. (2000) reported that L of ten versus 700 lux did not affect breast muscle weight. More information on laying hens is warranted since broilers and turkeys are bred for meat as opposed to laying hens who were selected for egg production.

1.12 Mortality

Through pathology examination, or necropsy, it is often possible to determine the cause of death. Mortality causes in poultry can be categorized as infectious, metabolic, skeletal, emaciation or dehydration, and other or no visible lesions. Infections include yolk sac infection, polyserositis, osteomyelitis, and peritonitis. Metabolic causes can include ascites. In pullets which is the topic of interest as well as other chicks, mortality is usually low with 97% to 98% livability (Lohmann Tierzucht, 2018). Within pullets and chicks, yolk sac infection is a common cause of death. The yolk sac is used for absorption of nutrients, minerals, antibodies, and fat-soluble vitamins for the chick (Rai et al., 2005). However, often times during the incubation process there can be an exposure to high counts of bacteria which can increase the risk of yolk sac infection (Cortés et al., 2004; Rai et al., 2005). Another possible cause for yolk sac infection is insufficient antibodies enclosed in the yolk sac, resulting in a compromised immune system during early chick life (Rai et al., 2005). Another common cause of death in chicks is emaciation or dehydration. Dehydrated chicks can be identified through the presence of dark, scaly legs on their carcasses (Verschuere, 2018). In addition, one concern about the effect of L on pullets is mortality due to cannibalism. Cannibalism is usually indicated by a severe wound on the body along with significant lack of feathers (Kjaer and Sørensen, 2002). Mentioned previously, studies have reported increased severe feather pecking (often precursors to cannibalism) in hens housed in high L (Hughes and Duncan, 1972; Kjaer and Vestergaard, 1999).

Light intensity may affect mortality due to its potential effects on the health of chickens' eyes and the incidence of accidents and trauma. However, even though constant light exposure can result in cataracts and blindness (Lauber and McGinnis, 1966), many studies on broilers reported no effect of L on mortality. Blatchford et al. (2009) observed that L did not have any effect on mortality in broiler birds housed in five, 50, or 200 lux. Deep et al. (2010) also reported no effect of L on broilers housed in one, ten, 20, or 40 lux. In another study, Olanrewaju et al. (2011) reported that broiler mortality was not affected by L at 0.2, 2.5, 5, 10, or 25 lux. Rault et al. (2017) also observed that L did not affect mortalities nor culls in broilers in five versus 20 lux. However, Deaton et al. (1981) reported lower mortality in broilers housed in 75 lux than those housed in five lux. Few studies have been conducted on L and mortality in layers, however, one study by Kjaer and Vestergaard (1999) reported higher mortality in laying hens housed in 30 lux than three lux from 16 to 46 wks.

1.13 Conclusion

The transition from conventional cages to alternative housing provides better welfare for laying hens. Added space and resources in these housing systems will increase the mobility of birds, allowing them to express more natural behaviours. While this addition might improve some aspects of welfare and potentially bone strength in laying hens, the risk of fractures and bone injuries due to crashes or failed landings on perches can compromise the health and well-being of the bird. Also, any issue in the health and welfare of a laying hen can lead to a decline in egg production during the production phase, rendering producers an economic loss. In pullets, this can be prevented by developing better bone structure and increasing bone strength during rearing so that laying hens will have a better structure when they enter the laying phase. Therefore, ensuring that pullets can navigate their surroundings successfully and safely is crucial in preventing bone injuries. High L may not only help birds see their surrounding environment better and move about more safely, but it may also increase bird activity which contributes to pullet musculoskeletal development. However, high L may also increase aggression and severe feather pecking, resulting in cannibalism. Therefore, it is important to find the optimal L to help with navigation without compromising the behaviour, health, and welfare of pullets. While many studies have been conducted on the effects of L on broilers, additional research is recommended to determine its effects on laying hens, especially during the pullet phase while they are still growing and developing their skeletal and musculoskeletal strength. Determining the optimal level of L on pullet growth in alternative housing systems can allow better understanding and control of the prevalence of broken bones in pullets, allowing adult laying hens to produce eggs at their full potential and thus creating a more efficient, productive, and welfare friendly system.

1.14 Objectives

The objectives of this project are to determine if L impacts:

- the activity levels and behavioural output of pullets,
- the ability of pullets in utilizing a non-cage floor-rearing system,
- the stress and fear responses of pullets,
- the body weights and mortality of the flock, and
- the overall health of the flock with reference to bone quality (keel bone damage and bone strength),

In addition, this project will evaluate if Lohmann LSL Lite and Lohmann Brown-Lite birds react differently to different light intensities.

1.15 Hypotheses

High L will increase bird activity through better visual acuity leading to increased behavioural expression and musculoskeletal development but increase fear and stress response through severe feather pecking for two S for birds which will differ in responses through genetic selection.

1. The behaviour of pullets raised in different light intensities will be affected. Exposure to brighter L will result in birds being more active. As a result, it is hypothesized that pullets reared in high L will perform more active behaviours. However, brighter L may also increase fear, stress, and aggression. Therefore, aggression, stress and fear responses will also increase.
2. Navigation of pullets raised in different light intensities will be affected. Pullets in brighter L will have fewer crashes and failed landings compared to birds in lower L. As a result, birds raised in lower L will have more keel bone fractures. Pullets reared in lower L will also have lower bone strength.
3. Due to genetic differences, birds of different S will react to their environment differently. As a result, the two S used in this study, the Lohmann LSL-Lite and Lohmann Brown-Lite pullets will have different measured outcomes.

2.0 Chapter 2: The impact of light intensity and strain on behaviour, jumping frequency and success, and welfare of egg-strain pullets reared in perchery systems from 0 to 16 weeks of age

The objectives of this work were to examine how light intensity, including the current recommended intensity for table-egg production systems in Canada in addition to two higher intensities, can affect layer pullets reared in floor pens containing a simple perchery system from 0 to 16 weeks of age. Chapter 2 focuses on the effects of different light intensity on layers pullets' behaviour, number of jumps between resources in the environment, success of those jumps, fear, and stress response.

2.1 Abstract

The objectives were to determine the impact of light intensity (L) and strain (S) on pullet navigation, behaviour, and welfare. A 3×2 factorial arrangement in a randomized complete block design (trial) tested three L (10, 30, or 50 lux, provided by white LED lights) and two Lohmann S (Brown-Lite (LB) and LSL-Lite (LW)). Pullets (n=1,800 per S) were randomly assigned to floor pens within light tight rooms (3 pens of each S per room, 4 rooms per L) containing a system of four parallel perches, a ramp, drinker line, and two tube feeders from 0 to 16 wk. Pullets were assessed for fear and stress levels with a novel object test (8 pen replicates per L×S) and blood heterophil/lymphocyte (H/L) ratios (12 pen replicates per L×S) at 15 wk. Behaviour was analyzed on a pen basis (4 pen replicates per L×S) via instantaneous scan sampling at 20 min intervals during light periods (12 h at 4 wk, 8 h at 8, 13, and 16 wk) and continuous sampling (24 h) for successful and failed jumps in the environment. The effect of L, S, and their interactions were analyzed using Proc Mixed (SAS 9.4) with room nested in L. Differences were significant when $P<0.05$. L did not affect fear, H/L, use of space, or jumping frequency. At 13 and 16 wk, pullets reared in 50 lux spent more time preening and jumping than those in 10 lux, whereas 10 lux pullets spent more time pecking at walls than those in 50 lux. LB pullets had a longer latency to peck at the novel object and higher H/L ratio than LW pullets. Throughout the experiment, LW pullets spent more time on perches resting and preening, while LB pullets spent more time expressing exploratory behaviours. LW pullets jumped between structures more than LB pullets, however jumping success did not differ. The results suggest that L between 10 and 50 lux do not impact pullet fear or stress response, however, 50 lux may increase bird activity and comfort. S influenced behaviour, jumping frequency, and fear responses, but did not interact with responses to L.

2.2 Introduction

The Canadian National Farm Animal Care Council Codes of Practice for Pullet and Laying Hens (2017) states that all conventional cages will be banned in Canada by 2036. Instead, all layers will be housed in alternative housing systems (furnished or non-cage). These systems provide more space and resources such as next boxes, perches, and pecking and scratching mats (Sandilands et al., 2009), and in some cases, dustbathing systems. These housing systems increase the freedom of mobility in layers, allowing them to express more natural behaviours. For these resources to be utilized to their fullest extent, the birds must be able to see well to so they can navigate their environment successfully. Even so, current literature shows that laying hens are more successful at using complex housing environments when reared in a similar type of housing, allowing learning to occur early in life (Wilkins et al., 2011). In fact, pullet navigation is important because pullets' bones are still developing as they learn to utilize and navigate perches (Whitehead, 2004). Currently, the National Farm Animal Care Council (2017) requires a minimum light intensity (L) of ten lux for birds housed in alternative housing systems, however the effect of a L of ten lux on successful pullet navigation has not been well studied.

Presently, the Canadian Codes of Practice requires layers in conventional cages to be housed in a minimum of five lux (NFACC, 2017). Low L of less than five lux keeps birds calm and reduces feather pecking, but may affect navigation (Hughes and Black, 1974; Widowski et al., 2013). Increasing L to more than 10 lux can improve poultry vision and may help pullets to navigate their surroundings more successfully, thereby preventing bone fractures and injuries (Taylor et al., 2003; Widowski et al., 2013). However, higher L can also increase aggressive behaviours, severe feather pecking, and cannibalism, which results in increased fear and stress within the flock (El-Lethey et al., 2000; Kristensen, 2008). In addition, strain (S) may differ in their navigational and behavioural qualities (Tauson and Abrahamsson, 1996; Pusch et al., 2018). This study was conducted to determine whether increasing L can aid pullets at navigating a complex environment without behavioural and welfare consequences. This chapter focused on the behaviour, navigation, and welfare parameters of two common egg-laying S.

The objectives of this study were to determine how L affects layer pullets' use of space, behaviour, and health. Specifically, these research questions were generated:

- How does L impact the behaviour of pullets?

    - Does higher L (30 and 50 lux) increase their active, nutritive, comfort, and exploratory behaviour as compared to 10 lux?
    - Does higher L (30 and 50 lux) increase their aggression?
- How does L impact the navigation or jumping behaviour of pullets?
    - Does higher L (30 and 50 lux, as compared to 10 lux) increase the number of jumps between resources in the environment?
    - Does higher L (30 and 50 lux) increase the accuracy or landing success of pullets?
- How does L impact the welfare of pullets?
    - Does higher L (30 and 50 lux, as compared to 10 lux) increase their fear and stress response?
- How does S impact the above measured parameters?

Three light intensities were tested: the current industry-recommended value of 10 lux, 50 lux, and a mid-range intensity of 30 lux. It was hypothesized that pullets reared in higher light intensities (30 and 50 lux) will be more active and able to navigate their environments better but have higher stress and fear levels than pullets reared in current industry standard's level of L (10 lux). Specifically, higher L (30 and 50 lux) will increase pullet activity, resulting in more jumps between resources in the environment. Because of higher L increasing visual acuity, pullets reared in 30 or 50 lux will also have higher landing success rates from jumps than pullets reared in 10 lux. However, higher L (30 or 50 lux) has also been shown to cause severe feather pecking within the flock. Therefore, it is also expected higher L (30 and 50 lux) will increase severe feather pecking or aggression levels within the flock, which will subsequently increase fear and stress responses. Finally, due to S differences, it was hypothesized that the two S used in this study will have different measured outputs.

2.3 Materials and Methods

The experimental procedures for this experiment were approved by the University of Saskatchewan Animal Care Committee, and all birds were cared for as specified in the Guide to the Care and the Use of Experimental Animals by the Canadian Council of Animal Care (2009).

2.3.1 Animal Housing and Husbandry

Research on the effect of varying light intensities during pullet rearing on use of space and behaviour were conducted over two 16-week blocked trials from May to August 2018 and 2019. Six individually controlled, light-tight rooms were used to test three light intensities: 10, 30, and 50 lux. In each trial, newly hatched Lohmann Brown-Lite (LB; n=900) and Lohmann LSL-Lite (LW; n=900) female pullets, obtained from a commercial hatchery (Clark's Poultry, Brandon, MB), were randomly assigned to one of the three L treatments (n=300 birds per L treatment × S), while light intensities were randomly assigned to each room. Each room contained six floor pens bedded with 7-10 cm depth of wheat straw. Birds were housed at an estimated stocking density of 6.5 birds/m^2 (50 pullets per pen), in accordance with the recommendations in the Lohmann Management Guide (Lohmann Tierzucht, 2018). Each pen (4.0 m × 2.3 m) was furnished with one perching system (height 0.56 m × width 1.16 m × length 2.18 m, spaced 30.0 cm apart) and one ramp (length 81.3 cm × width 48.3 cm at an angle of 38°, Figure 2.1). The perching system consisted of four wooden perches. Each of the four perches was a rectangle (length 3.8 cm × height 3.5 cm) with the top corners angled to allow for easy grasping (Figure 2.2), while the ramp was made of 14 gauge wire with 2.54 cm × 2.54 cm dimensions. Perches were placed in pens prior to chick arrival, whereas ramps were added at 14 days of age to prevent pullets trapping toes or legs in the ramp wires. Each pen contained two small pan feeders (36 cm diameter and 113 cm circumference), which were switched to larger pan size (44 cm diameter and 138 cm circumference) at six weeks of age (wks), and one drinker line with six nipples (Lubing Systems LP, Cleveland, TN, USA). All birds had *ad libitum* access to water and commercial feed appropriate for their stage of development (Lohmann Tierzucht, 2018; Table 2.1). During the first week, pullets had access to supplemental feeders and waterers. Birds were wing-banded at eight wks for identification purposes. Additionally, birds were vaccinated at the hatchery for Marek's Rispens, HVT-IBD, and Poulvac ST (second trial only). The birds were also vaccinated for Newcastle Bronchitis at two, six, and ten wks via coarse spray, for *Salmonella typhimurium* at nine and eleven weeks via coarse spray, and for Newcastle Bronchitis and *Salmonella enteriditis* at fifteen weeks via intramuscular injection.

For logistical and animal husbandry specifics, eight 11-watt white light-emitting diode (LED) lamps were used to illuminate each room (2821 K, Greengage Lighting Ltd., Edinburgh, UK; Figure 2.3). The light bulbs were placed directly over the pens, such as L is similar in all pens.

Figure 2.1 Perching system (height 0.56 m × width 1.16 m × length 2.18 m, spaced 30.0 cm apart) with ramp (length 81.3 cm × width 48.3 cm at an angle of 38°).

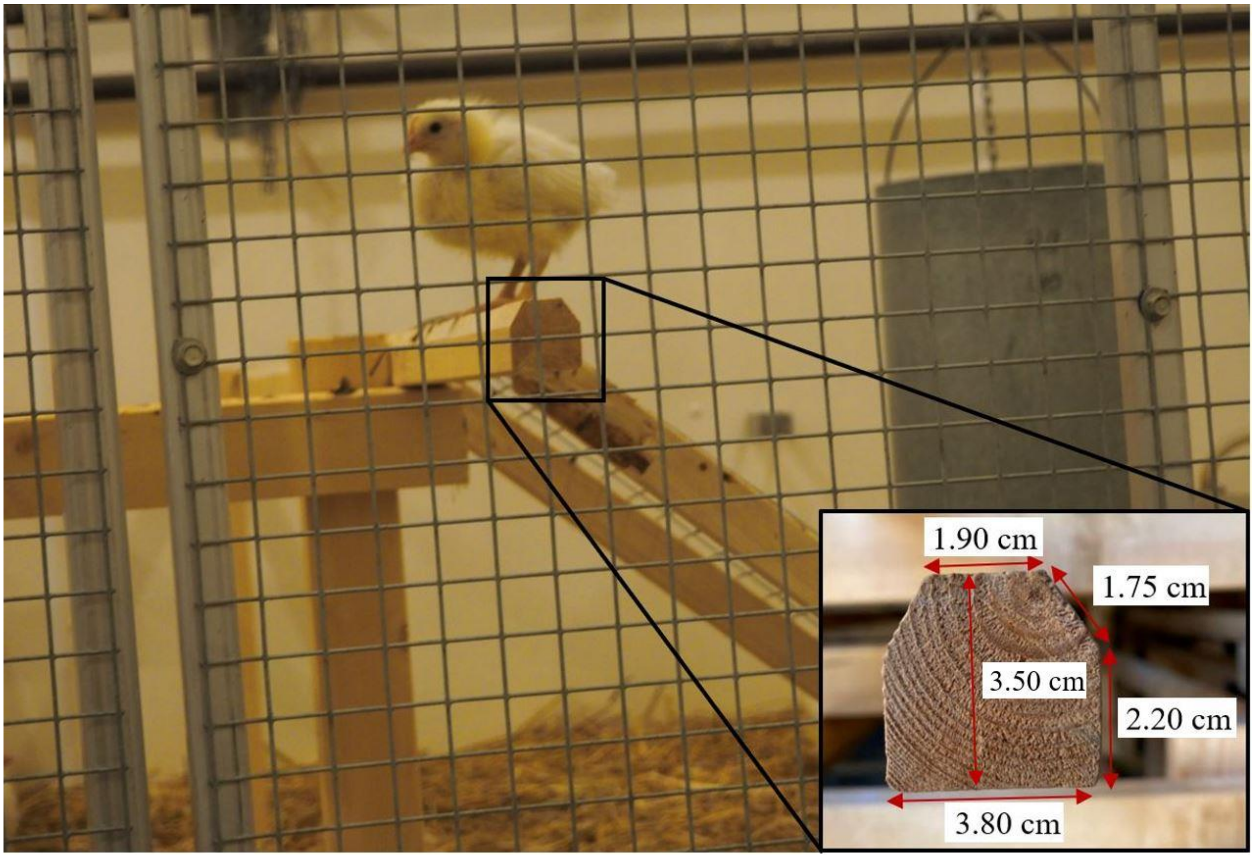

Figure 2.2 Dimensions of wooden perches.

Table 2.1 Feed ingredients and calculated nutrient content of diets for pullet diets until 16 weeks of age.

Ingredients: (%)	Starter (0 – 4wks)	Grower (5 – 10wks)	Developer (10 – 16wks)
Barley	10.00	0.00	18.0
Wheat	42.90	57.24	56.43
Soybean meal -48	0.00	6.74	2.50
Corn	12.00	0.00	0.00
Peas/Lentils	10.00	18.88	6.05
Meat meal restricted	9.50	0.00	0.00
Canola meal	7.00	9.08	10.00
Corn distillers	5.52	3.74	1.61
Tallow	1.00	0.00	0.00
Oat hulls	0.00	0.00	1.50
Canola oil	0.00	1.00	1.00
Mono calcium phosphate	0.00	0.49	0.36
Limestone	0.79	1.68	1.68
Salt	0.00	0.00	0.23
Sodium bicarbonate	0.00	0.21	0.14
Choline chloride	0.08	0.00	0.00
Enzyme – Endofeed[1]	0.02	0.03	0.03
Ronozyme P-CT[2]	0.00	0.03	0.03
DL-Methionine	0.08	0.08	0.07
L-Lysine HCL	0.16	0.00	0.03
L-Threonine	0.00	0.02	0.00
Mono calcium carbonate	0.28	0.00	0.00
Potassium chloride	0.08	0.00	0.00
Biotin	0.02	0.00	0.00
Amprolium 25%[3]	0.05	0.03	0.03
DG-200mg Selenium	0.04	0.14	0.15
V8V[4]	0.08	0.08	0.08
M2M[5]	0.07	0.08	0.08
Termin-8[6]	0.00	0.15	0.00

Calculated composition (%)			
ME (kcal/kg)	2738.00	2750.00	2725.00
Crude protein (%)	19.20	19.10	16.00
Calcium (%)	0.96	0.92	0.88
Chloride (mg/kg)	0.70	0.20	0.20
Non-phytate P (%)	0.43	0.40	0.36
Sodium (%)	0.17	0.17	0.16
Arginine (%)	1.13	1.03	0.78
Isoleucine (%)	0.66	0.61	0.49
Lysine (%)	0.99	0.98	0.56
Methionine (%)	0.39	0.37	0.29
Met + Cys (%)	0.73	0.72	0.67

Threonine (%)	0.64	0.55	0.43
Tryptophan (%)	0.18	0.19	0.16

[1] β-glucanase, 700 activity units/g and xylanase enzymes 2,250 activity units/g (GNC Bioferm Inc., Bradwell, Canada)

[2] Phytase enzyme, 2500 FYT/g (DSM Nutritional Products, Heerlen, the Netherlands)

[3] Coccidiostat

[4] Supplied per kilogram of diet: vitamin A (retinyl acetate + retinyl palmitate), 11000 IU; vitamin D3, 2200 IU; vitamin E (dl-α-topheryl acetate), 30 IU; menadione, 2.0 mg; thiamine, 1.5 mg; riboflavin, 6.0 mg; niacin, 60 mg; pyridoxine, 4 mg; vitamin B12, 0.02 mg; pantothenic acid, 10.0 mg; folic acid, 0.6 mg; and biotin, 0.15 mg; ethoxyquin, 0.625 mg; calcium carbonate, 500 mg

[5] Supplied per kilogram of feed: iron, 80 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.8 mg; and selenium, 0.3 mg

[6] Pathogen control (Salmonella spp., molds) (Anitox, Lawrenceville, USA)

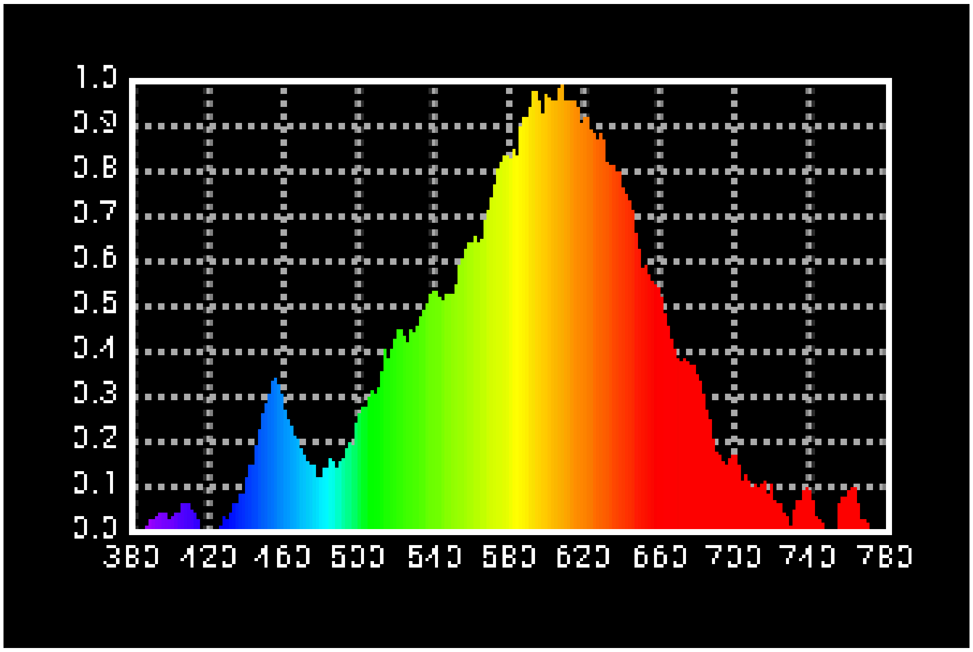

Figure 2.3 Spectrum of LED light at 10 lux (X axis = wavelength (nm); Y axis = relative sensitivity).

Wavelength was similar in all rooms. Birds were given twenty-three hours of light and one hour of dark (23L:1D) for the first week; the hours of light were decreased every week until the seventh week, at which lights remained at a constant 8L:16D until the end of the trial. L was set at 50 lux for the first week, after which L was adjusted according to the room-appropriate intensity treatment. Light intensity was measured with a lux meter every two weeks (ExTech LT300, ExTech Intstruments, Montreal, Quebec, Canada) and any variances corrected back to planned intensity. Dawn and dusk periods were simulated over a 15-minute period. Room temperature was initially set at 33°C and gradually decreased daily until 20°C where it was maintained. Heat was provided via hot water pipes running along the walls of the rooms, and all rooms were ventilated via a negative pressure inlet-fan system. Birds were checked a minimum of twice daily throughout the trial.

2.3.2 Data Collection

2.3.2.1 Behaviour

The behaviour of birds in one pen per S per room was recorded with infrared cameras (Panasonic WV-CF224FX; Panasonic Corporation of North America, One Panasonic Way 7D-4, Secaucus, NJ, USA) for 24 hour periods at four, eight, 13, and 16 wks. The cameras captured the entire area of the pen. The Genetec Omnicast Software (Genetec Inc., Montreal, Quebec, Canada) was used for video playback for observations. Instantaneous scan sampling was conducted at 20-minute intervals during the light period (12 h at four wks, 8 h at eight, 13, and 16 wks) according to the ethogram presented in Table 2.2. Each behaviour was also recorded with pullet location.

The 24-hour recordings were also used to conduct continuous behaviour sampling to determine how successful birds were at navigating their environment during jumps and flights between pen equipment. Successes and failures from jumps and flights landing onto perches, ramps, drinker lines, top of feeder bins, and the floor were recorded. Both takeoff and landing locations were recorded, and jumping success was determined when a pullet jumped from one part of the pen environment or equipment to another and landed successfully. A failed landing was defined as when the pullet was unable to successfully land on its destination. The success rate of jumping and landing in terms of percentage was also determined based on the number of success and failed jumps.

Table 2.2 Behavioural ethogram for pullets, adapted from (Webster and Hurnik, 1990; Estevez et al., 2002; Nicol et al., 2009; de Haas et al., 2010; Ericsson et al., 2014; Hunniford and Widowski, 2018).

Behaviour	Definition
Active behaviour	
Standing	Body in upright and idle position (Nicol et al., 2009)
Walking	Taking at least two successive steps (Webster and Hurnik, 1990)
Jumping or Flying	Both feet in the air with wings flapping (de Haas et al., 2010)
Resting behaviour	Lying down or crouching with breast on floor, or head tucked under wing, otherwise inactive (Ericsson et al., 2014)
Comfort behaviour	
Preening	Manipulating own feathers with beak while standing or laying (Nicol et al., 2009)
Wing or Leg stretching	Extending wing or leg out to the side or behind body and returning wing or leg back under body without taking a step forward (Nicol et al., 2009)
Tail wagging	Moving tail side-to-side without moving rest of body (Nicol et al., 2009)
Head shaking	Head moving side to side or up and down rapidly, body immobile (Nicol et al., 2009)
Head scratching	Extending leg forward and upward to scratch head or neck (Nicol et al., 2009)
Feather ruffling	Raising or shaking out feathers of wings and body (Ericsson et al., 2014)
Dustbathing	Rubbing body against floor and performing full body shake (Ericsson et al., 2014)
Wing flapping	Extending wings away from body and flapping up and down rapidly but without flight (Nicol et al., 2009)
Nutritive behaviour	
At the feeder	Standing or sitting with head extended into feeder (Webster and Hurnik, 1990)
At the drinker	Pecking at nipple drinker (Ericsson et al., 2014)
Exploratory behaviour	
Gentle pecking	Pecking at other birds which does not cause harm or damage to plumage (Nicol et al., 2009)
Wall pecking	Pecking at pen walls (Nicol et al., 2009)
Object pecking	Pecking at perch, ramp, feeder tube (not feed pan), drinker (away from nipples) (Nicol et al., 2009)
Litter pecking	Pecking at straw or litter (Nicol et al., 2009)
Ground scratching	Scratching movements on ground while crouching slightly (Nicol et al., 2009)
Head sweeping	Rubbing beak from side to side (Nicol et al., 2009)
Aggressive behaviour	
Aggressive pecking	Pecking at other birds directed at the head and neck but may include feet; causes the recipient to flinch or escape the environment (Hunniford and Widowski, 2018)

Fighting	Sparring, leaping, and wing flapping towards an opponent and can include pecking (Estevez et al., 2002)
Unidentified	Behaviour unidentifiable; action of bird cannot be seen

Post observations, these jumps were categorized into jumps upward, downward, and across. Jumps from the perch to the top of the feeder bin, perch to ramp and drinker line, from the ramp to the perch, floor, drinker line, or top of feeder bin, from the floor to the top of the feeder bin, from the drinker to the perch and top of feeder bin, and from the top of the feeder bin to the perch, floor, or drinker line were also recorded, but too infrequently to justify analyses. One observer was used for both types of sampling, and prior to beginning observations, inter-observer reliability was tested by having a second observer watch the same footages and calculating for percent agreement for each behaviour and obtaining an average minimum of 80% consistency across data.

2.3.2.2 Novel Object Test

The effect of L on fear responses of the two S of pullets reared in 10, 30, or 50 lux was assessed using a novel object test at 15 wks. The novel object used was a foil tie dye balloon weight (Unique 4927; Fancy Dress Worldwide, Worcester, UK, Figure 2.4), and it was placed on the pen floor, approximately two feet from the entrance. Pullets housed in two pens per S per room were evaluated by measuring the time taken for three birds to peck at the object. All pens per room were tested by live observation at the same time with four different testers assigned to each pen randomly, and with each pen being observed individually. A maximum time of 900 seconds (15 minutes) per observation was allotted and an average latency to peck the object for all three pecking times were recorded in seconds and used for analysis. Tests began at 8 a.m. and concluded at 9:30am.

2.3.2.3 H/L Ratio

Blood was collected from two birds per pen per room (n=72 birds per trial) at 15 wks for analysis of heterophil to lymphocyte (H/L) ratio to assess bird stress response. Two millilitres of blood were collected from the brachial vein in an Ethylenediamine tetraacetic acid (EDTA) anti-coagulation vacutainer using a 22-gauge needle. Two smear stains from each bird were created the same day blood was collected. After drying, slides were stained using PROTOCOL^{TM} Hema 3^{TM} (Fisher Scientific, Ottawa, Canada). A light microscope (Microscope B-290TB; Optika ©, Bergamo, Italy) fitted with 100× field of view with oil magnification was used to count to one hundred heterophil or lymphocyte cells. H/L ratios were determined by dividing the number of heterophils by the number of lymphocytes.

Figure 2.4 Novel object used for fear test. Object was placed on the pen floor , approximately 61 cm from the entrance. Pullets were evaluated by measuring the time taken for three birds to peck at the object for up to 900 seconds (15 minutes).

2.3.3 Statistical Analyses

The experiment was designed as a 3 (L) × 2 (S) factorial arrangement, with room nested within L, in a randomized complete block design. Room was the replicate unit for L (2 repetitions per L treatment per trial), pen was the replicate unit for S (3 replicates per S per room per trial), and trial was treated as a block. Each age was analyzed separately. All data were checked for normality using Proc Univariate (SAS® 9.4, Cary, NC, USA), and any data not meeting normality assumptions (such as percentage data) were log transformed (data log +1) prior to analyses. An analysis of variance (ANOVA) test was conducted using Proc Mixed (SAS® 9.4, Cary, NC, USA) to analyze differences among group means in the sample. A Tukey's range test was used to separate means. For all statistical analyses, significance was declared when $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

2.4 Results

2.4.1 Behaviour

The results from scan sampling are described below, and reported in Table 2.3 to Table 2.6. Light intensity had minor effects on pullet behaviour while S had many effects. No interactions between L and S were observed at 13 and 16 wks.

2.4.1.1 Active Behaviours

Active behaviours include standing, walking, and jumping or flying. The percentage of time spent in active behaviours for each recorded period is reported in Table 2.3, 2.4, 2.5, and 2.6.

At four wks, there was an interaction between L and S on wing-assisted jumping or flying (P<0.001, Table 2.3). Strains reacted differently to L in their percentage of time spent flying and jumping. LB pullets reared in 30 lux spent numerically more time (0.19% of a 12 h photophase) jumping or flying compared to those reared in 10 lux (0.06%) or 50 lux (0.00%), while for LW pullets, a peak was noted at 10 lux (0.15%) compared to 30 (0.05%) or 50 lux (0.09%, Table 2.7). Despite the peak, L impacted all the LW birds similarly.

At eight wks, L influenced walking behaviour (Table 2.4). Pullets reared in 50 lux spent more time walking than pullets reared in 10 lux, while pullets in 30 lux were intermediate (P=0.027; Table 2.4). There was no effect of L on walking or any other active behaviours at 13 and 16 wks.

Table 2.3 Average percentage of time (%) spent on each behaviour by Lohmann Brown- Lite (LB) and Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 12 hours of light (20 minutes scan sampling) at four weeks of age (4 pen replicates per L × S).

	Light Intensity (L)				Strain (S)			L × S	
	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Standing	22.07	19.62	20.16	0.457	19.10	22.13	0.047	0.510	0.741
Walking	4.67	5.46	5.65	0.164	5.05	5.47	0.450	0.122	0.239
Jumping or flying	0.11^{ab}	0.12^{a}	0.04^{b}	0.036	0.08	0.09	0.486	<0.001	0.016
Resting	10.19	11.99	12.10	0.404	8.10	14.75	<0.001	0.608	0.942
Preening	4.83	6.65	6.05	0.074	5.21	6.48	0.072	0.648	0.344
Comfort[2]	0.76	0.95	0.97	0.454	0.76	1.02	0.019	0.597	0.080
At the feeder	11.43	11.79	11.03	0.862	10.60	12.24	0.043	0.833	0.804
At the drinker	3.37	2.86	2.71	0.230	2.80	3.16	0.123	0.284	0.147
Gentle pecking	0.14^{b}	0.19^{ab}	0.31^{a}	0.029	0.20	0.22	0.765	0.022	0.031
Litter directed[3]	19.79	19.25	19.98	0.968	23.04	16.31	<0.001	0.632	0.953
Wall pecking	2.47	1.32	1.31	0.176	1.41	1.99	0.288	0.818	0.258
Object pecking[4]	0.29	0.25	0.08	0.157	0.26	0.16	0.052	0.363	0.038
Aggression[5]	0.02	0.01	0.02	0.921	0.01	0.03	0.327	0.377	0.010
Unidentified	19.87	19.54	19.61	0.887	23.38	15.96	0.001	0.986	1.179

[1] SEM – Standard error of mean.

[2] Wing or leg stretching, tail wagging, head shaking, head scratching, feather ruffling, dustbathing, and wing flapping.

[3] Behaviour directed towards ground, including litter pecking, ground scratching, and head sweeping.

[4] Pecking at perch, ramp, drinker, and feeder bin.

[5] Aggressive pecking and fighting.

Table 2.4 Average percentage of time (%) spent on each behaviour by Lohmann Brown- Lite (LB) and Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 8 hours of light (20 minutes scan sampling) at eight weeks of age (4 pen replicates per L × S).

	Light Intensity (L)				Strain (S)			L × S	
	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Standing	14.46	14.63	16.43	0.490	15.74	14.60	0.331	0.754	0.636
Walking	4.67^{b}	5.75^{ab}	5.78^{a}	0.027	5.21	5.59	0.361	0.997	0.201
Jumping or flying	0.11	0.10	0.42	0.240	0.20	0.22	0.916	0.885	0.078
Resting	21.64	21.05	20.22	0.780	16.45	25.49	<0.001	0.689	1.247
Preening	11.09	12.76	11.90	0.532	10.27	13.56	0.007	0.432	0.631
Comfort[2]	1.07	1.27	2.41	0.213	1.36	1.81	0.400	0.912	0.303
At the feeder	10.94	9.81	11.16	0.122	10.63	10.65	0.892	0.791	0.369
At the drinker	3.94	4.02	4.09	0.943	3.77	4.26	0.088	0.681	0.132
Gentle pecking	0.34	0.42	0.49	0.743	0.18	0.65	0.001	0.508	0.081
Litter directed[3]	17.24	17.52	17.45	0.959	20.97	13.83	<0.001	0.985	0.883
Wall pecking	3.47	2.52	2.16	0.084	3.11	2.32	0.078	0.092	0.355
Object pecking[4]	0.33	0.41	0.34	0.655	0.26	0.46	0.028	0.192	0.048
Aggression[5]	0.01	0.02	0.09	0.155	0.04	0.04	0.877	0.709	0.017
Unidentified	10.69	9.72	9.79	0.913	12.37	7.76	<0.001	0.048	0.721

[1] SEM – Standard error of mean.

[2] Wing or leg stretching, tail wagging, head shaking, head scratching, feather ruffling, dustbathing, and wing flapping.

[3] Behaviour directed towards ground, including litter pecking, ground scratching, and head sweeping.

[4] Pecking at perch, ramp, drinker, and feeder bin.

[5] Aggressive pecking and fighting.

Table 2.5 Average percentage of time (%) spent on each behaviour by Lohmann Brown- Lite (LB) and Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 8 hours of light (20 minutes scan sampling) at 13 weeks of age (4 pen replicates per L × S).

	Light Intensity (L)				Strain (S)			L × S	
	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Standing	24.87	26.65	25.03	0.729	25.85	25.19	0.659	0.652	0.713
Walking	3.88	4.05	3.99	0.912	4.08	3.86	0.602	0.986	0.195
Jumping or flying	0.02	0.05	0.12	0.451	0.09	0.04	0.433	0.779	0.030
Resting	13.78	14.99	14.78	0.732	11.77	17.26	<0.001	0.314	0.760
Preening	12.07^{b}	12.45^{ab}	14.72^{a}	0.023	11.16	14.99	<0.001	0.649	0.585
Comfort[2]	1.23	1.35	1.89	0.289	1.64	1.34	0.188	0.186	0.154
At the feeder	8.75	9.12	8.79	0.910	9.34	8.43	0.018	0.661	0.376
At the drinker	3.05	3.68	3.49	0.142	3.49	3.32	0.583	0.244	0.137
Gentle pecking	0.41	0.30	0.56	0.133	0.37	0.47	0.229	0.955	0.045
Litter directed[3]	17.48	16.20	16.79	0.658	18.58	15.06	<0.001	0.564	0.526
Wall pecking	4.12^{a}	3.40^{ab}	2.46^{b}	0.010	4.43	2.22	<0.001	0.385	0.404
Object pecking[4]	0.61	0.38	0.51	0.149	0.39	0.60	0.033	0.915	0.052
Aggression[5]	0.01	0.02	0.04	0.544	0.02	0.03	0.737	0.243	0.011
Unidentified	9.72	7.37	7.74	0.134	9.38	7.18	0.010	0.195	0.564

[1] SEM – Standard error of mean.

[2] Wing or leg stretching, tail wagging, head shaking, head scratching, feather ruffling, dustbathing, and wing flapping.

[3] Behaviour directed towards ground, including litter pecking, ground scratching, and head sweeping.

[4] Pecking at perch, ramp, drinker, and feeder bin.

[5] Aggressive pecking and fighting.

Table 2.6 Average percentage of time (%) spent on each behaviour by Lohmann Brown- Lite (LB) and Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 8 hours of light (20 minutes scan sampling) at 16 weeks of age (4 pen replicates per L × S).

	Light Intensity (L)				Strain (S)			L × S	
	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Standing	32.82	32.99	31.67	0.799	32.27	32.71	0.767	0.438	0.747
Walking	4.91	4.72	4.95	0.841	4.65	5.07	0.395	0.898	0.187
Jumping or flying	0.07	0.05	0.04	0.390	0.07	0.04	0.155	0.179	0.010
Resting	8.59	8.77	8.66	0.996	6.92	10.42	0.001	0.956	0.522
Preening	12.48^{b}	13.20^{b}	15.73^{a}	0.003	12.37	15.24	<0.001	0.930	0.516
Comfort[2]	0.86	0.64	0.75	0.578	0.68	0.82	0.300	0.190	0.067
At the feeder	8.10	7.65	7.39	0.394	7.22	8.20	0.015	0.927	0.314
At the drinker	3.34^{a}	2.52^{b}	3.12^{a}	0.005	2.95	3.04	0.620	0.838	0.113
Gentle pecking	0.59	0.36	0.43	0.425	0.77	0.16	<0.001	0.462	0.088
Litter directed[3]	15.29	16.34	15.95	0.554	16.10	15.62	0.464	0.825	0.354
Wall pecking	5.17^{a}	3.52^{ab}	3.24^{b}	0.031	5.61	2.34	<0.001	0.762	0.476
Object pecking[4]	0.85	0.75	1.06	0.679	1.11	0.66	0.093	0.798	0.138
Aggression[5]	0.01	0.03	0.03	0.682	0.03	0.02	0.746	0.605	0.009
Unidentified	6.93	8.45	6.98	0.144	9.25	5.65	<0.001	0.121	0.525

[1] SEM – Standard error of mean.

[2] Wing or leg stretching, tail wagging, head shaking, head scratching, feather ruffling, dustbathing, and wing flapping.

[3] Behaviour directed towards ground, including litter pecking, ground scratching, and head sweeping.

[4] Pecking at perch, ramp, drinker, and feeder bin.

[5] Aggressive pecking and fighting.

Table 2.7 Interaction between light intensity (10, 30 and 50 lux) and strain (Lohmann Brown-Lite (LB), and Lohmann LSL-Lite (LW)) on jumping and flying (%) at four weeks of age (4 pen replicates per L × S).

	Light Intensity		
	10	30	50
LB	0.06^{bc}	0.19^{a}	$9.71E\text{-}17^{c}$
LW	0.15^{ab}	0.05^{bc}	0.09^{abc}

Behaviours were observed over 12 hours of light using video footage and 20 minutes scan sampling (n = 4 pen replicates per light intensity × strain).

Strain affected standing behaviours only at four wks. LW pullets spent more time standing than LB pullets (22.13% vs 19.10%, *P*=0.047, Table 2.3). There was no effect of S on any of the other active behaviours and at any of the other recorded weeks.

2.4.1.2 Inactivity – Resting

There was no effect of L on resting behaviour (percentage of time). However, S had an effect. At all recorded weeks, LW spent more time resting than LB (*P*<0.001 at four wks, Table 2.3, *P*<0.001 at eight wks, Table 2.4, *P*<0.001 at 13 wks, Table 2.5, *P*=0.001 at 16 wks, Table 2.6).

2.4.1.3 Comfort Behaviours

Comfort behaviours included preening, wing or leg stretching, tail wagging, head shaking, head scratching, feather ruffling, dustbathing, and wing flapping. There was an effect of L on preening behaviour. At four wks, a trend was noted for more time spent preening by pullets reared in 30 lux, followed by 50 and 10 lux (*P*=0.074, Table 2.3). At 13 wks, pullets reared in 50 lux (14.72%) spent more time preening than pullets in 10 lux (12.07%), with pullets in 30 lux (12.45%) being intermediate (*P*=0.023, Table 2.5). At 16 wks, pullets reared in 50 lux (15.73%) spent more time preening than those in 10 (12.48%) and 30 (13.20%) lux (*P*=0.003, Table 2.6).

There was a trend for LW pullets to spend more time preening than LB pullets at four wks (*P*=0.072, Table 2.3). This trend became significant for the rest of the recording periods (*P*=0.007 at eight weeks, *P*<0.001 at 13 weeks, and *P*<0.001 at 16 wks). LW pullets (1.02%) also spent more time expressing other types of comfort behaviours (wing or leg stretching, tail wagging, head shaking, head scratching, feather ruffling, dustbathing, wing flapping) than LB pullets (0.76%) at four wks (*P*=0.019, Table 2.3).

2.4.1.4 Nutritive Behaviours

Nutritive behaviours include spending time at the feeder and drinker. Light intensity had an effect on drinking behaviour at 16 wks. Pullets reared in 10 (3.34%) and 50 (3.12%) lux spent more time at the drinker than pullets reared in 30 lux (2.52%, *P*=0.005, Table 2.6).

There was an effect of S on nutritive behaviours. At four and 16 wks, LW pullets spent more time at the feeder than LB pullets (12.24% vs 10.60%, *P*=0.043, Table 2.3 and 8.20% vs 7.22%, *P*=0.015, Table 2.6). However, at 13 wks, LB pullets spent more time at the feeder than LW pullets (9.34% vs 8.43%, *P*=0.018, Table 2.5). There was no effect of S on time spent at the feeder at eight wks, however there was an effect on time spent at the drinker, where a trend was noted for LW pullets to spend more time at the drinker than LB (*P*=0.088, Table 2.4).

2.4.1.5 Exploratory Behaviours

Exploratory behaviours included gentle pecking at other pullets, litter directed pecking, or pecking at walls or objects in the environment. Litter directed pecking included litter pecking, ground scratching, and head sweeping, and objects in the environment include the pecking at the perch, ramp, drinker lines (without consumption) or feeder bin (without consumption).

There was an interaction of L and S on gentle pecking at four wks. LB and LW pullets reacted differently to the light intensities (*P*=0.022, Table 2.8). For LW pullets, time spent gentle pecking was highest in 50 lux (0.43%) and was significantly expressed more than LW in 10 lux (0.07%) with pullets in 30 lux (0.17%) as intermediate. LB pullets spent the same amount of time gentle pecking in 10 (0.21%), 30 (0.21%), and 50 (0.19%) lux.

An interesting behaviour was wall pecking. At four wks, L had no effect on wall pecking behaviour. At eight wks, pullets reared in 10 lux tended to spend more time pecking at walls than those in 30 and 50 lux (*P*=0.084, Table 2.4). Then at 13 wks, this pattern was statistically significant. Pullets in 10 lux (4.12%) spent more time pecking at walls than pullets in 50 lux (2.46%), with pullets in 30 lux (3.40%) as intermediate (*P*=0.010, Table 2.5). This difference was also observed at 16 wks (*P*=0.031, Table 2.6).

Strain impacted different forms of exploratory behaviour. At four, eight, and 13 wks, LB pullets spent more time performing litter directed behaviours than LW pullets (*P*<0.001 for all three ages, Table 2.3, 2.4, 2.5). At four wks, an interaction for gentle pecking was observed (mentioned previously). At eight wks, LW pullets spent more time gentle pecking at their peers more than LB pullets (*P*=0.001, Table 2.4), however at 16 wks, LB pullets spent more time gentle pecking than LW pullets (*P*<0.001, Table 2.6). There was no effect of S on gentle pecking at 13 wks. At eight wks, there was a trend for LB pullets to spend more time pecking at walls than LW (*P*=0.078, Table 2.4). However, this trend became statistically significant at 13 and 16 wks (*P*<0.001 at both weeks, Table 2.5, 2.6). There was a trend for LB pullets to spend more time pecking at objects in the environment (perch, ramp, drinker lines, feeder bins) than LW pullets at four wks (*P*=0.052, Table 2.3). At eight and 13 wks, LW pullets were observed to spend more time performing this behaviour than LB pullets (*P*=0.028 and *P*=0.033, respectively, Table 2.4, 2.5).

Table 2.8 Interaction between light intensity (10, 30 and 50 lux) and strain (Lohmann Brown-Lite (LB), and Lohmann LSL-Lite (LW)) on gentle pecking behaviour at four weeks of age (4 pen replicates per L × S).

	Light Intensity		
	10	30	50
LB	0.21^{ab}	0.21^{ab}	0.19^{ab}
LW	0.07^{b}	0.17^{ab}	0.43^{a}

Behaviours were observed over 12 hours of light using video footage and 20 minutes scan sampling (n = 4 pen replicates per light intensity × strain).

Then, at 16 wks it was similar to the pattern at four weeks; there was a trend for LB pullets to spend more time pecking at objects in the environment than LW pullets (*P*=0.093, Table 2.6).

2.4.1.6 Aggressive Behaviours

The overall average percentage of aggressive behaviour was low, at 0.03%. Aggressive behaviours included aggressive pecking and fighting. Light intensity and S had no effect on aggressive behaviours at any recording periods (Table 2.3, 2.4, 2.5, 2.6).

2.4.1.7 Unidentified Behaviours

Unidentified behaviours are those in which the behaviour of the birds could not be seen. An interaction between L and S was observed at eight wks. A larger percentage of LB pullets at 10 lux could not be observed during behaviour observations than LW pullets at 10 or 30 lux (*P*=0.048, Table 2.4), likely because of feather color against wheat straw. The percentage of pullets that could not be observed was also lower for LW pullets reared in 10 lux treatment, compared to LB pullets in 30 lux treatment (Table 2.9). Light intensity had no effect on the identification of pullet behaviour, whereas there was a S effect; LB pullets were consistently more difficult to identify than LW pullets throughout all recording periods.

2.4.1.8 Location of Pullets

The locations of pullets were also recorded to determine whether L and S had an effect on their whereabouts within the pen. At four wks, pullets reared in 30 lux spent more time on top of drinker lines than pullets in 10 or 50 lux (0.37 vs 0.18 and 0.16%, *P*=0.047, Table 2.10). Light intensity did not affect pullet location at eight, 13, and 16 wks (Table 2.4, 2.5, 2.6).

Pullet location was influenced by S. At four wks, LW pullets tended to spend more time located on the perch than LB pullets (15.12 vs 12.00%, *P*=0.058) and conversely, LB pullets tended to spend more time located on the floor than LW pullets (86.95 vs 84.14%, *P*=0.091, Table 2.10). These trends became significant for the remainder of the weeks; LW pullets spent more time on the perch than LB pullets, and LB pullets spent more time on the floor than LW (all *P*<0.001, Table 2.10).

At eight wks, LB pullets were observed spending more time on top of drinker lines than LW pullets (0.46 vs 0.24%, *P*=0.040, Table 2.10).

Table 2.9 Interaction between light intensity (10, 30 and 50 lux) and strain (Lohmann Brown-Lite (LB), and Lohmann LSL-Lite (LW)) on unidentified behaviours (%) at eight weeks of age (4 pen replicates per L × S).

	Light Intensity		
	10	30	50
LB	14.68^{a}	12.10^{ab}	10.38^{abc}
LW	6.71^{c}	7.42^{bc}	9.19^{abc}

Behaviours were observed over eight hours of light using video footage and 20 minutes scan sampling (n = 4 pen replicates per light intensity × strain).

Table 2.10 Average percentage of time (%) spent at each location by Lohmann Brown-Lite (LB) and Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 12 hours of light at four weeks, and eight hours of light (20 minutes scan sampling) at eight, 13, and 16 weeks of age (4 pen replicates per L × S).

	Light Intensity (L)				Strain (S)			L × S	
Location	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
4 weeks of age									
Perch	12.69	14.31	13.69	0.762	12.00	15.12	0.058	0.790	0.769
Floor	86.50	84.54	85.60	0.602	86.95	84.14	0.091	0.771	0.783
Drinker Line	0.18^{b}	0.37^{a}	0.16^{b}	0.047	0.19	0.29	0.128	0.918	0.039
Ramp	0.63	0.75	0.55	0.627	0.85	0.43	0.016	0.362	0.090
Top of Feeder Bin	0.00	0.03	0.00	0.387	0.00	0.02	0.331	0.387	0.010
8 weeks of age									
Perch	33.84	35.58	32.87	0.603	26.66	41.54	<0.001	0.427	1.729
Floor	65.33	63.68	66.54	0.318	72.45	57.92	<0.001	0.873	1.711
Drinker Line	0.42	0.26	0.38	0.467	0.46	0.24	0.040	0.185	0.052
Ramp	0.33	0.34	0.28	0.833	0.39	0.25	0.063	0.234	0.039
Top of Feeder Bin	0.08	0.14	0.28	0.364	0.07	0.26	0.024	0.054	0.054
13 weeks of age									
Perch	32.79	32.87	33.88	0.760	25.43	40.94	<0.001	0.862	1.916
Floor	66.40	66.13	65.26	0.561	73.76	58.11	<0.001	0.255	1.924
Drinker Line	0.34	0.55	0.33	0.211	0.44	0.38	0.671	0.222	0.054
Ramp	0.37	0.31	0.32	0.776	0.36	0.31	0.620	0.895	0.035
Top of Feeder Bin	0.09	0.15	0.25	0.271	0.05	0.27	0.009	0.599	0.044
16 weeks of age									
Perch	32.43	32.53	34.96	0.303	27.16	39.46	<0.001	0.300	1.430
Floor	66.81	66.64	64.26	0.313	72.19	59.61	<0.001	0.565	1.454
Drinker Line	0.33	0.50	0.45	0.339	0.42	0.43	0.798	0.106	0.039
Ramp	0.43	0.28	0.29	0.284	0.23	0.44	0.016	0.846	0.045
Top of Feeder Bin	0.00	0.05	0.04	0.307	0.01	0.06	0.108	0.454	0.016

SEM[1] – Standard error of mean.

LW pullets spent more time on top of feeder bins than LB pullets at eight (0.26 vs 0.07%, *P*=0.024) and 13 (0.27 vs 0.05%, *P*=0.009) wks, but not at 16 wks.

One interesting observation was the use of ramps throughout each observation period. At four wks, LB pullets were observed spending more time on ramps than LW pullets (0.85% vs 0.43%, *P*=0.016), however this difference was reduced to a trend at eight wks (0.39% vs 0.25%, *P*=0.063), and then switched to LW pullets being on ramps more than LB pullets at 16 wks (0.44% vs 0.23%, *P*=0.016, Table 2.10). There was no S difference in ramp usage at 13 wks.

2.4.2 Jumping Frequency and Success Rate

The following sections report the average number of jumps performed by pullets over 24 hours at each observation period (four, eight, 13, and 16 wks). The jumps were evaluated based on whether the landing was successful or a failure, and a total number of successful and failed jumps was calculated. A successful jump was considered when a pullet jumped from one part of the pen environment or equipment to another and landed successfully. A failed landing was observed when the pullet was unable to land successfully. From these numbers, the success rate of jumping and landing in terms of percentage was determined. For all recorded weeks, no significant effect of the interaction between L and S was found on pullet jumps and success percentage.

2.4.2.1 Jumps Upward

Jumps directed upwards were observed most often for three jump destinations. The first was from the floor to the drinker line which can sway side to side, and which height increased with age of the pullets. The second is from the floor to the top half of the ramp, and the third is from the floor to the perch. Light intensity had an effect only at four wks, and only for the number of jumps from the floor to the ramp. Pullets reared in 50 lux (average 1.74 jumps per pullet over 24 hours at four wks) performed more successful jumps from the floor to the ramp than pullets reared in 10 lux (0.91 jumps), and pullets in 30 lux were intermediate (1.17 jumps, *P*=0.048, Table 2.11). There was no difference in the success percentage of jumps from the floor to the ramp (Table 2.12). Light intensity did not affect the frequency or success percentage of jumps upwards at eight, 13, or 16 wks (Table 2.13, 2.14, 2.15, 2.16, 2.17, 2.18).

Strain had many effects on jumps directed upwards. At four and 16 wks, LW pullets performed more jumps from the floor to the drinker line and to the ramp than LB pullets (7.75

jumps vs 1.62 jumps from floor to drinker line, 1.77 jumps vs 0.78 jumps from floor to ramp, both *P*<0.001, Table 2.11). LW pullets also performed more jumps than LB pullets from the floor to drinker line at 13 wks (3.68 jumps vs 2.43 jumps, *P*=0.025, Table 2.15). However, at four wks, LW pullets had more failed landings from the floor to drinker (0.17 failed landings vs 0.78, *P*=0.001) and to the ramp (0.03 failed landings vs 0.01, *P*=0.033) than LB pullets. This was in contrast to observations noted at 16 wks when there was no difference in failed landings between the two S. However, these failures at four wks were numerically small and did not affect overall percentage of landing success (Table 2.12).

Across all recording periods, LW pullets performed more jumps from the floor to the perch than LB pullets (average 8.59 jumps per pullet over 24 hours vs 1.15 at four wks, 15.22 vs 5.49 at eight weeks, 15.00 vs 6.78 at 13 weeks, 15.16 vs 6.25 jumps at 16 weeks, all *P*<0.001, Table 2.11, 2.13, 2.15, 2.17). However, although numerically small, at eight wks, LW pullets perform more failed landings than LB pullets (0.05 vs 0.02 failed landings, *P*=0.001, Table 2.13). This pattern also tended to occur at 13 wks, again, with numerically small occurrences (0.02 vs 0.01 failed landings, *P*=0.077). Despite more failures, total percentage of success in landings did not differ between the two S (Table 2.12). In fact, at four wks, LW had a higher percentage of successful landings (99.12%) than LB (95.18%, *P*=0.006, Table 2.12).

2.4.2.2 Jumps Downward

Similar to jumps upwards, jumps directed downwards were observed most often at three locations: jumps from the drinker to the floor, from the ramp to the floor, and from the perch to the floor. Light intensity did not affect these jumps at any recording periods, with the exception of a trend at 16 wks. Although numerically minute, pullets reared in 10 lux (0.01%) tended to have more failed landings when jumping from the perch to the floor than pullets reared in 30 (0.00%) and 50 (0.00%) lux (*P*=0.057, Table 2.17). Consequently, this affected the success rate of jumps for pullets reared in 10 lux. A trend was observed at 16 wks when pullets reared in 10 lux (99.96%) had a lower successful landing percentage than pullets in 30 (100.00%) or 50 (100.00%) lux (*P*=0.073, Table 2.18).

Table 2.11 Average number of successful jumps per bird directed upward, downward, and across by Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 24 hours at 4 weeks of age (4 pen replicates per L × S).

			Light Intensity (L)				Strain (S)			L × S	
From	To		10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Jumps upward											
Floor	Drinker	Success	4.28	4.66	5.11	0.736	1.62	7.75	<0.001	0.710	0.735
		Failure	0.09	0.11	0.07	0.877	0.01	0.17	0.001	0.647	0.024
Floor	Ramp	Success	0.91^{b}	1.17^{ab}	1.74^{a}	0.048	0.78	1.77	<0.001	0.802	0.194
		Failure	0.01	0.02	0.02	0.675	0.01	0.03	0.033	0.679	0.005
Floor	Perch	Success	4.88	5.19	4.54	0.729	1.15	8.59	<0.001	0.921	0.804
		Failure	0.05	0.06	0.08	0.457	0.05	0.08	0.173	0.591	0.010
Jumps downward											
Drinker	Floor	Success	4.22	4.52	5.05	0.755	1.57	7.62	<0.001	0.701	0.725
		Failure	0.00	0.01	0.00	0.289	0.00	0.01	0.065	0.289	0.001
Ramp	Floor	Success	0.32	0.44	0.53	0.418	0.36	0.50	0.248	0.597	0.063
		Failure	0.00	0.00	0.00	-	0.00	0.00	-	-	0.000
Perch	Floor	Success	5.75	6.05	6.56	0.365	2.54	9.69	<0.001	0.580	0.776
		Failure	0.00	0.00	0.01	0.766	0.01	0.00	0.129	0.098	0.002
Jumps across											
Perch	Perch	Success	5.91^{b}	8.41^{a}	7.29^{ab}	0.033	6.39	8.02	0.016	0.794	0.480
		Failure	0.08	0.05	0.03	0.337	0.05	0.05	0.770	0.687	0.011
Total jumps											
		Success	26.27	30.43	30.81	0.242	14.40	43.94	<0.001	0.667	3.265
		Failure	0.23	0.25	0.21	0.829	0.12	0.33	0.001	0.716	0.033

[1] SEM – Standard error of mean.

Table 2.12 Percentage of successful jumps per bird by Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 24 hours at 4 weeks of age (4 pen replicates per L × S).

		Light Intensity (L)				Strain (S)			L × S	
From	To	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Jumps upward										
Floor	Drinker	96.19	98.49	98.81	0.189	97.86	97.80	0.996	0.290	0.624
Floor	Ramp	98.14	98.78	99.28	0.702	98.55	98.92	0.721	0.416	0.536
Floor	Perch	96.77	97.17	97.51	0.897	95.18	99.12	0.006	0.690	0.701
Jumps downward										
Drinker	Floor	100.00	99.91	99.98	0.198	100.00	99.92	0.080	0.198	0.024
Ramp	Floor	100.00	100.00	100.00	-	100.00	100.00	-	-	0.000
Perch	Floor	99.97	99.83	99.82	0.479	99.77	99.98	0.066	0.212	0.057
Jumps across										
Perch	Perch	98.92	99.36	99.59	0.265	99.29	99.29	0.982	0.731	0.140
Total jumps		98.99	99.20	99.29	0.476	99.07	99.25	0.378	0.543	0.096

[1] SEM – Standard error of mean.

Table 2.13 Average number of successful jumps per bird directed upward, downward, and across by Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 24 hours at 8 weeks of age (4 pen replicates per L × S).

			Light Intensity (L)				Strain (S)			L × S	
From	To		10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Jumps upward											
Floor	Drinker	Success	2.75	3.19	3.71	0.337	3.04	3.39	0.758	0.129	0.271
		Failure	0.02	0.02	0.03	0.954	0.02	0.03	0.264	0.940	0.004
Floor	Ramp	Success	0.31	0.37	0.40	0.515	0.36	0.36	0.971	0.936	0.030
		Failure	0.00	0.00	0.00	0.387	0.00	0.00	0.331	0.387	0.001
Floor	Perch	Success	10.30	10.38	10.38	0.880	5.49	15.22	<0.001	0.778	1.169
		Failure	0.04	0.04	0.03	0.834	0.02	0.05	0.001	0.456	0.006
Jumps downward											
Drinker	Floor	Success	2.58	2.97	3.38	0.372	2.94	3.01	0.884	0.116	0.239
		Failure	0.00	0.00	0.00	-	0.00	0.00	-	-	0.000
Ramp	Floor	Success	0.11	0.09	0.19	0.343	0.09	0.17	0.121	0.064	0.032
		Failure	0.00	0.00	0.00	-	0.00	0.00	-	-	0.000
Perch	Floor	Success	7.58	7.28	7.53	0.937	3.39	11.53	<0.001	0.817	0.921
		Failure	0.00	0.00	0.00	0.447	0.00	0.00	0.329	0.385	0.001
Jumps across											
Perch	Perch	Success	8.40	10.34	9.67	0.193	8.98	9.97	0.143	0.615	0.380
		Failure	0.01	0.02	0.01	0.234	0.01	0.01	0.615	0.415	0.004
Total jumps											
		Success	32.03	34.62	35.27	0.479	24.29	43.66	<0.001	0.812	2.463
		Failure	0.07	0.08	0.07	0.651	0.05	0.10	0.003	0.519	0.009

[1] SEM – Standard error of mean.

Table 2.14 Percentage of successful jumps per bird by Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 24 hours at 8 weeks of age (4 pen replicates per L × S).

		Light Intensity (L)				Strain (S)			L × S	
From	To	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Jumps upward										
Floor	Drinker	97.98	99.15	99.18	0.569	99.26	98.28	0.360	0.559	0.516
Floor	Ramp	100.00	99.22	100.00	0.387	100.00	99.48	0.331	0.387	0.260
Floor	Perch	99.62	99.65	99.67	0.920	99.68	99.61	0.326	0.230	0.059
Jumps downward										
Drinker	Floor	100.00	100.00	100.00	-	100.00	100.00	-	-	0.000
Ramp	Floor	100.00	100.00	100.00	-	100.00	100.00	-	-	0.000
Perch	Floor	99.98	100.00	100.00	0.447	100.00	99.99	0.329	0.385	0.007
Jumps across										
Perch	Perch	99.95	99.82	99.90	0.351	99.90	99.88	0.838	0.449	0.033
Total jumps		99.76	99.76	99.79	0.865	99.79	99.76	0.600	0.656	0.028

[1] SEM – Standard error of mean.

Table 2.15 Average number of successful jumps per bird directed upward, downward, and across by Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 24 hours at 13 weeks of age (4 pen replicates per L × S).

			Light Intensity (L)				Strain (S)			L × S	
From	To		10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Jumps upward											
Floor	Drinker	Success	2.59	3.33	3.26	0.349	2.43	3.68	0.025	0.361	0.278
		Failure	0.01	0.02	0.00	0.200	0.01	0.01	0.383	0.951	0.004
Floor	Ramp	Success	0.11	0.18	0.17	0.380	0.14	0.17	0.427	0.377	0.021
		Failure	0.00	0.00	0.00	0.447	0.00	0.00	0.329	0.385	0.001
Floor	Perch	Success	10.69	11.02	10.96	0.749	6.78	15.00	<0.001	0.711	0.933
		Failure	0.01	0.02	0.02	0.251	0.01	0.02	0.077	0.432	0.004
Jumps downward											
Drinker	Floor	Success	2.56	3.19	3.00	0.445	2.37	3.46	0.034	0.440	0.250
		Failure	0.00	0.00	0.00	-	0.00	0.00	-	-	0.000
Ramp	Floor	Success	0.16	0.11	0.18	0.574	0.12	0.18	0.266	0.912	0.025
		Failure	0.00	0.00	0.00	-	0.00	0.00	-	-	0.000
Perch	Floor	Success	8.10	8.22	8.35	0.939	3.83	12.62	<0.001	1.000	0.953
		Failure	0.00	0.00	0.00	-	0.00	0.00	-	-	0.000
Jumps across											
Perch	Perch	Success	6.66	7.10	6.79	0.669	7.79	5.92	0.003	0.530	0.311
		Failure	0.00	0.01	0.00	0.554	0.00	0.01	0.181	0.520	0.002
Total jumps											
		Success	30.88	33.14	32.71	0.591	23.46	41.03	<0.001	0.780	2.084
		Failure	0.02	0.04	0.02	0.209	0.02	0.04	0.053	0.353	0.007

[1]SEM – Standard error of mean.

Table 2.16 Percentage of successful jumps per bird by Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 24 hours at 13 weeks of age (4 pen replicates per L × S).

		Light Intensity (L)				Strain (S)			L × S	
From	To	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Jumps upward										
Floor	Drinker	99.68	99.39	99.91	0.172	99.72	99.60	0.579	0.956	0.108
Floor	Ramp	96.88	100.00	100.00	0.447	97.92	100.00	0.329	0.385	1.042
Floor	Perch	99.95	99.84	99.88	0.381	99.90	99.88	0.687	0.779	0.031
Jumps downward										
Drinker	Floor	100.00	100.00	100.00	-	100.00	100.00	-	-	0.000
Ramp	Floor	100.00	100.00	100.00	-	100.00	100.00	-	-	0.000
Perch	Floor	100.00	100.00	100.00	-	100.00	100.00	-	-	0.000
Jumps across										
Perch	Perch	100.00	99.90	99.95	0.559	100.00	99.90	0.179	0.526	0.038
Total jumps		99.94	99.88	99.95	0.281	99.93	99.91	0.605	0.337	0.018

[1] SEM – Standard error of mean.

Table 2.17 Average number of successful jumps per bird directed upward, downward, and across by Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 24 hours at 16 weeks of age (4 pen replicates per L × S).

			Light Intensity (L)				Strain (S)			L × S	
To	From		10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
<u>Jumps upward</u>											
Floor	Drinker	Success	2.81	3.54	3.37	0.379	2.57	3.91	0.009	0.592	0.266
		Failure	0.00	0.01	0.01	0.635	0.00	0.01	0.143	0.358	0.002
Floor	Ramp	Success	0.08	0.05	0.11	0.191	0.05	0.12	0.014	0.301	0.015
		Failure	0.00	0.00	0.00	-	0.00	0.00	-	-	0.000
Floor	Perch	Success	10.08	10.79	11.24	0.157	6.25	15.16	<0.001	0.128	0.985
		Failure	0.01	0.02	0.03	0.119	0.01	0.02	0.154	0.474	0.004
<u>Jumps downward</u>											
Drinker	Floor	Success	2.75	3.51	3.31	0.356	2.55	3.84	0.010	0.600	0.260
		Failure	0.00	0.00	0.00	0.447	0.00	0.00	0.329	0.385	0.001
Ramp	Floor	Success	0.14	0.06	0.10	0.150	0.07	0.13	0.115	0.267	0.019
		Failure	0.00	0.00	0.00	-	0.00	0.00	-	-	0.000
Perch	Floor	Success	8.68	8.81	9.23	0.800	4.32	13.49	<0.001	0.901	0.982
		Failure	0.01	0.00	0.00	0.057	0.00	<0.01	0.083	0.057	0.001
<u>Jumps across</u>											
Perch	Perch	Success	6.65	7.41	6.66	0.310	8.04	5.77	<0.001	0.427	0.318
		Failure	0.00	0.01	0.00	0.820	0.00	0.00	0.992	0.480	0.002
<u>Total jumps</u>											
		Success	31.21	34.17	34.03	0.172	23.86	42.41	<0.001	0.416	2.086
		Failure	0.02	0.03	0.04	0.208	0.02	0.04	0.032	0.534	0.005

[1] SEM – Standard error of mean.

Table 2.18 Percentage of successful jumps per bird by Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux over 24 hours at 16 weeks of age (4 pen replicates per L × S).

		Light Intensity (L)				Strain (S)			L × S	
From	To	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Jumps upward										
Floor	Drinker	99.91	99.86	99.77	0.715	99.96	99.73	0.116	0.462	0.069
Floor	Ramp	100.00	100.00	100.00	-	100.00	100.00	-	-	0.000
Floor	Perch	99.94	99.81	99.74	0.197	99.81	99.85	0.681	0.598	0.039
Jumps downward										
Drinker	Floor	100.00	100.00	99.95	0.447	100.00	99.97	0.329	0.385	0.017
Ramp	Floor	100.00	100.00	100.00	-	100.00	100.00	-	-	0.000
Perch	Floor	99.96	100.00	100.00	0.073	100.00	99.97	0.098	0.073	0.010
Jumps across										
Perch	Perch	99.97	99.92	99.97	0.693	99.96	99.94	0.758	0.543	0.023
Total jumps		99.95	99.91	99.89	0.352	99.93	99.90	0.320	0.761	0.014

[1] SEM – Standard error of mean.

In terms of S effects, LW pullets performed more jumps than LB from the drinker to the floor at four, 13, and 16 wks, however there was a trend for LW pullets to have numerically small but more failed landings than LB pullets at four wks (0.01 vs 0.00 failed landings, *P*=0.065, Table 2.11). This affected landing success rate, where LB pullets tended to have a higher success percentage (100.00%) at jumping down from drinker lines to the floor than LW pullets, although, the numbers were very small (99.92%, *P*=0.080, Table 2.12). Across all recording periods, there was no effect of S on jumps from the ramp to the floor, however there was an effect on jumps from the perch to the floor. At all observation weeks, LW pullets performed more jumps down from the perch to the floor than LB pullets. A trend was noted for LW pullets to have a higher jumping success rate (99.98%) from the perch to the floor than LB pullets (99.77%) at four wks (*P*=0.066, Table 2.12). Another trend was also noted for LW pullets to have more failed landings (<0.01) than LB (0.00) at 16 wks (*P*=0.083, Table 2.17). However, this difference was numerically small and did not affect jumping success rates (*P*=0.098, Table 2.18).

2.4.2.3 Jumps Across

Jumps across were those that are within the same plane of elevation. In this study, jumps across consists of jumping between rungs of the perchery system, and the effect of L on these jumps was noted only at four wks. Pullets reared in 30 lux performed more jumps (average 8.41 jumps per pullet over 24 hours) than pullets reared in 10 lux (5.91 jumps), with pullets in 50 lux as intermediate (7.29 jumps) (*P*=0.033, Table 2.11). Light intensity had no effect on jumps across perches at all other observation periods. There was also no effect of L on success percentage in all recorded weeks.

Strain had an interesting influence on the number of jumps across different rungs of the perchery system. At four wks, LW pullets performed more jumps (average 8.02 jumps per pullet over 24 hours) than LB pullets (6.39 jumps) (*P*=0.016, Table 2.11). However, at 13 and 16 wks, LB pullets performed more jumps (7.79 and 8.04 jumps) than LW (5.92 and 5.77 jumps) (*P*=0.003 and P<0.001, Table 2.15, 2.17). Regardless, jumping success rate between the two S did not significantly differ throughout all observation weeks (Table 2.12, 2.14, 2.16, 2.18).

2.4.2.4 Total Jumps

Total jumps consisted of the accumulated number of jumps within the environment, including jumps upwards, downwards, and across. Although L had some effect on the number of jumps in each direction, overall, it did not impact the total jumps performed by pullets.

Strain influenced total jumps by pullets in the pen. Throughout all recording periods, LW pullets performed more jumps than LB pullets. Specifically, at four wks, LW pullets performed 3.05 times more jumps than LB (P<0.001, Table 2.11), followed by 1.80 times more jumps than LB at 8 weeks (P<0.001, Table 2.13), 1.75 times at 13 weeks (P<0.001, Table 2.15), and 1.78 times at 16 wks (P<0.001, Table 2.17). LW pullets also had more failed landings than LB pullets at all weeks, specifically crashing 2.75 times more at four wks (P=0.001), and 2.00 times more at eight (P=0.003), 13 (trend only, P=0.053), and 16 wks (P=0.032). However, these failed landings were numerically small. Despite failing more, this did not affect overall jumping success percentage between the two S.

2.4.3 Novel Object Test

Light intensity did not affect the latency to peck at the novel object, however LB pullets took a significantly longer time to peck at the novel object (676 s) than the LW pullets (212 s, P<0.001, Table 2.19). There was no interaction between L and S (Table 2.19).

2.4.4 H/L Ratios

Heterophil/lymphocyte ratios are summarized in Table 2.20. H/L ratios were not affected by L, nor was there an interaction between L and S. However, S had an effect on ratios; LW birds had a significantly lower H/L ratio (0.13) than LB birds (0.26, P<0.001, Table 2.20).

2.5 Discussion

2.5.1 Interactions Between Strain and Light Intensity

Behavioural observations are an important tool in assessing an animal’s response to its environment. To help understand whether S react differently to varying L, two S were used in this study. Interactions were observed only at four weeks and eight wks. At four wks, the interaction was on time spent flying or jumping. These activities were more common in LB pullets reared in 30 lux and were significantly more than those reared in 10 lux and 50 lux. For LW pullets, this behaviour was observed most in 10 lux.

Table 2.19 Latency to peck at novel object (seconds) by Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux at 15 weeks of age (8 pen replicates per L × S).

Light Intensity (L)				Strain (S)			L × S	
10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
397	497	437	0.436	676	212	<0.001	0.415	41.9

[1] SEM – Standard error of mean.

Table 2.20 Heterophil/lymphocyte ratios of Lohmann Brown-Lite (LB) and Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in 10, 30, and 50 lux at 15 weeks of age (12 pen replicates per L × S).

Light Intensity (L)				Strain (S)			L × S	
10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
0.20	0.18	0.20	0.507	0.26	0.13	<0.001	0.922	0.011

[1] SEM – Standard error of mean.

On the contrary, Moinard et al. (2004b) reported no difference in jumping accuracy among five, 10, and 20 lux in Lohmann Brown hens at 25 wks. The disagreement between results may be due to the age of birds used during the time of study, or due to L. Moinard et al. (2004b)'s light intensities were below 20 lux, while the present study only had one similar L treatment (10 lux). Therefore, results from the study of Moinard et al. (2004b) cannot be extended to this present study and it is probable that higher L up to 30 or 50 lux may yield different results, such as the case of this present study.

Strains reacted differently to L for gentle pecking behaviour at four wks. Light intensity had no effect on LB pullets, whereas LW pullets reared in 50 lux were observed to peck at their peers more than those reared in 10 lux. Gentle feather pecking is socially orientated and increases at four to eleven wks (Hughes and Duncan, 1972; Riedstra and Groothuis, 2002). In fact, gentle feather pecking has been suggested to be a form of allopreening which can reduce aggression and create and maintain social bonds (Riedstra and Groothuis, 2002). Results from this study indicate that at four wks, LW pullets reared in 50 lux spent more time performing gentle feather pecking behaviour which can in turn have positive welfare attributes.

At eight wks, a different interaction was noted. Although L did not impact behaviour observations, a larger percentage of LB pullets could not be identified, particularly at 10 lux. In contrast, LW pullets in the same L did not have the same issue. Therefore, this indicates that the barrier to identifying pullet behaviour was not based on the L setting, but rather S. In fact, LB pullets' behaviours were consistently more difficult to identify in all recorded weeks, regardless of L. One possible explanation could be the dark feather colour of LB pullets which matches the straw bedding on the floor. So, even though it is possible to identify the presence of a LB pullet, it is challenging to indicate specifically what behaviour the pullet is performing. On the other hand, the white feather colour of LW pullets would provide a contrast against the ground litter. As a result, low incidence behaviours such as those involved in interactions could have affected the results obtained. Other than the interaction at eight wks, there was no effect of L on unidentified behaviours at the other recorded weeks, indicating that L did not affect the ability for the observer to identify behaviours among pullets.

2.5.2 Light Intensity Effects

Several studies have reported an overall increase in active behaviours with increasing L in laying hens (Hughes and Black, 1974; Boshouwers and Nicaise, 1987; O'Connor et al., 2011). Results of the present study partially support this claim. Overall active behaviours did not change between the intensity treatments, however there was an effect specifically on the percentage of time pullets spent walking. At eight wks, the amount of time spent walking increased with L. Pullets reared in 50 lux spent more time walking than pullets reared in 10 lux, with pullets in 30 lux as intermediate. An increase in time spent performing active behaviours such as walking may indicate a state of positive well-being (Newberry, 1995; Bizeray et al., 2000). However, walking may also increase during distress (Dawkins, 2003). Results of the present study agreed with Kjaer and Vestergaard (1999) who found increase in walking behaviour in ten-week-old pullets reared in 30 lux compared to three lux. This data shows that at eight wks, pullets reared in higher L of 50 lux are spending more time mobilizing and navigating around the environment. At 13 and 16 wks, there was no difference in percentage of time spent walking between pullets housed in different L treatments. This lack of trend occurring at all recording periods suggests a change in behaviour with age, as discussed by Kozak et al. (2016) who reported decreasing active behaviours with age. Explanations for this pattern may be due to the lack of nutritional stress in pullets before entering the laying phase (Kozak et al., 2016), higher amounts of energetic movements and capacity in younger animals than older (Dial and Jackson, 2011), or social organization whereby the actions of pullets are influenced by others performing the same behaviour (McBride et al., 1969).

The results of this study revealed no effect of L on resting behaviour. This is in contrast with a study by Alvino et al. (2009a) who reported increase in resting behaviour in broilers housed in five lux compared to 50 and 200 lux. Several explanations can be made to describe this disagreement in result. Firstly, the L treatments in Alvino et al. (2009a) were drastically different, whereas the present study used light intensities that were only 20 lux apart. In a more comparable study, Deep et al. (2012) conducted an experiment on broilers housed in one, 10, 20, or 40 lux. The authors reported an increase in resting behaviour in broilers housed in one lux, however noted no difference in resting behaviour for the other L treatments, which is in agreeance with this present study. Interestingly, Boshouwers and Nicaise (1987) reported that resting is independent of L ranging between 0.5 and 120 lux in laying hens, which would also be in agreeance with this study. Secondly, Alvino et al. (2009a) studied broilers, whereas the present study was conducted on layer pullets. A study by Lambertz et al. (2018) revealed more time spent sitting or lying in purebred male broilers than crossbred dual-purpose laying hens. Therefore, it is possible that due to genetic selection and broilers having higher meat to carcass ratio than laying hens, broilers spend more time resting than laying hens in general, unless otherwise influenced by L, such as in the case of the study by Alvino et al. (2009a).

For comfort behaviours, a general pattern was observed for pullets to spend more time preening when reared in higher L (30 or 50 lux) than 10 lux, especially at 13 and 16 wks. Based on the results obtained, L between the range of 10 and 30 lux may not be different enough to result in an increase in the expression of comfort behaviour within the flock, however a L of 50 lux may result in this increase. Taylor et al. (2003) reported increase in frustration, measured by increased vocalizations in 59-week-old laying hens tasked to complete a jumping task in 0.8 lux compared to 1.5, 6.0, and 40.0 lux. From their study, L between 1.5 and 40 lux did not affect discomfort levels, which is in partial agreement with the present study. In the present study, comfort levels expressed via preening behaviour did not differ between 10 and 30 lux, which is comparable to the study by Taylor et al. (2003) ranging between 1.5 and 40.0 lux. However, perhaps when L is increased up to 50 lux, an increase in comfort behaviour may be observed, such as is the case of this present study where time spent preening increased. Preening can be visually motivated, as reported by Widowski et al. (1992) who observed increased preening in 24-week-old laying hens reared in fluorescent versus incandescent lighting. Therefore, it is also possible for time spent preening in this study to be affected by L.

No differences in overall nutritive behaviour were observed, however at 16 wks, pullets reared in L of 10 and 50 lux spent more time at the drinker than pullets reared in L of 30 lux. Prescott and Wathes (2002) stated that dim conditions (less than one lux) can be too faint for birds to see and can lead to starvation and dehydration. Deep et al. (2013) reported high levels of mortality in broilers when L was at 0.1 lux. Based on the outcomes of this study, L at 10 lux is not too dim for pullets to lose their visual acuity to the extent of starvation and dehydration (Chapter 3). Although pullets reared in 30 lux spent less time at the drinker than pullets reared in 10 and 50 lux, this nutritive behaviour does not affect overall body weight (Chapter 3) and was likely due to random chance.

Light intensity affected time spent pecking at the walls of the pen. At four wks, there was no difference in wall pecking behaviour between pullets reared in different light intensities. At eight wks, a trend was observed for time spent wall-pecking to decrease with increasing L. The negative linear relationship became significant at 13 wks and remained at 16 wks. This observation is supported by Kjaer and Vestergaard (1999) who also observed a reduction in gentle pecking (another type of exploratory behaviour) in pullets housed in 30 lux compared to three lux at 10 wks. The authors suggested that, during early development, low L (three lux) may lead to a reduced ability to identify environmental cues and thus causing them to increase the time spent exploratory pecking for compensation (Kjaer and Vestergaard, 1999). The type of exploratory behaviour that was reported by the authors is not similar to the one in this present study. A possible explanation for this could be the different type of behaviour recorded; the authors reported observations in severe feather pecking behaviour, whereas wall pecking behaviour was not included in their study. In fact, wall pecking behaviour is rarely found or reported in other studies. Perhaps then, exploratory behaviour in the context of Kjaer and Vestergaard's study could be extended to this study, but as a different form of exploration. Another form of exploratory behaviour is litter-directed, however this study revealed no effect of L on this behaviour, which is in contrast with other studies who reported increase in litter-directed behaviours with increase in L (Davis et al., 1999; Deep et al., 2012). It is possible that the light intensities used in this study allow similar litter-directed behaviours across treatments, however more research is warranted in this area.

There was no effect of L on aggressive behaviour or severe feather pecking. Unlike Kjaer and Vestergaard (1999), who reported two to three times more severe feather pecking behaviour in 10-week-old pullets reared in 30 lux compared to three lux, this present study showed no difference in aggression. Rather, the results are supported by Hartini et al. (2002) who reported no effect of L between a range of five to 80 lux on cannibalism on laying hens. It is therefore also possible that this study's L treatment range of 10 lux to 50 lux are not different enough to result in aggressive behaviour in pullets reared in floor pens from zero to 16 wks.

The success of pullet jumps was high through all observation periods. A few differences were noted at four wks. Pullets reared in 10 lux performed less jumps from the floor to the ramp and across the perch than pullets reared in 50 or 30 lux respectively. Although these findings were not consistent for the remainder for the observation periods, it may possibly indicate the importance of higher L for jumping between resources in the environment during an early age of four weeks. A study by Taylor et al. (2003) demonstrated the importance of high L for jumping. In their study, laying hens were tested for their latency to jump from one perch to another at 0.8, 1.5, 6.0, or 40.0 lux, and the latency to jump was significantly greater at light intensities below six lux. For illuminance to fall below what is required for visual acuity levels resulting in latency to jump and increased vocalizations, the environment would have to be below five lux (Taylor and Scott, 2002). In the present study, light intensities were not severe enough to affect the incidence of failed landings. However, similar to Taylor et al. (2003), in the present study, pullets reared in 50 lux performed more jumps from the floor to the ramp than those in 10 lux, and pullets in 30 lux performed more jumps between perches than those in 10 lux at four wks. This may indicate that with regards to the number of jumps, it is perhaps that higher L up to 30 or 50 lux may increase jumping, and better prepare pullets for navigating a complex environment.

The observation of increase in “jumps between” with L was reported only at four wks. This may be explained by Kozak et al. (2016) who stated that young pullets (10-16 wks in their study) express the largest amount of high-intensity physical activity (aerial ascent) compared to other age periods (17-24 and 25-37 wks). Therefore, the results of this study suggest that L higher than 10 lux can increase jumping behaviour of pullets within the environment, especially during the early stage of life, such as at four wks. However, despite a greater number of jumps in higher light intensities of 30 and 50 lux, the results from the present study indicate that L between 10 and 50 lux does not affect jumping success rate or pullet jumping accuracy. This is in agreeance with a study by Moinard et al. (2004b) using L of five, 10, or 20 lux. Additionally, this conclusion may only apply to targeted landings that are unobstructed, for instance an uncrowded perch.

In this study, it was hypothesized that fearfulness would increase with increasing L. This is because several studies reported a positive relationship between L and aggression or severe feather pecking (Kjaer and Vestergaard, 1999; Kristensen, 2008). As such, an increase in aggressive behaviour or severe feather pecking would simultaneously result in an increase in fear within the flock. Results from the novel object testing in this study revealed no difference in fear levels. This is in agreeance with Olanrewaju et al. (2007) who reported no difference in tonic immobility in broilers, another indicator of fear, with light intensities between the range of 0.2 and 20 lux. Additionally, behaviour observations from this study support the results of the novel object test. Severe feather pecking or aggressive pecking is often associated with fear (Hughes and Duncan, 1972; Bolhuis et al., 2009), which was minimal in the present study. Therefore, L of 10 to 50 lux do not affect the fear levels of pullets.

Similar to fear response, stress levels were also hypothesized to increase with increasing L. Heterophil/lymphocyte ratios are a reliable indicator of pullet stress (Maxwell and Robertson, 1998). Results from the present study suggest that L had no effect on pullet stress, and this is supported by Lien et al.'s (2007) work on broilers housed between one and 10 lux. Olanrewaju et al. (2011) also reported no difference in stress levels in broilers housed between 0.2 and 25 lux, as measured by blood corticosterone levels. Furthermore, O'Connor et al. (2011) reported no differences in corticosterone and heterophil/lymphocyte ratio of laying hens housed between five and 150 lux, however other differences were noted; hens housed in five lux had lower egg production than those in 150 lux. The present study was conducted on pullets only up to 16 wks, and so was unable to confirm egg production levels between L treatments. Nonetheless, this study affirms previous studies' findings, that L at a range of 10 and 50 lux does not affect heterophil/lymphocyte ratios of pullets.

2.5.3 Strain Effects

Overall, LW pullets spent more time on perches than LB pullets, which is in agreeance with several studies (Tauson and Abrahamsson, 1996; Wall and Tauson, 2007). LW pullets also consistently spent more time resting than LB pullets in all recorded weeks. This agrees with Mohammed and Said (2016) who also found that 30-week-old Lohmann Selected Leghorn hens ate, preened, nested, and rested more than Lohmann Brown hens. This could be due to the fact that most resting behaviour occurs on perches, which is correlated to high resting behaviour and high perch use in LW than LB pullets (Braastad, 1990; Mohammed, 2012).

LW pullets also expressed a higher incidence of comfort behaviour than LB pullets, which can be linked to high perch use. A study by Jensen (2019) reported that comfort behaviours were more frequently performed by laying hens when located on perches and platforms than the floor of furnished cages. On the other hand, LB pullets spent more time performing exploratory behaviours than LW pullets. Pusch et al. (2018) explained that this behavioural difference between the two S may relate to reactivity level (as defined by the degree of hormonal and behavioural responses) of the birds. LW pullets exhibit more reactive, flighty responses while LB pullets are more proactive and exploratory (Fraisse and Cockrem, 2006). In the same way, this difference could explain other results from the study. LB pullets had a higher latency to peck at the novel object than LW pullets. The novel object test as carried out in the present study assessed the response of the flock towards a novel object in the environment (Jones, 1996). By assessing the level of response, it is possible to measure fear levels when a novel item is introduced into their home environment, whereby the longer time taken to peck at the novel object, the more fearful the flock (Hughes and Black, 1974; Jones, 1996). The theory of reactivity extends to this study such that although LB pullets took a longer response time towards the novel object, it may not be due to their fear levels, but rather due to their low hormonal and behavioural response to an acute stressor, such as the introduction of a foreign object into the environment.

This is further supported by the low aggression levels within the flock. Aggression is often associated with fear, however there were no differences between the two S used (Hughes and Duncan, 1972). Moreover, the physiological results indicative of stress response via heterophil/lymphocyte ratio from this study can be explained by Pusch et al. (2018) who observed the different ways for Brown- and White- feathered S react to the same stressor. LW pullets are more reactive physiologically and quick to react behaviourally to stress, while the physiological responses of LB pullets are lower with slower behavioural reaction than LW pullets (Fraisse and Cockrem, 2006; Pusch et al., 2018; Peixoto et al., 2020). Therefore, although LB pullets have a higher latency to peck at the novel object and a higher heterophil/lymphocyte ratio than LW pullets, this may be due to genotypic traits, as opposed to LB pullets experiencing fear and stress.

The differences between S may also explain the number of jumps and flights within the environment. LW pullets performed more successful and non-successful jumps within the environment than LB pullets in all recorded weeks despite no difference in percentage of success. Jumping frequency can be explained by the body type between S. While LB pullets have larger body weight, LW pullets are comparatively lighter and can generate enough energy to navigate vertically and diagonally within a complex environment (Moinard et al., 2004b; Tobalske and Dial, 2007). A bird must be able to generate lift in relation to the force of gravity and with precise trajectory in order to fly with the right velocity and land accurately (Provine et al., 1984; Moinard et al., 2004b). For LB pullets that are heavier than LW pullets, this ability for flight manoeuvre and performance decreases with increasing body size (Marden, 1994; Moinard et al., 2004b). This may also explain why LB pullets were observed to spend more time on ramps than LW pullets at four wks. However, despite the lower number of jump performances, the results of this study found equal chances of success in jumping between LB and LW pullets, indicating that LB pullets still safely utilize perches, although to a lower degree.

2.6 Conclusion

The objective of this study was to determine the effect of L and S on behaviour, navigation, and welfare of Lohmann Brown-Lite and LSL-Lite pullets reared in floor pens containing a perchery system from 0 to 16 wks. The results of the present study demonstrated that L within a range of 10 and 50 lux resulted in minor changes in behaviour, with a small increase in preening noted at higher L. Light intensity at a range of 10 and 50 lux using white LED lights did not affect aggressive behaviour, nor fear and stress levels of pullets up to 16 wks. With respect to navigation, fewer jumps were recorded in 10 lux than 30 or 50 lux for several jumping behaviours at four wks, which many indicate that increasing L settings to up 30 or 50 lux, especially at a young age of four weeks, may help pullets perform more jumps within the environment, and subsequently prepare them for navigating complex environments. However, L at 10 lux is also sufficient for pullets to jump within their environment safely.

LW pullets spent more time resting and preening, and also performed more jumps in the environment, whereas LB pullets spent more time performing litter-directed behaviours and were reported to peck at walls more than LW pullets who spent more time on perches. LB pullets had a longer response to the fear test and a higher H/L ratio. Despite this, there was no difference in aggression levels between the two S. This may indicate genetic differences in behaviour between LB and LW pullets.

Overall, the results of the present study suggest that LB and LW pullets are able to effectively navigate and express their natural behaviours without fear or stress in floor pens with a perchery system in an environment illuminated with white LED lights at 10, 30, or 50 lux. For industry, while Canada transitions to alternative housing systems for laying hens, the current regulations on L set by the National Farm Animal Care Council (2017) do not negatively affect the navigation or welfare of pullets. However, higher light intensities at 30 or 50 lux may increase jumps within the environment which can better prepare pullets for navigating a complex environment.

3.0 Chapter 3: The impact of light intensity on body weight, keel bone quality, breast muscle weight, tibia bone strength, and mortality of pullets housed in perchery systems from 0 to 16 weeks of age

Chapter 3 focuses on the effects of light intensity (10, 30, and 50 lux) on the growth and bone health of pullets reared in floor pens containing a perchery system with a specific focus on body weight, keel bone quality and integrity, tibia bone strength, and mortality. Keel bone quality indicators included keel bone damage such as deviations and fractures, and keel bone integrity included breast muscle weight. The data presented in this chapter will help better understand the impact of light intensity on bone health of pullets in floor pens during the rearing phase.

3.1 Abstract

The study aimed to determine the effect of light intensity (L) on body weight (BW), keel bone damage (KBD), tibia bone strength, and mortality of Lohmann Brown-Lite (LB) and Lohmann LSL-Lite (LW) pullets. Three L (10, 30, or 50 lux, provided by white LED lights) and two strains (S) were used in a randomized complete block design. LB and LW (n=1,800 per S) were randomly assigned to floor pens (50 pullets per pen; 12 pen replicates per L×S) from 0 to 16 weeks of age (wk). Each floor pen contained a system of four parallel perches, ramp, two tube feeders, and a drinker line. Pullet BW was collected on a pen basis at 0, 8, and uniformity at 16 wk. Mortality and causes were recorded daily. At 16 wk, 10 pullets per pen (12 pen replicates per L×S) were palpated for KBD (deviations, fractures). An additional 9 pullets per pen (12 pen replicates per L×S) were euthanized and the keel bones, breast muscle, and right tibiae were removed to assess KBD, breast muscle weight, and bone strength, respectively. Bone strength was assessed using a 3-point-bending test. The effect of L, S, and their interactions were analyzed using Proc Mixed (SAS 9.4) with Tukey's range test used to separate means. Differences were significant when $P<0.05$. There was no effect of L, and no interactions between L and S, for any of the measured parameters. LB pullets were heavier than LW at 8 and 16 wk. S had no effect on KBD from either palpated or dissected keel bones. LB pullets had a higher breast muscle weight than LW, however relative to BW, LW pullets had a higher breast muscle mass. LB pullet's tibiae were heavier than LW, however LW pullets' tibiae were longer, thicker, and had a higher bone strength than LB when corrected for bone size. LW also had higher mortality than LB during the first week. Overall, the results indicate that L, within a range of 10 to 50 lux, does not affect pullet keel bone health or musculoskeletal development, however S may play a role in these parameters.

3.2 Introduction

By the year 2036, all laying hen housing systems in Canada must utilize either furnished cages or non-cage housing systems (NFACC, 2017). These housing systems support nesting, perching, and foraging behaviour, and improve welfare of the flock by allowing more freedom for laying hens to express their natural behaviour compared to conventional cage housing systems (Sandilands et al., 2009). Vision plays an important role in helping these birds utilize a more spacious environment, and light intensity (L) can help with vision and navigation (McFadden, 1993). Currently, industry standards require a minimum of 10 lux for egg-strain layers kept in such housing systems (NFACC, 2017). However, if light intensities were higher than 10 lux, it may help these birds, especially pullets, with navigation. Therefore, this study was designed to understand whether L higher than 10 lux, (30 and 50 lux), can improve navigation of pullets reared in a non-cage housing system (floor pen) containing a perch as environmental enrichment.

If there were unsuccessful landings on environmental resources such as perches, it could result in bone damage (Sandilands et al., 2009). Not only is this painful for pullets, but damage to the bone structure could lead to negative long-term effects during the laying phase, and have negative implications for welfare (Sandilands et al., 2009). Increasing L in non-cage housing systems may help mitigate this issue. Keel bone damage is an example of bone damage and is a major problem for commercial laying hens and is generally classified into deviations and/or fractures (Fleming et al., 2004; Casey-Trott et al., 2015). Keel bone deviations are abnormally shaped structures of the keel bone, which in appearances can be bent, S-shaped, twisted, or curved (Fleming et al., 2004; Lay et al., 2011; Casey-Trott et al., 2015). Keel bone fractures are sharp bends or fragmented sections of the keel bone, mostly observed at the tip of the keel bone (Einhorn, 2005; Casey-Trott et al., 2015). Keel bone damage is caused by various factors, namely crashes into the environment or strong muscular contractions, such as flying (Sandilands et al., 2009; Harlander-Matauschek et al., 2015). As such, the assessment of keel bone damage will be a good indicator for the effect of L on bone health. To date, only few studies evaluated the effect of L in laying hens. Gregory et al. (1993) reported fewer healed breaks in laying hens housed in 15 lux versus 0.5 or two lux. However, the laying hens were housed in battery cages (Gregory et al., 1993). Other studies that reached conclusions with respect to the possible effects of L include Prescott and Wathes (2002) and Taylor et al. (2003) who stated that very low L at less than one lux can compromise visual acuity, which may affect environmental collisions.

Additionally, the keel bone anchors the breast muscles (*Pectoralis major* and *Pectoralis minor*) which are used for wing motion (Fleming et al., 2004; Casey-Trott et al., 2015). Exposed keel bones can negatively impact breast muscle quality and subsequently flight success (Harlander-Matauschek et al., 2015). Since L may play a role in activity levels and keel bone damage in pullets, breast muscle mass can be correlated with level of exercise (Casey-Trott et al., 2017). McKee et al. (2009) reported heavier filets in broilers housed in one lux versus 150 lux. Yahav et al. (2000) reported no effect of L on breast muscle weight in turkey toms housed in ten versus 700 lux. However, it is difficult to make comparisons since turkeys and broilers have been selected for breast yield while laying hens have been selected for egg production. More research is warranted in this area, especially how L can affect pullet breast muscle mass.

High L can improve pullet vision and increase activity which in turn can improve bone quality and strength (Lewis and Morris, 2006; Casey-Trott et al., 2017). More importantly, exercise can directly impact pullets since their bones are still developing (Whitehead, 2004). Similar to keel bone health, the effect of L on bone strength is not well studied in laying hens. In broilers, Newberry et al. (1986) reported leg disorders were not affected by L ranging 0.1 to 100 lux. A more recent study by Rault et al. (2017) observed no difference in leg strength (measured by a latency to lie test) in broilers housed in 20 lux versus five lux. However, given that these studies were conducted on broilers, and laying hens are more active in nature (Bizeray et al., 2000; Norring et al., 2016), research on how L can affect activity levels and consequently bone strength of pullets is much needed.

Finally, it is important to assess whether L can impact pullet growth. Two factors to consider are body weight and mortality. In literature, few studies reported no effect of L on body weight of broilers housed in five versus 75 lux (Deaton et al., 1981) or six versus 180 lux (Newberry et al., 1988). In laying hens, few studies did not report body weight, however observed that feeding behaviour increased with L ranging less than one lux to 200 lux (Davis et al., 1999; Prescott and Wathes, 2002). In one study with smaller L treatment range (three versus 30 lux), L did not affect time spent eating (Kjaer and Vestergaard, 1999). With respect to mortality, many studies reported no effect of L in broilers (Blatchford et al., 2009; Deep et al., 2010; Olanrewaju et al., 2011; Rault et al., 2017). In layers, Deaton et al. (1981) reported lower mortality in hens housed in 75 lux versus five lux, whereas Kjaer and Vestergaard (1999) observed lower mortality in hens housed in three lux than 30 lux, caused by cannibalism.

This study was designed with the intention of understanding how L can affect pullet bone health, integrity, and strength without inhibiting regular performance indicators such as body weight and mortality. Therefore, the objectives of this study were to determine whether L influences layer pullet performance levels such as body weight and mortality, keel bone damage, breast muscle mass, and bone strength. This study will also examine whether strain (S) influences these factors. The hypotheses of this study were that pullets reared in high L (30 or 50 lux) would have better bone quality and strength than pullets reared in 10 lux. Specifically, brighter lighting (30 or 50 lux) will encourage more navigation within the environment (Chapter 2) and as a result, there will be less keel bone damage for pullets reared in 30 or 50 lux than pullets reared in 10 lux. Increased navigation will also result in increased activity and muscle mass, which will result in stronger tibia and heavier breast muscle mass, respectively. Finally, due to genetic differences, it was also hypothesized that the two S used in this study would react to their environment differently and have different measured outcomes.

3.3 Materials and Methods

The experimental protocol for this experiment was approved by the University of Saskatchewan Animal Care Committee. All birds were cared for as specified in the Guide to the Care and the Use of Experimental Animals by the Canadian Council of Animal Care (2009).

3.3.1 Experimental Design

Two 16-week experiments, blocked by trial, were conducted from May to August 2018 and 2019 to study the effect of L during pullet rearing on body weight, bone quality, and mortality. The experiment was designed as a 3 (L) × 2 (S) factorial arrangement, with room nested within L, in a randomized complete block design. The L treatments were 10, 30, or 50 lux, and the S tested were Lohmann Brown-Lite (LB) and Lohmann Selected Leghorn Lite (LW) (n=300 birds L × S).

3.3.2 Animal Housing and Husbandry per Trial

Newly hatched LB (n=900 per trial) and LW (n=900 per trial) female pullets, sourced from a commercial hatchery (Clark's Poultry, Brandon, MB), were randomly assigned to one of the three L treatments. All pullets were housed in six environmentally controlled rooms containing six pens each (4.0 m × 2.3 m) from 0 days to 16 weeks of age (wks) at an estimated stocking density of 0.15 m^2/bird, in accordance with the recommendations in the Lohmann Management Guide

(Lohmann Tierzucht, 2018). Each pen was bedded with 7-10 cm depth wheat straw and contained one perching system (height 0.56 m × width 1.16 m × length 2.18 m, spaced 30 cm apart) placed prior to chick arrival, and one ramp (length 81.3 cm × width 48.3 cm, at an angle of 38°) placed at 14 days of age to prevent pullets' toes and legs from getting caught in ramp wires. The perching system consisted of four wooden perches. Each of the four perches was a rectangle (length 3.8 cm × height 3.5 cm) with the top corners angled to allow for easy grasping (Figure 2.2), while the ramp was made of 14 gauge wire with 2.54 cm × 2.54 cm dimensions. Each pen also included two pan feeders (0.36 m diameter and 1.13 m circumference, and 0.44 m diameter and 1.38 m circumference at six wks) and one drinker line with six nipples (Lubing Systems LP, Cleveland, TN, USA). During the first week, supplemental feeders and waterers were used. All birds had *ad libitum* access to water and commercial feed appropriate for their stage of development, in accordance with the Lohmann Management Guide (Lohmann Tierzucht, 2018). Additionally, all feeders and drinkers were raised regularly to minimize feed wastage and water spillage. At eight wks, all pullets were wing-banded for identification purposes. All pullets were vaccinated at the commercial hatchery for Marek's Rispens, HVT-IBD, and Poulvac ST (second trial only). The birds were also vaccinated for Newcastle Bronchitis at two, six, and ten wks via coarse spray, for *Salmonella typhimurium* at nine and eleven weeks via coarse spray, and for Newcastle Bronchitis and *Salmonella enteritidis* at 15 weeks via intramuscular injection.

Each room was illuminated with eight 11-watt white light-emitting diodes (LED) lamps (2,821 K, Greengage Lighting Ltd., Edinburgh, UK). The light bulbs were placed over the pens, such that L is similar in all pens. Wavelength was similar in all rooms. For the first seven days, the pullets were on a 23 hours of light and one hour of dark (23L:1D) daily schedule. The photoperiod was gradually decreased every week until the seventh week, at which lights remained at a constant of 8L:16D until the end of trial. Light intensity was set at 50 lux for the first week, and then lux levels were adjusted according to the room appropriate intensity treatment. Light intensity was measured with a lux meter every two weeks (ExTech LT300; ExTech Instruments, Montreal, Quebec, Canada) and adjusted if necessary. Dawn and dusk periods were simulated over a 15-minute period. Temperature was 33°C on the first day and gradually decreased until 20°C by seven wks, at which room temperatures remained constant. All rooms were ventilated through a negative pressure inlet-fan system, and heat was provided via hot water pipes running along the walls of the rooms. Birds were checked a minimum of twice daily throughout the trial.

3.3.3 Data Collection per Trial

3.3.3.1 Body Weight

Birds were weighed on a pen basis to assess body weight changes at zero, eight, and 16 wks. In addition, all birds were weighed individually at 16 weeks to assess flock uniformity as affected by S and L.

3.3.3.2 Keel Bone Assessment

At the end of the trial (16 wks), ten pullets per pen (12 pen replicates per L × S, n=720) were palpated to assess for the presence of fractures or deviations in the keel bone (Casey-Trott et al., 2015). Two trained assessors of keel bone damage examined birds for the presence of a fracture or deviation. The two observers had previously received training to identify keel bone deviations and fractures through the palpation method at the University of Guelph (T. Widowski).

An additional nine pullets per pen (12 pen replicates per L × S, n=648) were euthanized at 16 wks to visually inspect for keel bone deviations and fractures. Pullets were euthanized via injection of T-61 (0.4 mL mebezonium iodide/tetracaine per kg; Intervet Canada Corp, Kirkland, QC, Canada) into the brachial vein. The right *Pectoralis major* and *Pectoralis minor* muscles were removed and weighed to determine if L affected keel bone integrity and breast muscle mass. Breast muscle mass were reported as absolute values and also calculated for breast muscle yield relative to pullet body weight. The remaining keel bones were scored for fractures and deviations. Similar to palpations, a mutual agreement in diagnosis between two assessors was recorded. All birds were also visually inspected for scratches or visible lesions.

3.3.3.3 Bone Strength Assessment

After keel bone removal, right tibiae were removed, cleaned of tendons and muscles using surgical suture scissors and tweezers and frozen until further assessment. At a later day, bones were thawed at 4°C for 24 hours before bone strength assessment. The weights of the bones, including bone head cartilage, were recorded. Measurements of the length and width perpendicular and parallel to the direction of the applied force were recorded using a 150 mm electronic caliper with digital display (Mastercraft 58-6800-4; Mastercraft Tools, Toronto, Canada). Post breakage, the internal widths at the inflection point of the tibia perpendicular and parallel to the direction of the applied force were also recorded. These measurements were used to calculate the distance between the neutral axis of the bone and the extreme outer fiber which are points along the plane of the bone (C, measured in cm), and the moment of inertia (Crenshaw et al., 1981). These calculations are shown below.

Equation 3.1 Calculation for distance between the neutral axis and extreme outer fiber of the bone.

$$C = \frac{1}{2}\ of\ the\ diameter\ \ OR\ \ \frac{D}{2}$$

D = outside diameter of the bone at the point of loading and parallel to the direction of the applied force

Equation 3.2 Calculation for moment of inertia.

$$Moment\ of\ inertia = 0.0491\ (BD^3 - \ bd^3)$$

B = outside diameter of the bone at the point of loading and perpendicular to the direction of the applied force

b = inside diameter of the bone at the point of loading and perpendicular to the direction of the applied force

D = outside diameter of the bone at the point of loading and parallel to the direction of the applied force

d = inside diameter of the bone at the point of loading and parallel to the direction of the applied force

The absolute value of each measurement was used for the calculations. In addition, relative values were calculated to adjust for body weight of the birds.

Bone breaking strengths were determined with an Instron Universal Testing machine (Instron 3366; Instron Corp., Norwood, MA, USA) fitted with a 50 kg load cell and loading rate of 30 mm/min. Bones were placed dorsal side up on supports placed 5 cm apart (Figure 3.1). The flexure load (N) was recorded, and the maximum flexure load was used as the ultimate breaking force required to break the tibia. The flexure load was converted to kilograms using the formula 1 N = 0.010971621 kg.

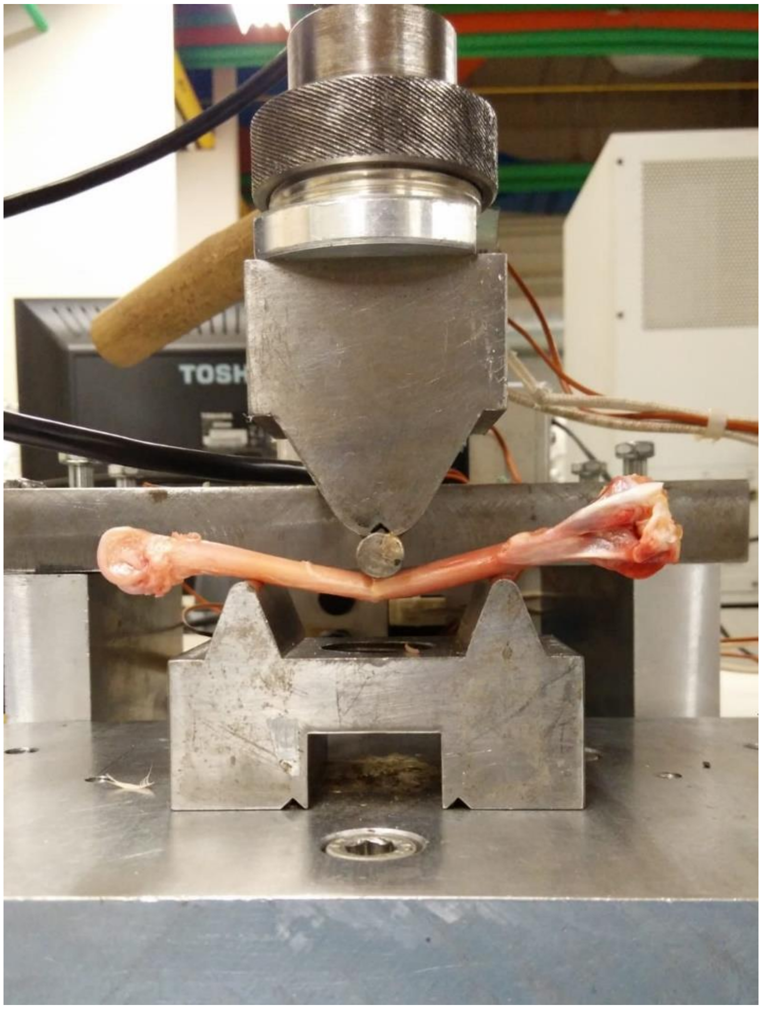

Figure 3.1 Bone strength assessment determined by Instron Universal Testing machine (Instron 3366; Instron Corp., Norwood, MA, USA) fitted with a 50 kg load cell and loading rate of 30 mm/min. Bones were placed dorsal side up on supports placed 5 cm apart.

It was then used to determine stress (kg/cm^2), which is the force necessary to break the bone relative to bone area (Crenshaw et al., 1981). Stress is the strength of each individual bone and considers not only the area over which the force is applied, but also the geometrical area; it is the force required to break the tibia relative to bone size (Crenshaw et al., 1981). The calculation for stress is shown below.

Equation 3.3 Calculation for stress.

$$Stress\left(\frac{kg^2}{cm}\right) = \frac{force\ (kg) \times length\ (cm) \times C\ (cm)}{4\ \times moment\ of\ inertia\ (cm^2)}$$

3.3.3.4 Mortality

Throughout the experiment, birds were monitored daily for morbidity or mortality. Any dead or culled pullets were submitted for necropsy and the cause of morbidity or death was determined by an independent diagnostic laboratory (Prairie Diagnostic Services, Saskatoon, SK, Canada). Light intensity was 50 lux for all treatments during the first week of age, therefore mortality was split into two age periods for analyses: Week 0-1 and Week 1-16.

3.3.4 Statistical Analyses

All data were checked for normality using Proc Univariate (SAS® 9.4, Cary, NC, USA) and percentage data were log transformed (data log +1) to achieve normality. All data were analyzed using Proc Mixed (SAS® 9.4, Cary, NC, USA) as a 3 (L) × 2 (S) factorial arrangement in a randomized complete block design with room as the replicate unit for light (2 repetitions per light treatment per trial) and nested within light, pen as the replicate unit for S (3 replicates per S per room per trial), and trial as block. A Tukey’s range test was used to separate means. For all statistical analyses, significance was declared when $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

3.4 Results

3.4.1 Body Weight

Pullet body weights are reported in Table 3.1. There was no effect of the interaction between L and S on body weight. Body weights were not affected by L either, however LB pullets were 11% and 25% heavier than LW when weighed at eight and 16 wks, respectively (P<0.001).

Table 3.1 Body weight of Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux at 0, 8, and 16 weeks of age.

	Light Intensity (L)				Strain (S)			L × S	
Weeks of age	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
0 (g)	34.04	34.17	33.92	0.405	33.97	34.11	0.360	0.528	0.176
8 (g)	753.92	743.08	744.33	0.419	785.50	708.72	<0.001	0.381	5.151
16 (kg)	1.32	1.31	1.31	0.739	1.46	1.17	<0.001	0.436	0.018
% within *x* of the mean									
5	56.31	59.52	56.82	0.240	55.47	59.64	0.016	0.977	0.848
10	87.49	89.37	89.76	0.293	86.25	91.50	<0.001	0.614	0.709
15	97.94	97.69	97.92	0.967	97.06	98.64	0.004	0.426	0.278
CV	6.61	6.27	6.29	0.263	6.86	5.92	<0.001	0.932	0.107

[1] SEM – Standard error of mean.

LW pullets were more uniform than LB pullets within 5, 10, and 15% of the mean, in addition to having a lower coefficient of variation ($P<0.001$, Table 3.1).

3.4.2 Keel Bone Assessment

The frequency of fractures and deviations is summarized in Table 3.2. The average percentage of deviations and fractures found was 5.78% and 0.63% respectively, but there was no effect of L, S, nor any interaction between L and S on keel bone quality. In terms of absolute *Pectoralis major* and *Pectoralis minor* weights, LB birds had significantly heavier pectoralis weight (1.12 times heavier for *P. major*; 1.13 times heavier for *P. minor*) than LW pullets (both $P<0.001$, Table 3.3). However, LW pullets had a heavier pectoralis weight relative to total body weight compared to LB (1.11 times more for *P. major* and 1.10 times more for *P. minor*, both $P<0.001$, Table 3.3).

3.4.3 Bone Strength Assessment

Bone strength results are summarized in Table 3.4. No effect of L on tibia bone size was observed, and LB pullets' tibiae were 1.31% heavier ($P<0.001$), 1.02% longer ($P<0.001$), and 1.18% wider ($P<0.001$) than LW pullets. When corrected for body weight, LB pullets' tibiae were still heavier ($P<0.001$), however, LW pullets' tibiae were 1.13% longer than LB pullets ($P<0.001$). There was a trend for LB pullets to have a thicker bone on the wide surface of the tibiae ($P=0.064$) however when corrected for body weight, LW pullets' tibiae were significantly thicker than LB pullets ($P<0.001$, Table 3.4). Relative to bone size, LW pullets had a higher bone strength than LB pullets (1,816 vs 1,277 kg/cm^2, $P<0.001$, Table 3.4).

3.4.4 Mortality

The effect of L and S on mortality is reported in Table 3.5. During the first week of age, L was 50 lux for all rooms, and L treatments (10, 30, and 50 lux) began at the end of the first week of age. Therefore, mortality was divided into two periods, which are the first week of age (Week 0-1) and the end of the first week of age until the end of trial (Week 1-16, Table 3.5). As a result, only the effect of S was analyzed for the first period (Week 0-1). During the first period (Week 0-1), LW pullets had 3.56 times more mortality than LB pullets ($P<0.001$). Specifically, the number of cases for yolk sac infection was 8.14 times higher in LW pullets than LB pullets ($P<0.001$, Table 3.5).

Table 3.2 Frequency of keel bone deviations and fractures (%) determined by palpation and dissection of Lohmann Brown-Lite (LB) and Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux at 16 weeks of age (12 pen replicates per light intensity × strain).

	Light Intensity (L)				Strain (S)			L × S	
	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Palpation									
Deviations	3.33	4.58	5.42	0.481	5.56	3.33	0.370	0.640	0.860
Fractures	1.25	2.50	0.00	0.361	0.83	1.67	0.787	0.437	0.557
Dissection									
Deviations	6.48	5.56	9.26	0.728	8.02	6.17	0.630	0.394	1.061
Fractures	0.00	0.00	0.00	-	0.00	0.00	-	-	0.000

[1] SEM – Standard error of mean.

Table 3.3 Pectoralis major and minor weights of Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux at 16 weeks of age (12 pen replicates per light intensity × strain).

	Light Intensity (L)				Strain (S)			L × S	
Weight	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
BW (kg)	1.32	1.30	1.31	0.403	1.45	1.17	<0.001	0.461	0.018
Pectoralis major (g)	62.89	61.59	61.09	0.539	65.28	58.43	<0.001	0.659	0.617
% BW	4.79	4.78	4.70	0.754	4.52	5.00	<0.001	0.314	0.052
Pectoralis minor (g)	19.61	20.01	19.70	0.841	20.99	18.55	<0.001	0.717	0.231
% BW	1.49	1.55	1.52	0.469	1.45	1.59	<0.001	0.548	0.018

[1] SEM – Standard error of mean.

Table 3.4 Tibia bone parameters of Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30, or 50 lux at 16 weeks of age (12 pen replicates per light intensity × strain).

	Light Intensity (L)				Strain (S)			L × S	
	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Absolute									
Tibia weight (g)	11.34	11.43	11.25	0.298	12.87	9.81	<0.001	0.928	0.188
Length (cm)	11.57	11.51	11.49	0.212	11.66	11.39	<0.001	0.708	0.022
Outer width (W[2], cm)	0.74	0.73	0.73	0.282	0.79	0.67	<0.001	0.213	0.007
Inner width (W[2], cm)	0.56	0.55	0.56	0.189	0.61	0.50	<0.001	0.095	0.007
Thickness (W[2], cm)	0.18	0.17	0.17	0.116	0.18	0.17	0.064	0.433	0.002
Outer width (N[3], cm)	0.59	0.59	0.59	0.499	0.63	0.55	<0.001	0.561	0.005
Inner width (N[3], cm)	0.45	0.45	0.45	0.372	0.49	0.41	<0.001	0.556	0.005
Thickness (N[3], cm)	0.14	0.14	0.14	0.722	0.14	0.14	0.657	0.687	0.001
Force (kg)	19.90	19.57	19.72	0.404	19.64	19.82	0.296	0.104	0.093
Relative[4]									
Weight (% BW)	0.87	0.88	0.86	0.668	0.93	0.81	<0.001	0.241	0.012
Length (cm/kg)	8.93	8.96	8.90	0.964	8.39	9.47	<0.001	0.214	0.114
Outer width (W[2], cm/kg)	0.57	0.56	0.56	0.998	0.57	0.56	0.571	0.141	0.006
Inner width (W[2], cm/kg)	0.43	0.43	0.43	0.870	0.44	0.42	0.021	0.203	0.005
Thickness (W[2], cm/kg)	0.14	0.13	0.13	0.309	0.13	0.14	<0.001	0.142	0.002
Outer width (N[3], cm/kg)	0.46	0.45	0.45	0.966	0.45	0.45	0.813	0.193	0.005
Inner width (N[3], cm/kg)	0.35	0.35	0.35	0.988	0.35	0.34	0.093	0.286	0.004
Thickness (N[3], cm/kg)	0.11	0.11	0.10	0.727	0.10	0.12	<0.001	0.160	0.002
Stress (kg/cm^2)	1523.35	1551.86	1553.63	0.832	1276.96	1816.23	<0.001	0.050	35.201

[1] SEM – Standard error of mean.

[2] W – wide. The diameters are perpendicular to the direction of the applied force.

[3] N – narrow. The diameters are parallel to the direction of the applied force.

[4] Relative – Tibia bone characteristics were adjusted for BW.

Table 3.5 Mortality (%) and its causes (%) of Lohmann Brown-Lite (LB) and Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 50 lux from zero days to one week of age, and 10, 30, or 50 lux from end of one to 16 weeks of age.

	Light Intensity (L)				Strain (S)			L × S	
	10	30	50	*P*-value	LB	LW	*P*-value	*P*-value	SEM[1]
Week 0 – 1									
Overall Mortality	-	-	1.78	-	0.78	2.78	<0.001	-	0.273
Infectious	-	-	1.39	-	0.33	2.45	<0.001	-	0.249
Yolk Sac Infection	-	-	1.28	-	0.28	2.28	<0.001	-	0.232
Polyserositis	-	-	0.06	-	0.00	0.11	0.139	-	0.039
Osteomyelitis	-	-	0.06	-	0.06	0.06	1.000	-	0.039
Peritonitis	-	-	0.00	-	0.00	0.00	-	-	0.000
Metabolic – Ascites	-	-	0.00	-	0.00	0.00	-	-	0.000
Skeletal – Rotated Tibia	-	-	0.00	-	0.00	0.00	-	-	0.000
Emaciation/Dehydration	-	-	0.31	-	0.33	0.28	0.866	-	0.094
Other/No Visible Lesions	-	-	0.08	-	0.11	0.06	0.541	-	0.047
Other	-	-	0.03	-	0.06	0.00	0.315	-	0.028
No Visible Lesions	-	-	0.06	-	0.06	0.06	1.000	-	0.039
Week 1 – 16									
Overall Mortality	0.50	0.75	1.00	0.691	0.39	1.11	0.015	0.155	0.150
Infectious	0.25	0.42	0.67	0.377	0.06	0.83	0.001	0.775	0.120
Yolk Sac Infection	0.08	0.25	0.33	0.369	0.06	0.39	0.026	0.650	0.075
Polyserositis	0.17	0.00	0.25	0.321	0.00	0.28	0.038	0.321	0.072
Osteomyelitis	0.00	0.17	0.00	0.331	0.00	0.11	0.102	0.071	0.039
Peritonitis	0.00	0.00	0.08	0.445	0.00	0.06	0.319	0.371	0.028
Metabolic – Ascites	0.00	0.08	0.00	0.428	0.00	0.06	0.315	0.365	0.028
Skeletal – Rotated Tibia	0.00	0.08	0.08	0.645	0.06	0.06	1.000	0.225	0.039
Emaciation/Dehydration	0.00	0.00	0.00	-	0.00	0.00	-	-	0.000
Other/No Visible Lesions	0.25	0.17	0.25	0.872	0.28	0.17	0.461	0.175	0.075
Other	0.00	0.17	0.08	0.373	0.06	0.11	0.566	0.718	0.047
No Visible Lesions	0.25	0.00	0.17	0.352	0.22	0.06	0.154	0.134	0.060

[1] SEM – Standard error of mean.

During the second period (Week 1-16), L did not affect mortality (average 0.75% mortality across all treatments). Mortality was 2.85 times higher in LW pullets than LB pullets, primarily due to yolk sac infection (*P*=0.026) and polyserositis (*P*=0.038, Table 3.5). No effect of interaction between L and S was found. Additionally, there was no effect of L on other causes of mortality, such as osteomyelitis, peritonitis, ascites, skeletal issues, emaciation, dehydration, or other causes of death (Table 3.5).

3.5 Discussion

3.5.1 Light Intensity

The objectives of this study were to understand how L and S can impact the body weight, bone health, and mortality of pullets reared in floor pens containing a perchery system from 0 to 16 wks. The results of this study indicated that L between 10 and 50 lux did not affect body weight of two different egg-strain pullets up to 16 wks. This is in agreeance with Dorminey et al. (1970) who evaluated the effect of L between one and 32 lux in White Leghorns. The authors reported no difference in body weight during the pullet phase of layers reared in floor pens (Dorminey et al., 1970). In Chapter 2, results of the current study show that pullets reared in 10 and 50 lux spent more time at the drinker than pullets reared in 30 lux. Despite this, body weight was not affected, suggesting that results were likely due to chance. Another possibility is that L between 10 and 50 lux does not affect pullet navigation to the extent where pullet hunger and thirst levels are compromised such that body weight is affected. Since the objective of this study was to understand whether brighter L can help pullets at navigating floor pens with a perchery system without affecting other factors, it is safe to say that L at 10, 30, and 50 lux will not negatively affect body weight.

Keel bone damage is caused by several factors. Crashes in non-cage systems, unequal wing-loading during wing-flapping, and perch use can all result in keel bone damage (Tauson and Abrahamsson, 1994; Sandilands et al., 2009; Stratmann et al., 2015a; b). Keel bone damage can be in the form of fractures or deviations. Deviations have been considered to be affected by perching behaviour (Tauson and Abrahamsson, 1996; Gunnarsson et al., 2000). In this study, there was no effect of L ranging from 10 to 50 lux on keel bone damage. There can be several reasons for this. Firstly, although L affected jumping frequency in the pullets, there was no effect on landing accuracy (Chapter 2). Since the landing success rate in the environment was similar across treatments, no differences in keel bone damage were observed between L. Another reason could be the age of pullets at the time of study. Ossification of the keel bone is a slow process which continues developing into the early stages of egg production at 28 to 40 wks (Buckner et al., 1949), unlike other long bones in birds which stop growing at onset of lay (Hurwitz, 1965; Hudson et al., 1993). At 16 wks, pullets are not yet photostimulated (conducted to initiate egg production), and the caudal portion of the keel is often still cartilaginous during this time (Casey-Trott, 2016). A study by Nicol et al. (2006) and review by Rufener and Makagon (2020) on the keel bone health of end-of-rearing pullets also confirmed no keel bone damage. Therefore, any damage to the keel bone at a young age would not be as severe than when the keel bone is fully developed and ossified. In the case of this study, the effect of L had no effect on keel bone damage either.

Despite no difference in keel bone damage resulting from L settings, when comparing keel bones from dissected and palpation samples, several differences between evaluation methods were found. Numerically, more fractures were recorded when keels were palpated, and more deviations were found from dissected keel bones (not statistically analyzed). Different pullets were used for keel bone palpation and dissection, therefore the differences in data could be random, or could also be false positives for fractures and false negatives for deviations from the palpation technique. However, even though the palpation technique can introduce reduced accuracy and repeatability, adequate training and practice can help increase the accuracy of palpation, which was done for this present study (Casey-Trott et al., 2015). Inter-observer reliability is also a key tool to increase the accuracy of palpation results, which was also practised in this study. Therefore, although differences in the incidence of keel bone damage were found between the two techniques used, this study utilized various tools to increase the accuracy of the results. Training was received by the assessors and inter-observer reliability was implemented. These tools were sufficient for the authors to see that L between 10 and 50 lux did not affect keel bone damage in pullets. However, for future research, more training on palpation to prevent false positives and negatives is warranted.

Bird flight requires the development of adequate pectoral muscle growth on the keel bone (Casey-Trott, 2016). In Red jungle fowl, a balanced ratio of breast muscle mass at 20% of body weight is necessary for flight (Duncker, 2000). In this study, breast muscle mass only made up 5.97% - 6.59% of the body weight in LB and LW pullets, respectively. Current modern laying hens have approximately twice the body weight of the ancestral Red jungle fowl, however, a similar breast muscle mass, resulting in a more exposed keel bone and reduced control in flights

(Jackson and Diamond, 1996; Fleming et al., 2004). Fleming et al. (2004) indicated that reduced breast muscle mass of modern layers could influence keel bone damage. One study by Lien et al. (2007) reported higher breast muscle mass in broilers housed in 11 lux as compared to one lux as an absolute weight, but not in terms of yield (% of body weight). However, the authors stated that the results were likely due to transitory feed consumption and not the effect of L (Lien et al., 2007).The results of this present study found that light intensities between 10 and 50 lux did not affect pullet *Pectoralis major* and *Pectoralis minor* weights at 16 wks. Another study comparing broilers housed in light intensities between one and 40 lux reported no differences in breast muscle mass (Deep et al., 2010), which is in accordance with the current study. Since no effect of L was found in pullet breast muscle mass, the results also support the absence of keel bone damage as influenced by L.

Finally, light intensities between 10 and 50 lux did not affect mortality or cause of mortality of pullets up to 16 wks. Many studies have been conducted on the effect of L on mortality in broiler chickens, where results have been contradictory. Downs et al. (2006) compared 2.7 and 10 lux, Kristensen et al. (2006) used L treatments ranging from five to 100 lux in their study, and Lien et al. (2007) compared 1.75 and 162 lux. These authors found no effect of L on mortality. On the other hand, Newberry et al. (1988) reported increased mortality in L of six lux versus 180 lux, whereas Ahmad et al. (2011) observed increased mortality in broilers housed in their highest L of 40 lux compared to five, 10, 20, and 30 lux. The authors reported that the contrast in results might be due to other management factors than L (Ahmad et al., 2011). The present study was conducted on layer pullets, which may have contributed to the disagreement between this study and those previously mentioned. Other studies of L in pullets have shown some association between L and the incidence of cannibalism, such as Kjaer and Vestergaard (1999) who found increased in cannibalism in birds housed in 30 lux versus three lux. On the other hand, several other studies reported no effect of L on cannibalism (Huber-Eicher and Audigé, 1999; Kjaer and Sørensen, 2002). The results of the present study agree with majority of literature; L between 10 and 50 lux did not cause cannibalism-based mortality, nor any other types of mortality.

3.5.2 Strain

The body weights of the pullets followed the Lohmann performance guide throughout the experiment (Lohmann Tierzucht, 2018). LB birds had consistently heavier body weights compared to LW birds, similar with other studies (Tauson et al., 1999; Vits et al., 2005; Singh et al., 2009). This may explain the heavier breast muscle mass and larger and heavier tibiae of LB pullets than LW pullets. Despite these differences in breast mass and bone size, there was no effect of S on keel bone damage on either S. Additionally, deviations are thought to be affected by perching behaviour (Tauson and Abrahamsson, 1996). In the present study, LW pullets spent more time on perches than LB pullets (Chapter 2), however, did not have more deviations than LB pullets. This indicates that both LB and LW pullets were able to navigate and utilize the floor pens, perchery system, and other environmental resources safely. Another reason may be due to the age of pullets. It was mentioned previously that ossification has not taken place at 16 wks, and therefore damage to the keel bone may not be as detrimental as during the laying phase (Whitehead, 2004; Casey-Trott, 2016). LW pullets have larger breast muscle mass yield (% of body weight) than LB pullets, this may be due to high activity levels (Chapter 2, Casey-Trott et al., 2017) or genetic differences (Fawcett et al., 2020).

Bone breaking strength is a gauge for skeletal system health that links to growth and egg production in layers (Rath et al., 2000). It is reported that higher breaking strength is an indication of healthier skeletal system, which can be achieved through many variables such as exercise (Fleming et al., 1994; Sandilands et al., 2009). Results from the present study reported higher bone strength in LW than LB pullets. It also reported longer and thicker tibiae in LW pullets than LB pullets. This may be correlated to the higher number of jumps and perching behaviour performed by LW pullets (Chapter 2). Several studies have reported the association of perching with bone formation because perching requires mechanical loading when birds utilize the perch and static loading for balancing on perches (Hughes and Appleby, 1989; Newman and Leeson, 1998; Casey-Trott et al., 2017). As such, it is possible that due to the higher amount of time spent perching and jumping within the environment, LW pullets developed a stronger musculoskeletal system than LB pullets. However, it is also possible that LW pullets spent more time perching because they have stronger bones, as opposed to having stronger bones because of high perching activity. A study by Riczu et al. (2004) reported higher absolute body weight, bone size, and strength in brown-feathered (Shaver 579) than white-feathered (Shaver 2000) end-of-lay hens housed in cages. Due to the lack of space for high intensity exercise such as jumping and flying in cages, the brown-feathered hens' naturally larger bones were stronger than the white-feathered hens. In the present study, both brown- and white-feathered birds had opportunities for flight and jumps between environmental structures, and since LW pullets performed higher levels of exercise than LB pullets, their tibiae were relatively stronger as well.

Finally, mortality was higher in LW than LB pullets. The highest causes of mortality were due to infections occurring early in life, namely yolk sac infection and polyserositis. Olsen et al. (2012) reported that mortality due to infections made up more than half of mortality causes in the first week. Yolk sac infection is acquired in-ovo as a result of translocation of bacteria from the intestine, air sacs, or bloodstream (Olsen et al., 2012). Polyserositis is inflammation of the serous membranes, also mainly caused by bacterial infection (Srinivasan et al., 2014). One reason for this may be the age of parent flock. Parents of the LW pullets were 31 and 30 wks for the two years the trial was conducted respectively, whereas parents of the LB pullets were 62 and 31 wks. In broilers, McNaughton et al. (1978) reported higher mortality when chicks were hatched from 29-week-old breeder hens than 59-week-old breeder hens. Another explanation could be that the parent flock may have been infected and vertical transmission and spread occurred during hatch and transport (Olsen et al., 2012).

3.6 Conclusion

The objectives of this study were to determine if L and S impacted the growth performance and bone health of pullets reared in floor pens containing a simple perchery system for up to 16 wks. The results of this study indicated that L from 10 to 50 lux did not affect body weight gain of pullets. These L settings also did not influence keel bone damage and bone strength. Light intensity did not affect mortality of egg-strain pullets.

LB pullets had a larger breast muscle mass, whereas LW pullets had a larger percentage of breast muscle compared to body weight, however in relation to keel bone integrity, neither caused keel bone deviations or fractions. This may be due to the early ossification stages of the keel bone, whereby any impact from crashes into the environment would not affect the caudal portion of the keel bone, which consists of cartilage at this age. In addition, although LB pullets had a heavier tibia, LW pullets had longer and thicker tibiae and performed more jumps within their environment, which may explain stronger tibiae than LB pullets. Finally, LW pullets had higher mortality than LB pullets.

Overall, the results suggest that current industry standards of 10 lux allow for visual acuity in pullets, allowing successful navigation throughout their environment. Indeed, providing perches during the rearing phase can prepare pullets to navigate complex environments (Tauson and Abrahamsson, 1994, 1996). However, other factors such as behaviour and welfare factors should be taken into consideration altogether (Chapter 2 and 4).

4.0 Chapter 4: Overall Discussion

4.1 Introduction

As consumer perceptions on where their food comes from change, concerns for the well-being of food animals are rising. In poultry, the table egg industry comes under scrutiny when the environment in which laying hens are raised in spark controversy, especially towards conventional cages. As a result, many countries began banning or phasing out conventional cages and implementing alternative housing which includes aviaries, furnished cages, and free-range systems. Canada is no exception to this. The Canadian National Farm Animal Care Council Codes of Practice for Pullets and Laying Hens (2017) requires table egg farms to use alternative housing by the year 2036. Alternative housing promotes welfare in laying hens by providing additional space and freedom to express more natural behaviours. For these housing systems to be utilized to their fullest potential, the birds must be able to see well so they can navigate their environment successfully. Methods to support this include manipulating environmental factors such as L to help with vision.

Currently, L settings for laying hens housed in alternative housing are recommended at a minimum of 10 lux (NFACC, 2017). Increasing L can increase active and exploratory behaviours (Kjaer and Vestergaard, 1999), which help birds explore and grow accustomed to their surroundings more quickly (Wilkins et al., 2011). In adult laying hens, increasing L can also result in aggression and fear within the flock, decreasing welfare (Hughes and Duncan, 1972; El-Lethey et al., 2000). Many types of research that have been conducted on L often use laying hens in the production phase as test subjects (Taylor et al., 2003; Moinard et al., 2004a; b; O'Connor et al., 2011). On the other hand, research using pullets reared in complex environments such as alternative housing systems are less common (Davis et al., 1999; Kjaer and Sørensen, 2002). Research on L and pullets is needed since pullets' bones are still developing and any potential damage to bone health can have long term effects (Regmi et al., 2015).

The purpose of this research was to understand how different L, including current industry standards, affected pullet behaviour, jumping success, and health with specific reference to bone quality, growth, and welfare of Lohmann LSL-Lite (LW) and Lohmann Brown-Lite (LB) pullets reared in floor pens containing a simple perchery system. White LED lights controlled at 10, 30, and 50 lux were tested. The effects of the L treatments on pullet growth were assessed using measures of body weight and mortality. Pullet health and well-being were determined using behaviour, jumping frequency and success, fear response, and physiological indicators of stress. Bone health was evaluated by measuring by the incidence of keel bone damage, breast muscle mass, and tibial bone strength.

4.2 Discussion

Light intensity is a management tool that is widely used in the poultry industry. Light intensity can affect biological rhythms and physiological aspects such as growth, behaviour, and reproduction (Lewis and Morris, 2006). In laying hens kept in conventional cages, L is recommended at a minimum of five lux to keep birds calm and reduce feather pecking which can lead to cannibalism (Hughes and Duncan, 1972; Kjaer and Vestergaard, 1999; NFACC, 2017). Alternative housing systems in Canada are required to utilize higher L, with a minimum of 10 lux, so that hens can better navigate their surroundings (NFACC, 2017). Further, for chicks who are just placed into their new surroundings, a minimum of 20 lux is required for at least the first seven days to allow them to easily locate feed and water (Kristensen, 2008; NFACC, 2017). Hence it is evident that L and increasing L play a role in navigation of hens and pullets.

Although the number of studies on L in broilers are plentiful (Kristensen et al., 2007; Alvino et al., 2009a; Deep et al., 2010), there is less research on layers (Taylor et al., 2003; Moinard et al., 2004a; b; O'Connor et al., 2011). Additionally, another limitation to available research on layers is the age studied. Many studies have been completed on laying hens during the production phase (Hill et al., 1988; Tucker and Charles, 1993; Lewis and Morris, 1999), whereas the present study focused on pullets. Articles published 60 to 70 years ago suggested that L as low as one lux was sufficient for growth in pullets (King, 1962; Morris, 1967). More recent research has argued for the importance of light management and L of at least five lux in raising broilers (Blatchford et al., 2009; Deep et al., 2010). In addition, Lewis and Morris (2006) reported that L must be at least 1.1-3.3 lux for extra-retinal light stimulation. As new studies brought to light the importance of vision to poultry health and well-being, L at a minimum of five lux during photoperiods was set as the new recommendation for layers, with a minimum of 20 lux during the first few weeks of life (Buyse et al., 1996; Collins et al., 2011). Birds in the previous era were mostly housed in conventional cages (Grover et al., 1972; Hartini et al., 2002), however, the addition of enrichment devices in alternative housing has resulted in the environment becoming more complex. Since vision plays a major role in a bird's life, it is crucial that the birds see well to utilize their environment safely. As such, information on pullets reared in alternative housing warrants further investigation.

One concern that may be associated with increasing L is cannibalism (Widowski et al., 2013). Cannibalism can develop from different causes including severe feather pecking and is a major health and welfare issue in the egg industry (Gentle and Hunter, 1990; McAdie and Keeling, 2000). Severe pecking may develop from dustbathing, which is a comfort behaviour, or from redirected litter pecking to relieve boredom (Blokhuis, 1986; Riedstra and Groothuis, 2002; Gilani et al., 2013). One way to reduce severe feather pecking is by lowering L settings to keep birds calm (Nicol et al., 2013; Widowski et al., 2013). Hence, the Canadian National Farm Animal Care Council Codes of Practice for Pullets and Laying Hens (2017) recommended setting L levels at five lux for birds housed in conventional cages. In alternative housing systems, L is recommended at a higher setting of minimum 10 lux to help birds with navigation in the environment (Taylor et al., 2003; NFACC, 2017). As such, the risk of severe feather pecking and cannibalism was possibly increased. For instance, Kjaer and Vestergaard (1999) reported increased feather pecking in 28-week-old hens housed in 30 lux than three lux. On the other hand, other studies reported no effect of L on aggression or severe feather pecking (Hughes and Black, 1974; Huber-Eicher and Audigé, 1999). Age may also play a factor in this behaviour. For example, Hartini et al. (2002) observed cannibalism in ISA Brown pullets reared in floor pens in 60-80 lux than five lux in as early as five weeks of age (wks). However, results from the present study disagreed with Hartini et al. (2002); there was no effect of L on severe feather pecking and fighting in pullets reared from 0 to 16 wks (Chapter 2). Aside from the different L treatments used, the study by Hartini et al. (2002) also explored the effects of other variables including whether or not the birds were beak trimmed or type of dietary fiber sources, which may explain the discrepancy in results between studies.

Fear and stress responses are a few of many good ways to assess the wellbeing of an animal. Measuring fear and stress levels can be reflective of an animal’s response to its surroundings. For instance, fear can be associated with severe feather pecking in laying hens and can also be correlated with stress levels (Hughes and Duncan, 1972; Jones, 1996; Campler et al., 2009). Multiple methodologies exist to assess fear and stress responses. Common methods include conducting a novel object test and measuring blood physiology through heterophil/lymphocyte ratios, respectively. In the present study, L did not affect either of these measurements, which could also be linked to the lack of aggressive behaviour observed (Chapter 2). This is not in accordance with a study by Hughes and Black (1974) who reported increased fearfulness in hens housed in 17-22 lux versus 55-80 lux (it was unclear why a range was provided for each treatment as it was not reported how L was measured). However, a more recent study by O'Connor et al. (2011) reported no difference in physiological stress indicators in 16-24 week-old laying hens housed in five versus 150 lux. Several different experimental factors can be found between the studies of Hughes and Black (1974) and O'Connor et al. (2011). For instance, Hughes and Black (1974) used pullets reared in cages while O'Connor et al. (2011) had 16-week-old hens kept in floor pens. Hughes and Black (1974) also regrouped their pullets multiple times throughout the study to test for stocking density effects which would destabilize the pecking order each time while O'Connor et al. (2011) measured the effect of high or low noise levels on the flock in addition to high or low L. Few studies have been conducted on the effect of L on pullet fear and stress levels, however other studies on broilers (Olanrewaju et al., 2007; Fidan et al., 2015; Rault et al., 2017) agreed with the results of the present study and that of O'Connor et al. (2011). In the case of this present study, the results in accordance with literature suggest that L does not affect the stress levels of the birds.

Preening may develop as a comfort behaviour, however it could also be a displacement behaviour. The results of the present study indicated that pullets reared in 50 lux spent more time preening than those reared in 10 lux. This was in contrast with O'Connor et al. (2011) and Davis et al. (1999), who both reported increased preening and dustbathing activities in low L (five and six lux, respectively) than high L (150 and 200 lux, respectively). In the present study, the highest L treatment was 50 lux, whereas studies by O'Connor et al. (2011) and Davis et al. (1999) used much higher L at 150 or 200 lux, which may explain the differences in behavioural results. O'Connor et al. (2011) inferred that hens in the five lux treatment performed more comfort behaviours because they were less engaged in other activities, more comfortable in low light, or sought additional comfort than those housed in the 150 lux treatment. On the other hand, the hens kept in high L (150 lux) spent more time performing active behaviours (O'Connor et al., 2011). In broilers, several authors reported increased time spent preening with L (Alvino et al., 2009a; Deep et al., 2012), which is in agreeance with the present study. Alvino et al. (2009a) suggested that higher L (such as 200 lux versus five lux as used in their study) improves visual acuity, which encourages the birds to maintain good plumage condition. Based on the results of this study, it is possible that L of 50 lux compared to 10 lux may increase time spent preening in the flock.

However, since the results of the present study only reported increased in time spent preening and not in any of the other comfort behaviours (wing or leg stretching, tail wagging, head shaking, head scratching, feather ruffling, dustbathing, wing flapping), it is possible that the results in the present study were due to chance. Additionally, the interpretation of the preening behaviour as a displacement behaviour can be excluded since L had no effect on fear or stress levels (Chapter 2).

Alternative housing systems also enable hens to perform more exploratory behaviours compared to when housed in conventional cages. Foraging substrates such as litter are provided for warmth and to absorb moisture, however they also have the added benefit of encouraging and promoting natural litter pecking and socializing behaviours (Johnsen et al., 1998). When factoring the effect of L on exploratory behaviours, this study yielded interesting results. Firstly, L had no effect on the amount of time spent towards litter-directed and object pecking in pullets (Chapter 2). However, at 13 and 16 wks, pullets reared in 10 lux spent more time pecking at the walls of the pen than those reared in 50 lux. Kjaer and Vestergaard (1999) found similar results when comparing the behaviour of birds kept at three or 30 lux, suggesting that low L (three lux) can reduce the ability to identify environmental cues and cause pullets to increase exploratory pecking for compensation. However, given that 10 lux in the present study did not affect litter-directed pecking and object pecking but specifically wall pecking, a possible hypothesis for this difference is wall colour; the walls of the pens used in the current study are white, allowing dirt (or fecal matter) to be easily visible for pullets. In L of 30 or 50 lux, the environment may have been bright enough for pullets to distinguish the matter on the walls. However, in 10 lux, the contrast of brown (fecal) matter against a white background may have attracted the pullets enough to affect their curiosity to peck at the spots on the wall (Newberry, 1999), but not enough that they would be able to tell simply by looking at the fecal spots. In fact, it is only at 13 and 16 weeks where these behaviours were observed and not at four and eight weeks, possibly indicating the length of time it takes for the walls of the environment to become soiled.

Another interesting result was the effect of L on gentle pecking behaviour. The two S used in this study reacted to the light intensities differently with respect to performance of this behaviour, however, only at four wks. LW pullets reared in 50 lux spent more time gently pecking at their peers than those reared in 10 lux. In contrast, LB pullets' time spent gentle pecking were not affected by L (Chapter 2). Although it is unclear why this is the case, gentle feather pecking without causing harm is also said to be a social behaviour (Craig, 1992; Ellen et al., 2008), and studies have also reported that due to different genetic lines white strain birds exhibit more feather pecking behaviours than brown strain birds (Biscarini et al., 2010; de Haas et al., 2013). Additionally, it is possible that the brown feather colour of LB pullets against brown litter in the pen may not have elicited curiosity within the flock, whereas in LW pullets, especially at 50 lux, any evidence of a spot or mark against a white feathered bird may have increased curiosity and gentle feather pecking.

In previous studies, it was reported that laying hens would be more active when L was increased from approximately 10 lux or less to 30 lux or more (Hughes and Black, 1974; Kjaer and Vestergaard, 1999; O’Connor et al., 2011). The results of the present study agreed with those previous studies; pullets reared in 50 lux spent a larger portion of time walking than those in 10 lux (Chapter 2). No effect of L on resting behaviour was observed. Interestingly, an interaction was observed with S. At four wks, LB pullets spent more time jumping and flying when reared in 30 lux than 10 or 50 lux while there was no effect of L on LW pullets performing these actions (Chapter 2). This is in contrast with Moinard et al. (2004b) who observed no effect of L (5, 10, or 20 lux) on jumping and flying behaviour. It also disagrees with Taylor et al. (2003) who reported only low L of less than six lux (versus 40 lux) hindered hens from jumping between perches. In this present study, since no clear pattern was established and this interaction was only observed once at four wks, it may be due to chance that LB pullets reacted to L in this way. However, it is also possible that L of 30 lux increased visual acuity and encouraged the LB pullets to perform more jumping and flying behaviours, whereas an even brighter environment of 50 lux may have encouraged the birds to engage in other types of activities. More research on whether different S behaviours can be influenced by L to an extent is warranted.

Looking at the frequency and success of jumps between resources in the environment, L also only affected jumps at four wks (Chapter 2). Pullets reared in 50 lux jumped up from the floor to the ramps more than those reared in 10 lux, and pullets reared in 30 lux jumped across perches more than those reared in 10 lux. This data agrees with the results of the active behaviours, where an increased percentage of time walking was observed in pullets reared in a higher L (50 lux vs 10 lux) at four wks. It also agrees with studies which reported increased activity with increased L (Boshouwers and Nicaise, 1987; Kjaer and Vestergaard, 1999; O’Connor et al., 2011). The age effect may explain expression of activity levels at different observation periods. Harlander-Matauschek et al. (2015) reported that laying hens expressed the most physical activities during their pullet rearing phase. Although the present study was conducted within pullet ages, active behaviours and jumping frequencies were more affected by L during the first observation period at four wks than towards the later stage in a pullet's life at 16 wks. It is also important to note that while pullets reared in 30 or 50 lux performed more jumps, percent success of jumps did not differ between any of the L treatments, including those reared in 10 lux (Chapter 2). This indicates that jumping behaviour of pullets in different light intensities was more highly associated with activity levels than being dependent on visual acuity; pullets reared in 10 lux were able to navigate as safely as those reared in higher (30 or 50 lux) light intensities. In addition, jumping behaviour results in the present study were similar to pullet location results, as affected by L; pullets reared in 30 lux spent more time on drinker lines than pullets reared in 10 or 50 lux at four wks only (Chapter 2). Standing or resting on top of drinker lines required wing assisted jumping or flying motion. Therefore, this data confirmed that during early pullet rearing (four wks), pullets reared in 30 or 50 lux spent more time performing active behaviours than pullets reared in 10 lux.

Exercise can increase muscle mass and improve bone strength (Whitehead, 2004). The switch from conventional cages to alternative housing alone increases bird activity which has positive impacts on the health of a pullet (Albentosa and Cooper, 2004; Rodenburg et al., 2005). However, there are also added risks of bone damage from crashes or failed landings in the environment, such as keel bone damage. A combination of factors typically causes keel bone damage, including crashes in non-cage systems (Stratmann et al., 2015a), perch use (Tauson and Abrahamsson, 1994; Sandilands et al., 2009), nutrition inadequacies (Whitehead, 2004; Fleming et al., 2006), reduced breast muscle mass (Fleming et al., 2004), and genetic factors (Whitehead, 2004; Stratmann et al., 2015b). Breast muscle mass is linked to keel bone integrity for controlled flight manoeuvres and the appropriate breast muscle mass of 20% of body weight is ideal (Duncker, 2000). Bone strength is associated with an increase in exercise (Whitehead, 2004; Regmi et al., 2015). Increasing L was previously mentioned to result in an increase in the time spent preening and performing active behaviours in the flock, but would it improve bird vision to help with navigation? The results of the present study indicated that L at 10 lux would not negatively affect the integrity of health of the keel bone compared to 30 or 50 lux (Chapter 3). Concurrently, there was no effect of L on breast muscle mass and tibia bone strength either (Chapter 3).

Two possible reasons exist for why L of 10, 30, or 50 lux did not affect pullet bone health as determined directly by keel bone damage, and indirectly by breast muscle mass and tibiae bone strength in this study. Firstly, age may have played a factor, especially for the assessment of keel bone damage. At 16 wks, pullet keel bones were still developing and have not yet fully ossified (Buckner et al., 1949; Casey-Trott, 2016). The cartilaginous keel bones would hence not be easily damaged during the rearing phase. Extending the duration of research into the laying phase may help answer this question, especially when activity and jumping frequency were affected by L. However (secondly), it was also possible that although L increased activity in the flock, the environment at 10 lux was bright enough for pullets to navigate their environment safely, thereby not affecting bone health. In addition, it was probable that total activity levels and jumping behaviours in each flock was not large enough to influence bone health. For instance, compared to a simple perchery system used in this study, birds housed in other types of alternative housing systems, such as aviaries with multiple tiers, will have increased opportunities for exercise which will improve bone strength (Newman and Leeson, 1998; Casey-Trott et al., 2017). Regardless, the results of the current study suggest that L at 30 or 50 lux increased the activity and jumping frequency of pullets reared up to 16 wks, however pullets reared in 10 lux would be able to jump around their environment safely just as well without compromising bone health.

Along with no effect on bone health, L did not affect pullet body weight (Chapter 3). On the other hand, Hartini et al. (2002) reported increased body weight in 10 week old pullets reared in high L (60 to 80 lux) versus dim L (five lux). Hartini et al. (2002) also observed that low L (five lux) resulted in more calm pullets which the authors suggested may be due to reduced environmental cues, reduced visual ability to locate feeders, or suppressed feeding appetites. This was not the case for the present study, as when comparing body weight with nutritive behaviour, L had no effect on feeding behaviour (Chapter 2). On the other hand, drinker behaviour was affected at 16 wks; pullets reared in 10 or 50 lux spent more time at the drinker than pullets reared in 30 lux (Chapter 2). Previous scientific literature reported increased feed and water consumption with increased L (Davis et al., 1999; Prescott and Wathes, 2002), therefore, data from the current study would suggest that the results were likely due to chance.

Throughout the study from 0 to 16 wks, mortality or cause of mortality was also unaffected by L (Chapter 3). At the beginning of this discussion, it was established that previous studies reported increased cannibalism, severe feather pecking, and aggression with increasing L (Kjaer and Vestergaard, 1999; Hartini et al., 2002). In contrast, aggression was not a concern in the present study, and neither was mortality resulting from cannibalism. Other possible mortality causes diagnosed reported no association with L either. Therefore, based on the result of this study, L of 10, 30 or 50 lux does not affect mortality in pullets reared to 16 wks.

The effects of L on floor-reared pullets provided some insight that L at 10, 30, or 50 lux enabled pullets to jump in their environment safely without negatively affecting bird health and welfare. The provision of perches during rearing can affect the spatial ability of hens. A study by Gunnarsson et al. (2000) comparing pullets reared with and without perches during the first eight wks reported pullets reared without perches took a longer time towards completing a spatial cognitive task of assessing feed presented at 80 cm above ground (Gunnarsson et al., 2000). Therefore, if L can play an assisting role in pullet jumps at a young age, it can better prepare pullets for navigating complex environments during the laying phase. In this study, L at 30 or 50 lux had the added benefit of increasing bird activity and comfort behaviour. The results of this study also reported that S reacted differently to different L, specifically for gentle feather pecking and jumping and flying behaviours. However, L aside, there were also several S effects observed in this study.

Throughout the study, LW pullets spent more time resting and preening (except at four wks) than LB pullets. They were also observed spending more time standing and performing comfort behaviours than LB at four wks (Chapter 2). LW pullets were observed to spend more time pecking at objects in the environment than LB pullets at eight and 13 wks (Chapter 2). LB pullets spent more time litter pecking (except at 16 wks), and wall pecking (13 and 16 wks) compared to LW pullets (Chapter 2). Other behaviours that had no pattern as affected by S across recording periods included percent of time spent feeding and gentle pecking, while behaviours unaffected by S were walking, drinking behaviour, and aggression (Chapter 2). Time spent at each location by pullets was also dependent on S. LW pullets spent more time on perches than LB pullets, and LB pullets spent more time on the floor than LW pullets (Chapter 2). LW pullets jumped within the environment more than LB pullets, however, total percent success did not differ between the two S (Chapter 2). Such behavioural differences may be explained by inherent genetic and character differences; LW pullets are thought to be more reactive to their environment in the sense that they exhibit large hormonal and behavioural responses, spending more time performing active behaviours while LB pullets are more exploratory (Murphy, 1977; Fraisse and Cockrem, 2006;

Pusch et al., 2018). Despite LW pullets performing more jumps in the environment, there was no effect of S on keel bone damage (Chapter 3). However, S influenced breast muscle weight. Although LB pullets had heavier body weight than LW pullets, LW pullets had a larger breast muscle yield (relative to body weight) than LB pullets (Chapter 3). According to Moinard et al. (2004a), increased body weight relative to wing area can reduce flight performance, or in the case of this present study jumping frequency. Such was the case for the LB pullets. Although performance was not hindered, LB pullets must be able to control the lift of its entire body in relation to the force of gravity, bring about correct trajectory to make a perfect landing, and generate enough braking force to maintain itself on the perch (Provine et al., 1984; Moinard et al., 2004a). Unfortunately for LB pullets who are heavier than LW pullets, this ability for manoeuvre decreases with increasing body weight (Marden, 1994; Tobalske and Dial, 2007). This may also explain why LB pullets had lower numbers in jumps in the environment than LW pullets and may also clarify why LB pullets were recorded to spend more time on the floor than LW pullets during light hours.

When comparing bone strength relative to bone size, the legs of LW pullets were thicker and able to withstand a higher amount of stress than LB pullets (Chapter 3). One explanation for this may be due to the high amounts of navigation within the environment compared to LB pullets. Increase in jumps stimulates muscle activity and increases bone tissue calcification, which in turn increases bone strength (Fleming et al., 1994; Tauson and Abrahamsson, 1996; Casey-Trott and Widowski, 2016). Results from this study indicated that due to genetic inclination, LW pullets performed more jumps, which may have contributed to a thicker tibia and higher strength than LB pullets. This may lead to better welfare for the bird overall because stronger bones can help reduce future risks of fractures and bone breakages (Gunnarsson et al., 2000; Casey-Trott and Widowski, 2016).

Furthermore, perching may also increase bone strength. When a bird mounts or dismounts a perch, mechanical loading is involved to generate lift (Moinard et al., 2004a; b). When a bird rests on top of a perch, static loading is required for the muscles to maintain a balance (Hughes and Appleby, 1989; Newman and Leeson, 1998). Several studies have reported that perching in addition to other active behaviours can increase bone quality and bone mineral density which may prevent fractures and injuries, especially when perches are placed during the pullet rearing phase (Hester et al., 2013; Janczak and Riber, 2015; Regmi et al., 2015). In the present study, in addition to higher levels of jumping between environmental structures, LW pullets spent more time on perches than LB pullets (Chapter 2), which may contribute towards bone formation and development of the musculoskeletal system (Newman and Leeson, 1998; Casey-Trott et al., 2017).

For fear and stress response between S, LB pullets had a higher response time to the novel object and a higher heterophil/lymphocyte ratio than LW pullets (Chapter 2). In theory, these higher values are indicative of higher levels of fear and stress, however, previous studies analyzed these measures based on the effect of a single treatment variable causing stress (Hughes and Black, 1974). Few studies have been published on the different effects of stress across S. Recent studies have suggested that LB and LW pullets may have different characteristics and therefore different responses to stressful situations (Fraisse and Cockrem, 2006; Pusch et al., 2018; Peixoto et al., 2020b). Conclusively, more research is warranted in this area.

Finally, mortality differed between S only during the first week of age. There were higher cases of mortality in LW pullets than LB pullets and these were discussed in Chapter 3. Mortality was largely due to yolk sac infection that occurred within the first week of age. Yolk sac infection occurs when high counts of bacteria are exposed to the intestines, air sac, or bloodstream of a chick, compromising its immune system (Rai et al., 2005; Olsen et al., 2012). This generally is related to either breeder flocks or incubator cleanliness, and therefore these differences may not related to S specifically. Rather, many variables from the parent flock (McNaughton et al., 1978) to incubation environment (Cortés et al., 2004; Rai et al., 2005) can result in yolk sac infection.

4.3 Conclusion

The overall objective of this study was to determine whether increasing L over the industry standard of 10 lux (to 30 or 50 lux) can help pullets navigate their environment without affecting other parameters such as growth, behaviour, and welfare. The results from this study indicated that the birds exposed to the industry standard (controlled treatment) of 10 lux did not differ from those given higher L (30 or 50 lux) in terms of jumping success. Light intensity at 30 or 50 lux also did not result in increased aggression, fear and stress levels which were concerns with higher L. However, L at 30 or 50 lux had mild effects on pullet behaviour and activity in other ways, such as pullets reared in the 50 lux treatment spent more time preening at 13 and 16 wks. Despite no difference in jumping success, pullets reared in 10 lux spent more time than those in 30 or 50 lux pecking at walls of the pen which may suggest slightly reduced visual acuity to distinguish between fecal spots on the wall than by looking at them. Pullets reared in higher L (30 or 50 lux) had higher activity levels than those reared at 10 lux at four (higher jumping frequency) and eight (more time spent walking) wks. Aside from that, body weight, aggression, keel bone quality and integrity (measured via breast muscle mass), tibiae bone characteristics and strength, and mortality were not affected by L. Strain on the other hand had many effects on these variables.

The original predictions of this study were to expect an increase in aggressive behaviour, fear and stress response with higher L. It was also predicted that jumping success would increase with L, and for keel bone damages to increase with decreasing L. The results of this study do not support the hypotheses, as the data suggests that the current industry standards of 10 lux recommended by the Canadian National Farm Animal Care Council Code of Practice for Pullets and Laying Hens (2017) is bright enough for pullets to perform jumps in a floor-reared environment safely. However, higher light intensities at 30 or 50 lux may increase jumps within the environment which can better prepare pullets for navigating a complex environment.

This research is important because it provided science-based information on the effect varying industry relevant L can have on the productivity, health, and welfare of layer pullets. This research benefits the Canadian table egg industry as it helps further establish the importance of the role of L in preparing pullets to navigate alternative housing environments. As commercial egg production systems begin to transition from caged to alternative housing, understanding how L can affect bird welfare becomes even more important. However, there is still limited research on the long-term effects of pullet L or possible carry over effects of pullet L into the laying phase. Following pullets into production and evaluating whether L has helped them familiarize and navigate their surroundings to prevent injuries and keel bone damage during the laying phase would be of interest and represents a research gap. Additionally, as mentioned earlier, research on the different characteristics of S, especially with regards to fear and stress responses warrant further investigation.

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