SPACE, WALKING ABILITY, AND BROILER CHICKEN BEHAVIOR AND WELFARE

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Dedicated to my friends, family, and Catniss
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LIST OF ABBREVIATIONS

D: Days
FPD: Footpad Dermatitis
H: Hours
ABSTRACT

Stocking density, space availability, and lameness are important aspects affecting broiler chicken behavior and welfare. Stocking density refers to the weight of broiler chickens per a set area of space typically measured as kg/m². Space availability is the amount of space per individual broiler chicken typically measured as m²/bird. Stocking density and space availability can contribute to lameness and other aspects of welfare such as footpad dermatitis, hock burn, and feather cleanliness. The behavior of broiler chickens can also be modified by stocking density, space availability, and lameness. All of these aspects are typically related to a change in activity levels which could be used as an indicator of animal welfare. To date, the majority of research that has examined the walking ability of broiler chickens has assessed how stocking density influences the development of gait problems when applied during the grower phase. However, not all broilers develop gait problems at the same point in time and it is unknown whether broilers that initially have sound gait develop gait problems at a similar rate to broilers that are initially classified as having affected gait. Further, the influence of stocking density on the progression of gait abnormalities of broilers with sound and poor gait is unknown. Finally, since space becomes more and more limited as broiler chickens increase in body weight and age, it is unknown how the provision of space during the finisher phase, when broiler chickens are gaining weight rapidly, can influence their walking ability and welfare outcomes.

Stocking density is calculated based on projected final weights of broiler chickens from the time they are placed on a commercial farm, and that projected stocking density remains the same from the chick placement date. As stocking densities are increased, there is also an increase in the prevalence of lameness. Age is also known to be related to walking ability, and as broiler chickens age, there is an increase in the prevalence of lameness. While it is known that increasing stocking density and aging are both contributing factors to broiler chicken lameness, there is no previous research on if reducing stocking density at a later age can help alleviate the prevalence of lameness. To address this knowledge gap, two studies were conducted. In the first study, 784 mixed-sex Ross 708 broiler chickens in commercial barns were placed into four treatment groups.

- **SOUND**: Consisted of broiler chickens that were considered to have sound gait (scores of 0 and 1) and the broilers were housed at farm stocking density (6lb/ft², 29.29 kg/m²),
• **AFFECTED**: Consisted of broiler chickens that were considered to have affected gait (scores of 2 or higher) and were housed at farm stocking density (6lb/ft\(^2\), 29.29 kg/m\(^2\))

• **MIXED-F**: Consisted of 50% of broiler chickens that were considered to have sound gait and 50% that were considered to have affected gait and were housed at farm stocking density (6lb/ft\(^2\), 29.29 kg/m\(^2\))

• **MIXED-L**: Consisted of 50% of broiler chickens that were considered to have sound gait and 50% that were considered to have affected gait and were housed at half of the farm stocking density (3lb/ft\(^2\), 14.65 kg/m\(^2\))

Broiler chickens were randomly selected at 33 days of age from each of four commercial barns for welfare assessments, which included gait scoring to assess walking ability, as well as the assessment of footpad dermatitis and hock burn. Broilers were then assigned to one of two gait categories based on their gait scores. Broilers were either considered to have sound gait meaning they had no or unidentifiable abnormalities, or affected gait meaning there were identifiable abnormalities. To separate treatment groups, custom-built pens (4 ft x 12 ft, 1.22 m x 3.66 m) were constructed. At 37 days of age welfare assessments were conducted again, and then the broiler chickens were placed back into the flock (Chapter 2). The behavior of the broiler chickens was recorded from the evening of day 33 to the morning of day 37 and video was analyzed using scan sampling. The proportion of broiler chickens performing target behaviors was recorded every 10 minutes in the morning (6:00 – 8:00) and evening (19:00 – 21:30). Better gait scores were observed at 37 days of age in broiler chickens in the MIXED-L group and broiler chickens in the SOUND group. The presence of hock burn was lower in broiler chickens in the SOUND group. Cleanliness scores were better for broiler chickens in the MIXED-L group and in broiler chickens in the SOUND. Stocking density impacted the proportion of broilers performing eating, drinking, sitting, and walking (P < 0.05). Walking ability impacted the proportion of broiler chickens standing, walking, and sitting (P < 0.05).

To continue investigating the implementation of housing changes later in the broiler chickens’ life, a second study was conducted using 705 mixed-sex Ross 708 broiler chickens. At 7 d, broiler chickens were randomly assigned to 1 of 16 pens (46-47 birds/pen). At 28 d, half of the pens doubled in size after welfare assessments were completed (measuring 8 ft x 10 ft, 3.05 m x 2.44 m, DOUBLE), while the other half remained at the original dimensions (8 ft x 5 ft, 2.44 m...
The DOUBLE pens had an expected stocking density of 15.2 - 15.5 kg/m² (3.11 - 3.17 lb/ft²) and an estimated space availability of 0.15 to 0.16 m²/bird while the SINGLE pens had an estimated stocking density of 30.4 - 31.1 kg/m² (6.23-6.37 lb/ft²) and an estimated space availability of 0.07-0.08 m²/bird. Welfare assessments consisting of scoring gait, feather cleanliness and for the presence of FPD and hock burn were conducted at 22 d, 28 d, and 38 d (Chapter 4). At 38 d, broiler chickens in SINGLE pens were less likely to have a score of 0 for FPD (Wald $\chi^2 = 15.45$, $P < 0.0001$), hock burn (Wald $\chi^2 = 7.26$, $P = 0.0071$), and feather cleanliness (Wald $\chi^2 = 11.77$, $P = 0.0006$) than broiler chickens in DOUBLE pens. However, broiler chickens in SINGLE pens were more likely to have a gait score of 0 compared to broiler chickens in DOUBLE pens (Wald $\chi^2 = 11.34$, $P = 0.0008$).

Broiler chicken behavior was recorded at 23-26 d (Period 1: before space increase), 28-31 d (Period 2: time of space increase), and 36-37 d (Period 3: after space increase). Behavior data were collected using focal sampling for two broiler chickens per each of the 16 pens in the morning, afternoon, and evening (Chapter 5). Broiler chickens housed in double pens had an increased frequency of leg extensions compared to broiler chickens housed in single pens ($P < 0.05$). Period had a significant impact on the frequency of eating, sitting, and walking and the durations of sitting, environmental pecking, standing, and walking ($P < 0.05$). Time of day had a significant impact on the frequency of eating, sitting, walking, preening, and leg extensions and the durations of sitting, eating, preening, and standing ($P < 0.05$). The interaction of age and time of day had a significant impact on the frequency of drinking and leg extensions and the durations of sitting, eating, and walking ($P < 0.05$). The interaction of age and treatment had a significant impact on the frequency of eating and walking and the duration of preening ($P < 0.05$). In conclusion, broiler chickens housed in DOUBLE pens did not exhibit a difference in behaviors compared to those in SINGLE pens, other than broilers in the DOUBLE pens performing leg extensions more often.

While the first study indicated that having more space available per broiler chicken led to better walking ability, the second study showed the opposite to be true as those with more space had reduced walking ability. This indicates that changing the stocking density through manipulating space in the finisher phase may impact welfare, but further investigation is needed. Future research should first examine the effects of adding space in the finisher phase with 3 treatment groups. While the two discussed here would remain the same, the third group should start with broilers in a pen that is already the size of the DOUBLE pens and remains that way for
the entire project. This will ensure that increasing space during the finisher period is beneficial rather than the additional space availability in general accounting for the differences in treatments. All treatment groups should also get fresh bedding with the pen increase to ensure the welfare measurement results are due to the changes in space availability rather than the provision of fresh litter.
CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

In recent years, the demand for broiler chicken production in the United States has increased. The estimated value of the broiler industry was $50.4 billion in 2022 (United States Department of Agriculture & National Agricultural Statistics Service, 2023), and the industry is projected to continue increasing. In 2014, there were reported to be 8.54 billion broiler chickens raised within the United States (National Agricultural Statistics Service, 2015). By 2022 this number increased to 9.17 billion broiler chickens (United States Department of Agriculture & National Agricultural Statistics Service, 2023). The numbers of chickens raised domestically has increased to meet consumption, which has increased from 86 pounds per person per year in 2006 to 98.8 pounds per person per year in 2022 (Dohlman et al., 2023). Chicken consumption is expected to rise to 106 pounds per person per year by 2032 (Dohlman et al., 2023). With the rise in demand, producers are required to increase the number of chickens they raise, whether that be through raising more chickens in less space (increasing the stocking density) or building more farms to increase production. As production has increased, the focus has primarily been on having the best meat yield per animal (Koknaroglu & Akunal, 2013). The concerns of animal welfare were not at the forefront of research while working towards meeting the supply demands; however, with the increase in attention from the general public on the welfare of animals in production there has been an increase in the demand for animal welfare related research (Koknaroglu & Akunal, 2013).

Many researchers believe that lameness is one of the most severe welfare problems impacting broiler chickens (Farm Animal Welfare Council, 1992; Gregory, 1998; Webster, 1995; Weeks et al., 2000) because lameness is a painful condition that influences broilers’ behavior, health, and other welfare aspects such as the presence of hock burn. Lameness is a multifactorial issue which is influenced by many factors including stocking density and space availability (Silvera et al., 2017; Son, 2013; Weeks et al., 2000). Lameness is defined as a broiler chicken’s inability to walk and the presence of lameness is identified through scoring gait (Granquist et al., 2019). Broiler chickens with a gait score of 3 or higher based on the scale by Kestin et. al (1992, Table 1.3) are considered to be lame, as prior research has indicated these scores are when broiler
chickens prefer food with analgesic which then increases movement (Caplen et al., 2013; Danbury et al., 2000; Hothersall et al., 2016). Stocking density is the amount of weight of broiler chickens per a predetermined area of space (kg/m²) or the number of birds in a predetermined area. As stocking density increases, there is an increase in the prevalence of lameness and other leg health issues (Buijs et al., 2009; Das & Lacin, 2014; G. S. Sanotra et al., 2001; Sørensen et al., 2000; Thomas et al., 2004).

Stocking density is also associated with the amount of useable space that is available to broiler chickens for various behaviors, and their behavior, and associated activity levels, can influence their walking ability. Prior research determined that when there was more space available, there was an increase in the amount of time broiler chickens spent doing play or locomotion behaviors, but there is still a lot to be understood about the impacts of space availability on welfare and behavior (Vas et al., 2023). Broiler chickens who had higher activity levels and were performing more active behaviors were more likely to have better walking ability (Silvera et al., 2017; Son, 2013; Weeks et al., 2000). Increasing the space allowance for broiler chickens may reduce the prevalence of lameness; however more research needs to be done to fully understand the impacts of space availability on walking ability. Particularly, there is little information available about whether changing the stocking density part way through the production phase, when broilers are rapidly gaining weight, can influence their walking ability. As the development of gait problems is not necessarily occurring at the same rate for every broiler chicken, it is important to understand how stocking density influences the progression of gait problems. If the space available remains the same from the beginning, the impacts it has on gait cannot be separated from other factors such as age and weight gain, because as broiler chickens age and grow, they begin to take up more of the available space and therefore may use the available space differently. In addition to the effects of stocking density during the finisher phase, it is unknown whether broilers that have better gait experience slower declines in walking ability compared to broilers with worse gait. This dissertation aims to fill the gaps identified above through two experiments.

1.2 Animal Welfare

In order to understand the factors that influence broiler chicken lameness, and the impacts of lameness on other welfare measures, an understanding of what animal welfare means is necessary. The American Veterinary Medical Association (AVMA) defines animal welfare as the
state an animal is in and the treatment the animal is receiving. Broom (1991, 1996) defined animal welfare as the way an individual animal is coping with its environment, meaning that the welfare of an animal is a reflection of how they are responding to stimuli or potential problems around them. Several frameworks have been developed to understand and assess animal welfare. One of these frameworks is the Five Freedoms which were based on the Brambell report (Brambell, 1965; Farm Animal Welfare Council, 1979).

Table 1.1. The Five Freedoms of animal welfare (Farm Animal Welfare Council, 1979)

<table>
<thead>
<tr>
<th>Freedoms</th>
<th>Provisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freedom from thirst, hunger, and malnutrition</td>
<td>By ready access to fresh water and a diet to maintain full health and vigor</td>
</tr>
<tr>
<td>Freedom from discomfort</td>
<td>By providing an appropriate environment including shelter and a comfortable resting area</td>
</tr>
<tr>
<td>Freedom from pain, injury, or disease</td>
<td>By prevention or rapid diagnosis and treatment</td>
</tr>
<tr>
<td>Freedom to express normal behavior</td>
<td>By providing sufficient space, proper facilities, and company of the animal’s own kind</td>
</tr>
<tr>
<td>Freedom from fear and distress</td>
<td>By ensuring conditions and treatment which avoid mental suffering</td>
</tr>
</tbody>
</table>

The Five Freedoms were developed to clearly analyze and investigate the welfare of animals (Farm Animal Welfare Council, 1993). The Five Freedoms were then used to formulate the Five Provisions of animal welfare (Mellor, 2016). The Five Provisions were developed because the provisions defined for each of the Five Freedoms were found to be more easily applied than the freedoms themselves (Mellor, 2016). The other issue with the Five Freedoms is that there was no way for animals to be completely free from negative experiences (Mellor, 2016). For example, there cannot be a total freedom of thirst or hunger as an animal must feel these in order to drink or eat (Mellor, 2016). The Five Provisions provide additional information on how to provide good welfare (Mellor, 2016, Table 1.2). The Five Provisions also take into account promoting positive welfare experiences rather than focusing only on minimizing the negative experiences.
Table 1.2. The Five Provisions of animal welfare (Mellor, 2016)

<table>
<thead>
<tr>
<th>Provisions</th>
<th>Animal Welfare Aims</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Good Nutrition</strong>: Provide ready access to fresh water and a diet to maintain full health and vigor</td>
<td>Minimize thirst and hunger and enable eating to be a pleasurable experience</td>
</tr>
<tr>
<td><strong>Good Environment</strong>: Provide shade/shelter or suitable housing, good air quality, and comfortable resting areas</td>
<td>Minimize discomfort and exposure and promote thermal, physical, and other comforts</td>
</tr>
<tr>
<td><strong>Good Health</strong>: Prevent or rapidly diagnose and treat disease and injury, and foster good muscle tone, posture, and cardiorespiratory function</td>
<td>Minimize breathlessness, nausea, pain, and other aversive experiences and promote the pleasures of robustness, vigor, strength, and well-coordinated physical activity</td>
</tr>
<tr>
<td><strong>Appropriate Behaviors</strong>: Provide sufficient space, proper facilities, congenial company, and appropriately varied conditions</td>
<td>Minimize threats and unpleasant restrictions on behavior and promote engagement in rewarding activities</td>
</tr>
<tr>
<td><strong>Positive Mental Experiences</strong>: Provide safe, congenial, and species-appropriate opportunities to have pleasurable experiences</td>
<td>Promote various forms of comfort, pleasure, interest, confidence, and a sense of control</td>
</tr>
</tbody>
</table>

The Five Domains Model of animal welfare was designed to incorporate new developments in the field of animal welfare and has continued to be updated as more information is gathered (Mellor et al., 2020). The Five Domains Model was first created in 1994 and has been updated several times (Mellor et al., 2020). The original five domains were nutrition, environment, health, behavior, and mental state (Mellor et al., 2020). The updates to the Five Domains Model have incorporated including positive affective state, increasing the number of species the model applied to, and expanding the application (Mellor et al., 2020). The update in 2015 allowed for the model to create a more in-depth system for scoring welfare that allowed the outcome to be scaled rather than binary (Mellor et al., 2020). Most recently, in 2020, the model added human-animal interactions to the behavior domain of welfare because humans have an impact on the welfare of an animal (Mellor et al., 2020). The animal may not voluntarily perform a behavior that is needed, which requires the caretaker to step in and therefore the human is impacting the welfare of the animal (Mellor et al., 2020). With this inclusion, more scales and scoring systems were added to the Five Domains to ensure the impact of humans is included in the measurement of animal welfare.

Another framework to investigate animal welfare is the three circles model (Fraser et al., 1997, Figure 1.1) which included the three main concepts of natural living, freedom from negative states, and overall basic health and biological functioning. The three circles model emphasizes that
while each aspect of welfare is its own category, there is overlap between all the circles and it is important to study their interactions.

The concepts within each of these frameworks provide starting points on what data to collect to assess the welfare status of an animal. The concepts can reduce bias through providing guidelines for the evaluation of welfare (Mellor et al., 2020) because animal welfare based research will use similar tools to assess and evaluate animal welfare.

![Diagram of three circles model of animal welfare](image)

**Figure 1.1.** The three circles model of animal welfare contains the three focus areas of basic health and biological functioning, affective state, and natural living (Fraser et al., 1997)

### 1.3 Lameness and its impact on broiler chicken welfare

Lameness impacts the ability of a broiler chicken to escape aversive experiences, perform natural behaviors, and reach food and water (Butterworth et al., 2002; Sanotra et al., 2002; Vestergaard & Sanotra, 1999; Weeks et al., 2000). Inability to access feed and water can lead to mortality (Tainika et al., 2023). Lameness is one of the most prevalent welfare concerns on commercial broiler farms with up to 90% of broilers having a detectable gait abnormality (Bassler et al., 2013; S. Kestin et al., 1992; Kittelsen et al., 2017). A range of 14-50% of broiler chickens experience lameness that is severe enough to compromise their welfare (Bassler et al., 2013; S. Kestin et al., 1992; Kittelsen et al., 2017).
Lameness is evaluated based on scoring a broiler chicken’s gait. Gait scoring was developed to measure walking ability to determine the prevalence of leg weakness (Kestin et al., 1992). The immobility that accompanies lameness and leg weakness can hinder a broiler chicken’s ability to experience aspects of good welfare (Kestin et al., 1992). Prior to the development of gait scoring systems, the prevalence of leg weakness was assessed based on skeletal abnormalities (Kestin et al., 1992). Developing subjective methods of gait measurement were necessary for walking ability to be scored on a larger number of birds (Kestin et al., 1992). Kestin et al. (1992) developed a subjective gait scoring system to assess abnormalities in gait (Table 1.3).

Table 1.3. Broiler chicken gait scoring system (Kestin et al., 1992)

<table>
<thead>
<tr>
<th>Gait Score</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No detectable abnormalities of gait</td>
</tr>
<tr>
<td>1</td>
<td>Slight gait abnormality but unidentifiable</td>
</tr>
<tr>
<td>2</td>
<td>Identifiable gait abnormality that does not impact movement</td>
</tr>
<tr>
<td>3</td>
<td>Identifiable gait abnormality that does impact movement</td>
</tr>
<tr>
<td>4</td>
<td>Severe gait abnormality, still able to walk but with increased difficulty</td>
</tr>
<tr>
<td>5</td>
<td>Incapable of walking</td>
</tr>
</tbody>
</table>

The prevalence of lameness is impacted by age and growth rate, as well as environmental and management factors, such as litter moisture, ammonia levels, stocking density and space availability which will be discussed later (Bradshaw et al., 2002; Buijs et al., 2009; Das & Lacin, 2014; De Jong et al., 2014; Knowles et al., 2008; G. S. Sanotra et al., 2001).

1.4 Footpad Dermatitis

Footpad Dermatitis (FPD) is a welfare concern where necrotic lesions form on the bottom of the foot (Shepherd & Fairchild, 2010). Based on behavioral observations, there is evidence to suggest that FPD is associated with pain (Martland, 1984, 1985; Shepherd & Fairchild, 2010). The presence of FPD can also influence walking ability when all of the weight of a broiler chicken is placed on the bottom of the foot, increasing the pain associated with the lesions and making it harder to walk (De Jong et al., 2014; Granquist et al., 2019; Kittelsen et al., 2017; Martland, 1984; Tullo et al., 2017). Footpad dermatitis can be assessed using the Welfare Quality (2009) scoring system. Footpad dermatitis is typically found on the pads of the foot, but occasionally occurs on
the toes as well (Welfare Quality Network, 2009). The scoring system (Welfare Quality Network, 2009) in Table 1.4 allows for the evaluation of the severity of footpad dermatitis.

Table 1.4. Broiler chicken footpad scoring system for presence of footpad dermatitis (Welfare Quality Network, 2009)

<table>
<thead>
<tr>
<th>Scores</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No evidence of footpad dermatitis</td>
</tr>
<tr>
<td>1</td>
<td>Minimal evidence of footpad dermatitis</td>
</tr>
<tr>
<td>2</td>
<td>Evidence of footpad dermatitis</td>
</tr>
</tbody>
</table>

1.5 Hock Burn

Hock burn is similar to FPD but is the presence of contact dermatitis on the back of the hock joint (Welfare Quality Network, 2009). The contact dermatitis lesions are believed to be caused by ammonia in litter causing a chemical burn (Gordon & Tucker, 1993; Haslam et al., 2006). The scoring system in Table 1.5 allows for the severity of the hock burn to be assessed.

Table 1.5. Broiler chicken scoring system for presence of hock burn (Welfare Quality Network, 2009)

<table>
<thead>
<tr>
<th>Scores</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No evidence of hock burn</td>
</tr>
<tr>
<td>1</td>
<td>Minimal evidence of hock burn</td>
</tr>
<tr>
<td>2</td>
<td>Evidence of hock burn</td>
</tr>
</tbody>
</table>

1.5.1 Age and Growth Rate

Age is significantly related to lameness; as age increases, walking ability decreases (Silvera et al., 2017; Sørensen et al., 2000). As broiler chickens age and their body weight increases, their walking ability declines and therefore the influence of age cannot be separated from the influence of body weight (Kestin et al., 2001; Sørensen et al., 2000; Weeks et al., 2000).

As there has been an increase in the demand for meat yield in chickens, there has been a push for broiler chickens to grow faster which is resulting in a higher prevalence of welfare concerns such as leg weakness, reduced activity levels, and increased prevalence of footpad dermatitis, hock burn, and skin lesions (Bessei, 2006; Bradshaw et al., 2002; Hartcher & Lum, 2020; Knowles et al., 2008). Based on the Ross 708 management guide, broiler chickens’ average
daily weight gain is about 0.1 kg per day (Aviagen, 2018). Weight gain is known to be associated nonlinearly with walking ability (Sørensen et al., 2000). Walking ability decreases as body weight increases until broilers have a severe impact on their locomotion and at that point the broilers have a harder time reaching feed, resulting in lower body weights (Sørensen et al., 2000).

The difference in growth rate of broiler chicken strains from different years has been evaluated by Zuidhof et al. (2014), and more modern strains of broiler chickens are growing even faster than those evaluated for their impact on gait. As the rapid weight gain of broiler chickens continues to increase, it can be presumed that the impacts on welfare become more severe. The increased growth has been selected to increase the weight of the breast which has also changed the center of gravity (Corr et al., 2003). While the meat yield per broiler chicken has been adjusted for rapid growth, other areas of the broiler chicken body have not, such as their legs (Corr et al., 2003), which may be why rapid growth negatively impacts their gait.

1.5.2 Environmental and Management Factors

Environmental conditions have a large impact on the welfare of a broiler chicken as a reaction to any external stimuli can increase stress levels and impair productivity, performance, and the ability to live in a positive state (Tainika et al., 2023). Management practices impact broiler welfare as they introduce potential stressors including litter moisture and stocking density (Tainika et al., 2023). As welfare is the way an animal copes with its environment, it is important to have a deep understanding of how changing environmental factors impact welfare (Broom, 1991, 1996).

Litter Moisture and Ammonia

Litter moisture may be affected by many different factors within a housing system including stocking density, ventilation, and design of the drinker (Shepherd & Fairchild, 2010). Feddes et al. (2002) reported that water consumption increased per bird as stocking density increased, which resulted in feces becoming more watery, and leading to increased litter moisture. The increase in stocking density makes maintaining litter moisture levels more challenging (Dawkins et al., 2004). Ammonia levels are highly connected to the moisture level of litter (Estevez, 2002). The moisture in the litter when paired with high temperatures promotes bacterial growth that leads to the production of ammonia (Estevez, 2002). Due to this, the problems that are
associated with wet litter are often combined with those of high ammonia levels and it can be
difficult to separate these two factors (Estevez, 2002).

Ammonia is an irritant that leads to an increase in the presence of FPD and hock burn (De
Jong et al., 2014; Gordon & Tucker, 1993; Haslam et al., 2006; Martland, 1984). As FPD worsens,
there is more pain (Martland, 1984, 1985; Opengart et al., 2018) which leads to the broiler chickens
walking abnormally resulting in higher gait scores. The litter quality can have a big impact on the
welfare of a broiler chicken as a part of their environment, and therefore it is important to have a
quick way to assess litter quality. The assessment of litter quality is used to identify the quality of
the litter, based partly on moisture levels (Table 1.6).

Table 1.6. Scoring system for litter in broiler chicken housing (Welfare Quality Network, 2009)

<table>
<thead>
<tr>
<th>Score</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Completely dry and flaky, easily moves with foot</td>
</tr>
<tr>
<td>1</td>
<td>Dry but does not easily move with foot</td>
</tr>
<tr>
<td>2</td>
<td>Foot leaves importing and will form a ball if compacted, but ball does not stay together well</td>
</tr>
<tr>
<td>3</td>
<td>Sticks to boots and sticks readily in a ball if compacted</td>
</tr>
<tr>
<td>4</td>
<td>Sticks to boots once compacted crust is broken</td>
</tr>
</tbody>
</table>

Stocking Density and Space Availability

The National Chicken Council (2022) states that stocking densities should be between
36.62 – 41.5 kg/m². However, the Global Animal Partnership’s (GAP) Animal Welfare Standards
(2020) have a maximum stocking density of 29.29 kg/m² for farms that want to be certified. GAP
is one of the many welfare certification programs available in the United States, and it is important
to understand their standards as the current dissertation conducted experiments on farms that were
GAP certified. This means that throughout the design and implementation of the experiments in
this dissertation, these standards had to be maintained and influenced the stocking densities that
could be evaluated. Previous studies have determined that broilers housed at lower stocking
densities had better walking ability than those housed at higher stocking densities (Sørensen et al.,
2000). As stocking density increases, there is less space for broilers to move around resulting in a
reduction of activity levels which is associated with increased prevalence of lameness (Silvera et
al., 2017; Son, 2013; Weeks et al., 2000). Therefore, allowing broilers the space to move at reduced
stocking densities can maintain walking ability (Sørensen et al., 2000). Space availability is often
discussed in relation to stocking density as the space available per broiler chicken is impacted by the stocking density. Space availability is the amount of space per broiler chicken which is typically measured as m²/bird (Vas et al., 2023).

Stocking density is a broiler chicken welfare concern as stocking density can have a direct impact on animal-based welfare measures. Studies have shown that as stocking density increases, walking ability on average within the flock decreases (Buijs et al., 2009; Das & Lacin, 2014; G. S. Sanotra et al., 2001; Sørensen et al., 2000; Thomas et al., 2004). In the study by Son (2013), the stocking densities examined were within the typical examined ranges, which are from 25 to 46 kg/m². The lowest stocking density was 30-32 kg/m², the middle stocking density was 36-38 kg/m², and the high stocking density was 42-44 kg/m² (Son, 2013). With each increase of stocking density, gait scores were significantly higher, meaning overall walking ability reduced as stocking density increased, which is consistent with other research (Buijs et al., 2009; Das & Lacin, 2014; G. S. Sanotra et al., 2001; Son, 2013; Sørensen et al., 2000; Thomas et al., 2004). There are many potential reasons as to why increased stocking density can be associated with a decrease in walking ability. All of the potential reasons are related, and it is hard to distinguish which one may exactly be leading to the decrease in walking ability. One of the potential reasons for the decrease in walking ability is that as stocking density increases, leg strength decreases (Buijs et al., 2009).

Another potential reason is the relationship between broiler chickens’ weight gain and stocking density increasing as they grow. Higher stocking densities appear to have more of an impact later in the broiler chickens’ lives as their body weights get closer to market weights (Anderson et al., 2021). As broiler chickens are beginning to reach market weight, they are also experiencing increased stocking density. While the space available per broiler chicken does not change with age, unless there are mortalities, the way a broiler chicken uses the space changes. Sørensen et al. (2000) found that broilers housed at lower stocking densities had better walking ability even though they had a higher average body weight, suggesting that increased body weight is not the sole cause of the increased presence of lameness. However, this finding should be examined more as the broiler chicken strains from more recently may produce different results. While the relationship between stocking density and lameness is well studied, there are still aspects that are missing further understanding. The available research has not delved into how the impact of stocking density changes if stocking density is changed as broilers age. In previous studies, researchers selected an estimated end stocking density at the start and they did not change the
estimated end stocking density throughout. Currently, there is no research on the impacts of decreasing stocking density later in the life of the broiler chickens and this dissertation aims to fill this gap.

The amount of space available for broiler chickens can have a direct impact on the behavior of a broiler chicken (Vas et al., 2023). As the amount of space per broiler chicken increases, the behaviors displayed by the broilers also change. In a study by Vas et al. (2023), space availability ranged from 0.058 - 0.076 m²/bird. Allowing for a small increase in space per individual, broilers are more likely to perform behaviors that would be space-consuming (Vas et al., 2023). Broilers with more space are more likely to spend time running and jumping (Vas et al., 2023). With there being an increase in space availability, there was more opportunity for large sections of space to open up within a barn. The large areas of free space opening up could trigger an increase in playful behaviors (Baxter et al., 2019, 2020; Liu et al., 2020; Rayner et al., 2020). This allows for there to be more opportunities for space consuming behaviors to occur such as longer jumps or running (Vas et al., 2023).

High stocking density can also lead to an increase in FPD and hock burn (Abudabos et al., 2013; Ventura et al., 2010). Increasing stocking density has an impact on factors related to ventilation and air control that can lead to changes in ammonia levels and litter quality (Dawkins et al., 2004; Feddes et al., 2002; Heier et al., 2002; Hester, 1994; Martrenchar et al., 2002). The increased prevalence of these problems could be due to there being more broilers in the same amount of space. If there are more broilers in an area, there will be more individuals defecating, which gives the litter less time to absorb and overall, the litter quality reduces (Ventura et al., 2010). As the litter quality reduces, the prevalence of FPD increases, and therefore walking ability may also decline (Martland, 1984, 1985; Opengart et al., 2018).

While there is not currently a lot of research directly discussing the relationship between space availability and the presence of leg health issues, such as lameness, it can be hypothesized from the information above that increasing the space availability per broiler chicken will reduce the presence of lameness. This dissertation will further evaluate the relationship between space availability and leg health issues.
1.6 Broiler chicken behavior

Lameness is associated with changes in behavior, which are used as animal-based measures of welfare. As the severity of lameness increased, broiler chickens spent less time eating and drinking compared to those that were not lame (Weeks et al., 2000), which could have led to welfare issues of thirst, hunger and potentially starvation. Broiler chickens that were impacted by lameness also performed abnormal eating behaviors, such as sitting while eating more frequently than those that did not have some degree of lameness (Riber et al., 2021). There is evidence that the presence of lameness also increased the amount of time broiler chickens spent sitting down as sitting is a less physically demanding posture for individuals with leg issues (Weeks et al., 2000). Broilers that showed signs associated with lameness also spent more time lying than those who did not show signs of lameness (Weeks et al., 2000). Overall broilers with increased gait scores performed less physically intense behaviors (Riber et al., 2021). Increased time spent sitting is also associated with an increase in the prevalence of FPD and hock burn as broiler chickens are spending more time in contact with litter (De Jong et al., 2014; Martland, 1984; Shepherd & Fairchild, 2010). Also, as FPD is associated with an increased risk of declining walking ability (Martland, 1984, 1985; Opengart et al., 2018), increased time spent sitting due to poor gait may lead to a further reduction in walking ability.

Behavioral observations are often used as a method to assess the welfare of broiler chickens. One of the aspects of the three circles model of welfare (Fraser et al., 1997) and the Five Freedoms model of welfare (Farm Animal Welfare Council, 1993) is the ability to express natural behaviors. The Five Provisions also consider natural behavior, but also include broiler chickens exhibiting behaviors associated with positive welfare (Mellor, 2016). For broiler chickens, the opportunities to express natural behaviors should include having chances to run, forage, flap their wings, preen, and rest undisturbed (Bergmann et al., 2017; Broom, 2001). Broiler chickens typically spend a majority of their day resting and that is not dependent on housing style or genetic line (Bergmann et al., 2017; Son, 2013). It is estimated that by 39 to 49 days of age, broilers are spending about 76-86% of their time resting (Weeks et al., 2000). As resting seems to take up the majority of a broiler chicken’s day, it is important for their welfare that they are provided quality places to rest (Weeks et al., 2000), which include locations with adequate litter quality. As discussed earlier, litter quality can have an impact on the prevalence of FPD and hock burn, and as contact time with litter increases the irritation from the litter will lead to an increase in FPD and hock burn (Gordon
& Tucker, 1993; Haslam et al., 2006; Martland, 1984). Therefore, the provision of good quality places to lay can impact not only time spent performing natural behaviors, but can also impact the presence of welfare concerns.

A study by Son (2013) also found that lying down is the most common behavior at all the stocking densities that were evaluated. There was a tendency that broilers spent a longer time lying in the highest stocking density (42-44 kg/m²) when compared to the lowest stocking density (30-32 kg/m²) (Son, 2013). As stated earlier, these stocking densities fall within the typical range as other studies have investigated stocking densities ranging from 21 – 48 kg/m²; however, the effects of lower stocking densities have only been studied in experimental pens (Feddes et al., 2002; Hongchao et al., 2014; Sørensen et al., 2000). Therefore, the impacts of lower stocking densities on broiler chicken behavior should be evaluated in commercial barns to ensure the results are transferable.

The other aspect of activity levels is locomotion behaviors. The percentage of time spent walking decreased as age increased and the prevalence of lameness increased (Weeks et al., 2000). It is suspected that the low percentage of walking in lame birds is only the walking that needs to be done out of necessity, such as walking to feed and water (Weeks et al., 2000) rather than optional movements such as moving to sit at another spot within a pen. This may be related to the reduced body weights of broilers with a severe gait impairment, as they may only be walking to feed when necessary, rather than grazing throughout the day (Kestin et al., 1994) resulting in lower body weights.

Some behaviors are characteristic of certain welfare concerns. For example, broiler chickens that were lame had increased amounts of lying with one of their legs extended away from their body at a right angle (Weeks et al., 2000). It is possible that this was done as a way for the individual to manage pain (Weeks et al., 2000). Extending the leg away from the body is a behavior that should always be looked for, as it may be indicative of lameness. Lame birds also typically preen themselves less often than birds with sound gait (Weeks et al., 2000).

Dustbathing is considered to be a natural behavior that broiler chickens perform at varying degrees. However, most studies report very low levels of dustbathing in broiler chickens (Bessei, 1992; Blokhuis. H. J. & Van Der Haar, 1990; Weeks et al., 1994, 2000). The percentage of time spent dustbathing decreased more when broilers presented signs associated with lameness
(Vestergaard & Sanotra, 1999). This reduction of dustbathing activity may be due to pain rather than a lack of motivation to participate in the behavior.

Space availability and stocking density can have an impact on all of the behaviors of a broiler chicken. As stocking density decreases, and space availability increases, broiler chickens spend more time exhibiting behaviors that are considered space consuming such as running or jumping (Vas et al., 2023). Previous research has focused on the impact of providing increased space availability at the beginning of an experiment and has not investigated if providing additional space during later production phases would yield similar results. This dissertation aims to investigate if broiler chickens will still use space available as they did in prior research if they are provided with additional space later in life.

**Play Behavior**

Play behaviors are typically thought of as a sign of a positive affective state. While there is limited research into the play behavior of domestic chickens, there are a few play behaviors that have been identified. Typically, these behaviors occur when the chickens are young and become less common as they age (Appleby et al., 2004; Nicol, 2015). Worm-running is a play behavior that occurs when one bird picks up a food item or object and the other birds chase the bird attempting to steal the item away from them (Cloutier et al., 2004; Kruijt, 1964; Liu et al., 2020). Worm-running was originally thought to be a feeding behavior; however, when food is abundant or the activity is performed with an inedible object it can be considered play behavior (Cloutier et al., 2004). Another play behavior is sparring which is the display of jumping and physical contact but there is no aggressive pecking or injuries (Diamond & Bond, 2003). Sparring is thought to be closely related to running in the early weeks of life (Rushen, 1982). Finally, the third type of play behavior is called frolicking. Frolicking is when a bird spontaneously runs with either raised or flapping wings and it is also socially contagious (Dawson & Siegel, 1967). Frolicking and sparring are expected to no longer be performed when broiler chickens are older (Dawson & Siegel, 1967). However, this was reported in 1967 when the strain of broiler chickens weas different than the broiler chicken strain today so this should be further researched in today’s broiler chickens.

Space availability and stocking density can directly impact the prevalence of play behavior. In pens with reduced stocking density, it is more likely that large sections of floor space will become open as broilers move through the pens. In these open spaces, broiler chickens are more
likely to begin performing play behaviors (Baxter et al., 2019, 2020; Liu et al., 2020; Rayner et al., 2020).

1.7 Summary

Broiler chickens experience many factors that may lead to welfare concerns as they are raised for production. The impacts of the environmental factors on welfare can be studied by using a mix of resource-based and animal-based measures. Welfare assessments on individual birds in the flock and behavioral observation are used for the animal-based measures while resource-based measures may be gathered through measurement tools such as a light meter or scoring systems such as the one for litter quality. Among these stressors and welfare concerns are stocking density and lameness. While it is known that increasing the stocking density of broiler chickens can have a negative impact on lameness (Buijs et al., 2009; Das & Lacin, 2014; G. S. Sanotra et al., 2001; Son, 2013; Sørensen et al., 2000; Thomas et al., 2004), little is known of what happens to broiler chickens if their stocking density and space allowance are changed at a later age.

The evidence above focused on the impacts on lameness within a research barn or on a commercial farm setting. However, when in the commercial barn setting, the same group of broiler chickens is not typically assessed as it can be hard to identify individuals in a commercial sized barn. Working in a commercial barn also allows for there to be more variability in scores when compared to experimental pens. There is a gap in understanding how gait changes in individuals in a commercial barn. Following the same individuals allows for an understanding of how gait changes over time and if the individuals that may start out with reduced walking abilities are continuing to decline or if broiler chickens tend to reach a certain gait score and remain there which is something currently missing from the research.

The objectives of this research were to:

1. Investigate the consistency of gait scores as broiler chickens age.

2. Investigate how stocking density influences broiler chicken welfare and behavior through manipulating the space availability in the finisher phase.
CHAPTER 2. CHARACTERIZING AGE-RELATED CHANGES IN WALKING ABILITY, WELFARE, AND BEHAVIOR OF INDIVIDUAL BROILER CHICKENS AT DIFFERENT STOCKING DENSITIES

2.1 Abstract

Lameness is a costly welfare concern of broilers which can lead to additional welfare concerns and lost productivity. While it is known that walking ability declines with age and increasing stocking density, little is known about whether individual birds initially classified as having sound or affected gait experience similar declines in walking ability. This study examined 1) how walking ability (gait) and welfare measures of individual broiler chickens that were originally classified as having affected or sound gait changed with age, and 2) the influence of stocking density on changes in gait and welfare measures. Four pens (4 ft x 12 ft, 1.22 m x 3.66 m), each representing a treatment group, were constructed in each of 4 commercial barns (2 barns/farm; n=4 pens/group). Broilers (Ross 708, 196 birds/barn) were randomly assigned to pens based on their gait score at 33d. The four groups were: SOUND, birds with sound gait housed at farm stocking density (6lb/ft\(^2\), 29.29 kg/m\(^2\), 56 birds); AFFECTED, birds with affected gait at farm density (56 birds); MIXED-F, mixed (sound and affected) gait at farm density (56 birds); and MIXED-L, mixed gait at low stocking density (3lb/ft\(^2\), 14.65 kg/m\(^2\), 28 birds). Sound gait was classified as a score of 0-1 on the Bristol Scale (Kestin et al., 1992), while affected gait was classified as a score of 2 and 3. Welfare measures (hock burn, footpad dermatitis, and feather cleanliness) were assessed at 33d, prior to moving birds into pens. Gait and welfare measures were scored again at 37d. Behavior was recorded from 33 days of age to 37 days of age and the proportion of broiler chickens performing behaviors of interest were determined using scan sampling every 10 minutes in the morning (6:00 – 8:00) and evening (20:00 – 21:30). Welfare measures were compared among conditions and time points using PROC LOGISTIC (SAS 9.4) to calculate odds ratios. Walking ability decreased in all groups from 33d to 37d. At 37 d, 78% of SOUND birds, 17% of AFFECTED birds, 49% of MIXED-F birds and 29% of MIXED-L birds experienced decreased walking ability. All groups experienced a decrease in other welfare measures; odds ratios indicated that all groups had low odds of having no hock burn, no footpad dermatitis, and clean feathers at 37d (P < 0.0001). The AFFECTED, MIXED-L, and MIXED-F birds had lower odds of having a 0 gait score at 37d vs. 33 d (P < 0.0001). By 37d, MIXED-L birds
were more likely to have clean feathers compared to all other groups (P < 0.0001), and SOUND birds were more likely to have clean feathers compared to AFFECTED (P < 0.0001) and MIXED-F (P < 0.0001) birds. In addition, AFFECTED birds were less likely to have no hock burn compared to SOUND birds (P < 0.0001), MIXED-F birds (P < 0.0001), and MIXED-L birds (P < 0.0001). Scan sampling data were analyzed using PROC GLIMMIX (SAS 9.4). Broiler chickens performed the behaviors of eating (P < 0.0001), drinking (P = 0.0109), and standing (P < 0.0001) more, and sitting (P < 0.0001) less, in the morning than in the evening. The proportion of broiler chickens in the MIXED-L group performing the behaviors of eating (P < 0.05) and drinking (P < 0.05) was higher than other treatment groups, and the proportion of broiler chickens sitting was lower than other treatment groups (P < 0.05). The proportion of broiler chickens in the AFFECTED treatment group sitting was higher than all other treatment groups (P < 0.05). These results indicated that the walking ability of all birds declined with age, but less so in birds housed at the lower stocking density. Therefore, walking ability, stocking density, and age influence the welfare of broiler chickens. Also, broilers housed at the lower stocking density presented more active behaviors when compared to those housed at the farm stocking density, and broiler chickens with affected gait presented fewer active behaviors than those with sound gait.

### 2.2 Introduction

Lameness, the inability to walk, is a costly welfare concern which impacts a high percentage of broiler chickens (Kestin et al., 1992; Knowles et al., 2008; Sanotra et al., 2003) and can lead to additional welfare concerns, such as pain (Danbury et al., 2000; Granquist et al., 2019), hunger, and dehydration (Butterworth et al., 2002). As broiler chickens’ walking ability declines, they spend more time sitting, and express natural and active behaviors less often (Riber et al., 2021; Vestergaard & Sanotra, 1999).

Stocking density, defined as the amount of weight of broiler chickens per a set amount of space, can influence welfare measures, including gait, footpad dermatitis (FPD), and hock burn (Abudabos et al., 2013; Dozier et al., 2006; Knowles et al., 2008; Ventura et al., 2010). However, results from several studies have indicated that factors other than stocking density can negatively influence lameness such as age (Silvera et al., 2017) and final body weight (Butterworth & Haslam, 2009) which change along with stocking density and can make it challenging to analyze their individual effects separately.
The decrease in walking ability resulting from a higher stocking density is compounded by the decrease in activity levels, which are known to negatively influence walking ability. For example, broiler chickens housed at higher stocking densities are more likely to spend time sitting and less likely to spend time walking or preening (Buijs et al., 2010; Hall, 2001; Norring et al., 2019; Riber et al., 2021; van der Eijk et al., 2022; Weeks et al., 2000). The influence of stocking density on eating behavior is less clear, with several studies reporting no effect of stocking density on eating behaviors (Buijs et al., 2010, 2011; Ventura et al., 2012), whereas others indicated that eating behavior decreased as stocking density increased (Simitzis et al., 2012; van der Eijk et al., 2022).

Research to date has demonstrated that stocking density and age negatively influence walking ability; however, there is currently no information available about how broiler chickens’ prior classification of gait scores impacts their future gait scores and scores for other welfare measures. It is also unknown whether broiler chickens housed within a group only containing one gait classification will behave differently than a mixed classification group as broiler chickens may be impacting one another’s behavior and welfare measures. For example, broiler chickens that have affected gait are associated with an increase in time spent sitting (Riber et al., 2021; Weeks et al., 2000), however broilers with sound gait may be walking into affected gait broiler chickens and disrupting the behavior of sitting. If broiler chickens are in a group consisting of only affected gait broilers, there may be more differences in behavior identified as other gait are not there to impact behavior. Finally, in prior studies examining the influences of stocking density, the stocking density was applied at the beginning of the study. Researchers do not currently have an understanding of how changing stocking density during the finisher phase may impact walking ability, other welfare measures, and behavior. If stocking density influences activity levels and walking ability, then providing a lower stocking density during the finisher phase, when broiler walking ability begins to deteriorate, may lead to an increase in activity levels and prevent the decrease in walking ability. It was hypothesized that broiler chickens initially classified as having sound gait were less likely to experience decreased walking ability and were more likely to have better outcomes for other welfare measures than broilers classified as having affected gait. Broiler chickens classified as having sound gait are also expected to spend more time performing active behaviors such as walking while spending less time sitting. Based on previous research, we also hypothesized that broiler chickens housed at the lower stocking density would have better walking
ability, less footpad dermatitis and less hock burn compared to broilers housed at the higher stocking density. Broiler chickens housed at the lower stocking density were also hypothesized to spend more time performing active behaviors such as walking. The aim of this study was to examine how walking ability, other welfare measures, and the behavior of individual broilers that had originally been classified as having affected or sound gait changed with age and changed with manipulating the stocking density during the finisher phase.

2.3 Methods

2.3.1 Animals and Housing

All of the procedures used in this study were approved by the Purdue Animal Care and Use Committee (PACUC 1909001946). Broiler chickens were housed in curtain sided commercial barns. Broilers were provided ad libitum standard commercial feed and water. The photoperiod was 18 hours of light and 6 hours of dark for the duration of the study.

2.3.2 Treatment Groups

This study was conducted on four commercial broiler chicken farms in the midwestern U.S. In each barn, 196 mixed sex, Ross 708 broiler chickens (784 birds total) were randomly selected for welfare assessments. The initial welfare assessment included gait scoring to assess walking ability based on the scale by Kestin et al. (1992), as well as assessment of footpad dermatitis, hock burn, and feather cleanliness (assessed based on the Welfare Quality Assessment Protocol for Poultry, 2009). All welfare measures were scored by one trained observer. Broilers were then assigned to one of two categories based on their gait scores. At 33 d, the randomly selected broilers had a range of gait scores from 0 to 3 and were therefore combined into groups of gait scores of 0-1 and gait scores of 2-3. Broilers with gait scores of 0 and 1 (indicating no abnormality or an abnormality that was not identifiable) were categorized as sound while broilers with scores of 2 or higher (indicating an identifiable abnormality that may be impacting function) were categorized as having impaired (affected) gait. Therefore, broilers were selected a priori as having sound or affected gait. The farms’ protocols stated that broilers with scores of 4 and 5 would be humanely euthanized by farm staff. Research personnel notified farm staff when birds with scores of 4 and 5 were found and they were not included in the statistical analyses.
Based on their gait score classification, broilers were randomly assigned to one of four treatment groups at 33 days of age, including:

- **SOUND**: gait scores of 0 and 1 based on the scoring system by Kestin et al., (1992) and housed at the farm’s stocking density (6lb/ft\(^2\), 29.29 kg/m\(^2\))

- **AFFECTED**: gait scores of 2 or higher based on the scoring system by Kestin et al., (1992) and housed at the farm’s stocking density (6lb/ft\(^2\), 29.29 kg/m\(^2\))

- **MIXED-F**: 50% of broiler chickens that were considered to have sound gait and 50% that were considered to have affected gait and housed at the farm’s stocking density (6lb/ft\(^2\), 29.29 kg/m\(^2\))

- **MIXED-L**: 50% of broiler chickens that were considered to have sound gait and 50% that were considered to have affected gait and housed at half of the farm’s stocking density (3lb/ft\(^2\), 14.65 kg/m\(^2\))

To separate the treatment groups from the rest of the flock, temporary pens (4ft wide x 12ft long; 1.22 m x 3.66 m) were constructed using PVC pipes, cable ties, and temporary fencing (Figure 2.1) in each of the four barns. Birds with sound gait were marked with non-toxic neon green livestock marker (Ideal®, Lansing, MI) on their heads, and broilers with affected gait were marked with neon pink livestock marker. Pens were adjacent to each other, and each pen included at least one feeder that provided *ad libitum* feed and two nipple drinkers. Between 1-2 feeders were available in each pen with adjacent pens sharing the second feeder. The Ross broiler management guide (Aviagen, 2018) requires broiler chickens with pan feeders have at least one feeder per 45-80 birds, and with the largest pens having 56 broilers per pen, feeder access was adequate. The order of treatment groups was randomized within each barn. At 37 days of age, welfare assessments were conducted again as described previously for all broilers in each pen.
2.3.3 Novel Object Test

The Novel Object Test (NOT, Welfare Quality Network, 2009) was conducted in each pen at 37 d. The NOT occurred prior to welfare assessments at the front edge of the pen farthest away from the wall. All NOTs within a barn were conducted at the same time by 4 different observers. The novel object was a dowel rod with a 2 cm diameter, wrapped in colored tape (Figure 2.2) and was placed on the ground at the front edge of the pen. The observer then stepped back about 1.5 m from the pen and counted the number of chickens within 0.5 m of the novel object every 10 s for 2 min.
Figure 2.2. The novel object used for NOT, a 2 cm diameter dowel rod wrapped in colored tape.

### 2.3.4 Behavioral Observations

#### Video Recording

Two video cameras (GoPro HERO 5 Black Model number: ASST1, GoPro Inc., San Mateo, CA) were suspended from the ceiling in each barn to cover the entirety of the four treatment pens. The video cameras were set to record at 720p video resolution (RES), 30 FPS, and wide field of view (FOV). Behavior was recorded on the mornings of days 34, 35, 36, and 37 and evenings on days 33, 34, 35, and 36 for all 16 pens. Video recordings were analyzed from 6:00 to 8:00 and 20:00 to 21:30.

#### Scan Sampling

Videos were sampled at 10 min intervals for both evening and morning recordings by the same trained observer. The number of broiler chickens doing each behavior listed in the ethogram (Table 2.1) was counted in each pen at each ten-minute mark. The proportion of broiler chickens
performing each behavior was calculated using the total number of broilers in each pen and used for statistical analysis.

Table 2.1. Ethogram of broiler chicken behavior

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eating</td>
<td>Bird is in front of feeder ingesting food (Branco et al., 2020)</td>
</tr>
<tr>
<td>Drinking</td>
<td>Bird is reaching head up to water line, may be tipping head back to swallow (Branco et al., 2020)</td>
</tr>
<tr>
<td>Sitting/Laying Down</td>
<td>Bird is lying in the litter while the head is resting on ground or erect (Baxter et al., 2019)</td>
</tr>
<tr>
<td>Standing</td>
<td>Bird is standing on both legs with no movement (Shynkaruk et al., 2023)</td>
</tr>
<tr>
<td>Walking</td>
<td>The bird is moving at a slow pace, at least 2 steps (Branco et al., 2020)</td>
</tr>
<tr>
<td>Running</td>
<td>Bird is moving at a fast pace (at least 3 steps, quicker than walking) (Branco et al., 2020; Shynkaruk et al., 2023)</td>
</tr>
<tr>
<td>Preening</td>
<td>The bird is cleaning and aligning feathers with beak (Baxter et al., 2019; Jacobs et al., 2019)</td>
</tr>
<tr>
<td>Environmental Pecking</td>
<td>Bird is pecking at an object in its environment (Shynkaruk et al., 2023)</td>
</tr>
<tr>
<td>Foraging</td>
<td>Bird is scratching with feet and pecking at ground (Baxter et al., 2019; Jacobs et al., 2021)</td>
</tr>
<tr>
<td>Aggressive Pecking</td>
<td>The bird is aggressively pecking any part of the body of another bird (Bailie et al., 2013)</td>
</tr>
<tr>
<td>Threat</td>
<td>One bird standing before another, stretching its neck, ruffling its feathers, spreading both wings, and looking downwards at the other bird (Bailie et al., 2013)</td>
</tr>
<tr>
<td>Lateral Lying</td>
<td>The bird is lying laterally with a stretched leg (Baxter et al., 2019)</td>
</tr>
<tr>
<td>Dust Bathing</td>
<td>The bird is bathing in the dust with the use of wings, head, neck, and legs; using beak to bring substrate closer and then performing head rubbing, vertical wing shakes and leg scratching (Baxter et al., 2019)</td>
</tr>
<tr>
<td>Leg Extension</td>
<td>The bird is stretching one wing and one leg of the same body hemisphere (Weeks et al., 2000)</td>
</tr>
<tr>
<td>Unknown</td>
<td>Cannot see the bird’s head</td>
</tr>
</tbody>
</table>

2.3.5 Statistical Analysis

All welfare assessment data analyses were completed using SAS 9.4 (Cary, N.C.). Gait scores of 4 or 5 were not included in analysis due to farm protocol to cull broiler chickens with those scores. Due to the frequencies of gait scores, a binomial system was used for scoring gait such that gait scores of a 2 and 3 were combined into a single category (AFFECTED), and gait
scores of 0 and 1 were combined into another category (SOUND). Hock scores of 2 and 3 were also combined into a single category, resulting in hock scores ranging from 0-2 rather than 0-3. Data were analyzed using PROC LOGISTIC to calculate odds ratios using methods similar to Dong et al. (2021). Data from the NOT were analyzed in R 4.3.1 (2023) using methods similar to Rasmussen et al. (in prep).

The proportion of broiler chickens performing each behavior was calculated for each scan sample at each 10 minute interval. Data were analyzed with a repeated measures analysis using PROC GLIMMIX in SAS 9.4 (Cary, N.C.). The model included the treatment group, time of day, and their interaction. Pen was included as a random effect and time of day nested within age was included as a repeated measures effect. The “other” behavior category condensed the behaviors that were not listed on the ethogram and behaviors that occurred too infrequently for analysis, including preening, environmental pecking, foraging, lateral lying, and leg extension. Drinking, standing, walking and “other” behaviors were analyzed using a lognormal distribution and then back-transformed to least squares means ± SE. Tukey’s test (Tukey, 1949) was used for post-hoc comparisons of LS means.

### 2.4 Results

#### 2.4.1 Novel Object Test

The proportion of broiler chickens within 0.5 m of the novel object was not influenced by the interaction of treatment and the time of the observation \( \chi^2 = 1.12, P = 0.98 \), but tended to be impacted by the observation time \( \chi^2 = 4.15, P = 0.077 \).

#### 2.4.2 Welfare Measures Results

_Treatment-related differences in welfare measures_

The frequencies of all scores for all welfare measures are presented in Table 2.2.
Table 2.2. Percentage of broiler chickens with each score for welfare parameters at 33 and 37 d of age in each treatment group (SOUND: broiler chickens with sound gait and housed at the farm stocking density, AFFECTED: broilers with affected gait and housed at farm stocking density, MIXED-L: broilers with mixed gait housed at half of the farm stocking density, and MIXED-F: broilers with mixed gait housed at farm stocking density).

<table>
<thead>
<tr>
<th>Welfare Parameter</th>
<th>Treatment group</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOUND</td>
<td>AFFECTED</td>
<td>MIXED-L</td>
<td>MIXED-F</td>
<td>33 d</td>
<td>37 d</td>
<td></td>
</tr>
<tr>
<td>Score</td>
<td>33 d</td>
<td>37 d</td>
<td>33 d</td>
<td>37 d</td>
<td>33 d</td>
<td>37 d</td>
<td></td>
</tr>
<tr>
<td>Gait</td>
<td>0</td>
<td>1.8</td>
<td>0</td>
<td>0</td>
<td>0.89</td>
<td>0</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>98.2</td>
<td>21.93</td>
<td>0</td>
<td>0</td>
<td>49.11</td>
<td>21.24</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>73.68</td>
<td>91.08</td>
<td>74.22</td>
<td>45.54</td>
<td>69.03</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>3.95</td>
<td>8.92</td>
<td>23.56</td>
<td>4.46</td>
<td>9.73</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
<td>0.44</td>
<td>0</td>
<td>1.33</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.89</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Footpad</td>
<td>0</td>
<td>55.86</td>
<td>31.58</td>
<td>62.5</td>
<td>33.18</td>
<td>66.96</td>
<td>46.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>20.72</td>
<td>38.16</td>
<td>17.86</td>
<td>39.01</td>
<td>14.29</td>
<td>25.66</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>23.42</td>
<td>30.26</td>
<td>19.64</td>
<td>27.8</td>
<td>18.75</td>
<td>27.43</td>
</tr>
<tr>
<td>Hock</td>
<td>0</td>
<td>84.68</td>
<td>51.75</td>
<td>81.25</td>
<td>22.42</td>
<td>89.29</td>
<td>45.13</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9.46</td>
<td>33.33</td>
<td>15.63</td>
<td>53.81</td>
<td>8.04</td>
<td>43.36</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.86</td>
<td>14.91</td>
<td>3.13</td>
<td>23.77</td>
<td>2.68</td>
<td>14.54</td>
</tr>
<tr>
<td>Feather Cleanliness</td>
<td>0</td>
<td>77.93</td>
<td>24.12</td>
<td>62.05</td>
<td>21.97</td>
<td>71.43</td>
<td>45.13</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>19.37</td>
<td>37.28</td>
<td>29.02</td>
<td>32.74</td>
<td>21.43</td>
<td>30.09</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.8</td>
<td>37.72</td>
<td>6.25</td>
<td>40.81</td>
<td>5.36</td>
<td>24.78</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.9</td>
<td>0.88</td>
<td>2.68</td>
<td>4.48</td>
<td>1.79</td>
<td>0</td>
</tr>
</tbody>
</table>

**Gait Scores**

Treatment differences were analyzed separately for 33 days and 37 days. Treatment groups were defined based on gait scores at 33 days, and analysis of gait scores confirmed that gait scores differed significantly among treatment groups, except for the two mixed groups which were significantly different from other treatment groups but not from each other (MIXED-F and MIXED-L) (Table 2.3).
Table 2.3. Differences in gait scores at 33 days among broiler chickens with sound gait and housed at the farm stocking density (SOUND), broilers with affected gait and housed at farm stocking density (AFFECTED), broilers with mixed gait housed at half of the farm stocking density (MIXED-L), and broilers with mixed gait housed at farm stocking density (MIXED-F)

<table>
<thead>
<tr>
<th>Treatment group comparison</th>
<th>Odds Ratio Estimate</th>
<th>95% CI Lower Limit</th>
<th>95% CI Upper Limit</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOUND vs AFFECTED</td>
<td>2828.5</td>
<td>613.6</td>
<td>13038.3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SOUND vs MIXED-L</td>
<td>123.1</td>
<td>29.02</td>
<td>522.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SOUND vs MIXED-F</td>
<td>115.5</td>
<td>27.87</td>
<td>478.4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>AFFECTED vs MIXED-L</td>
<td>0.04</td>
<td>0.02</td>
<td>0.09</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>AFFECTED vs MIXED-F</td>
<td>0.04</td>
<td>0.02</td>
<td>0.08</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MIXED-L vs MIXED-F</td>
<td>0.94</td>
<td>0.60</td>
<td>1.47</td>
<td>0.7804</td>
</tr>
</tbody>
</table>

At 37 days there were differences in gait scores based on treatment groups (Table 2.4). SOUND birds had higher odds of having a gait score of 0 than AFFECTED birds and MIXED-F birds. AFFECTED birds had lower odds of having a gait score of 0 compared to MIXED-L birds and MIXED-F birds. Broilers in the MIXED-L group had higher odds of having a score of 0 compared the MIXED-F birds. SOUND birds did not differ from MIXED-L birds.
Table 2.4. Differences in gait scores at 37 days among broiler chickens with sound gait and housed at the farm stocking density (SOUND), broilers with affected gait and housed at farm stocking density (AFFECTED), broilers with mixed gait housed at half of the farm stocking density (MIXED-L), and broilers with mixed gait housed at farm stocking density (MIXED-F)

<table>
<thead>
<tr>
<th>Treatment group comparison</th>
<th>Odds Ratio Estimate</th>
<th>95% CI Lower Limit</th>
<th>95% CI Upper Limit</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOUND vs AFFECTED</td>
<td>12.64</td>
<td>7.35</td>
<td>21.74</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SOUND vs MIXED-L</td>
<td>1.42</td>
<td>0.81</td>
<td>2.48</td>
<td>0.2182</td>
</tr>
<tr>
<td>SOUND vs MIXED-F</td>
<td>7.32</td>
<td>4.30</td>
<td>12.46</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>AFFECTED vs MIXED-L</td>
<td>0.11</td>
<td>0.06</td>
<td>0.21</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>AFFECTED vs MIXED-F</td>
<td>0.58</td>
<td>0.37</td>
<td>0.90</td>
<td>0.0146</td>
</tr>
<tr>
<td>MIXED-L vs MIXED-F</td>
<td>5.15</td>
<td>2.77</td>
<td>9.56</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**Footpad condition**

At 33 days, there were no significant differences in footpad scores. At 37 days, SOUND birds had lower odds of having a footpad score of 0 compared to MIXED-L birds. No other differences were found among treatment groups at 37 days (Table 2.5).
Table 2.5. Differences in footpad scores at 37 days among broiler chickens with sound gait and housed at the farm stocking density (SOUND), broilers with affected gait and housed at farm stocking density (AFFECTED), broilers with mixed gait housed at half of the farm stocking density (MIXED-L), and broilers with mixed gait housed at farm stocking density (MIXED-F)

<table>
<thead>
<tr>
<th>Treatment group comparison</th>
<th>Odds Ratio Estimate</th>
<th>95% CI Lower Limit</th>
<th>95% CI Upper Limit</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOUND vs AFFECTED</td>
<td>0.88</td>
<td>0.62</td>
<td>1.24</td>
<td>0.4696</td>
</tr>
<tr>
<td>SOUND vs MIXED-L</td>
<td>0.61</td>
<td>0.40</td>
<td>0.94</td>
<td>0.0235</td>
</tr>
<tr>
<td>SOUND vs MIXED-F</td>
<td>0.77</td>
<td>0.54</td>
<td>1.09</td>
<td>0.1383</td>
</tr>
<tr>
<td>AFFECTED vs MIXED-L</td>
<td>0.69</td>
<td>0.45</td>
<td>1.06</td>
<td>0.0942</td>
</tr>
<tr>
<td>AFFECTED vs MIXED-F</td>
<td>0.87</td>
<td>0.62</td>
<td>1.24</td>
<td>0.4488</td>
</tr>
<tr>
<td>MIXED-L vs MIXED-F</td>
<td>1.26</td>
<td>0.82</td>
<td>1.93</td>
<td>0.2915</td>
</tr>
</tbody>
</table>

**Hock lesions**

Broiler chickens at 33 days in the MIXED-L treatment group had higher odds of having a score of 0 than broiler chickens in the MIXED-F treatment group for hocks (OR = 2.23, 95% CI [1.13, 4.41], P = 0.0215).

At 37 days of age, SOUND birds had higher odds of having a hock score of 0 compared to AFFECTED birds (Table 2.6). AFFECTED birds had lower odds of having a hock score of 0 compared to MIXED-L birds and MIXED-F birds. No differences were found among SOUND, MIXED-L and MIXED-F broilers.
Table 2.6. Differences in hock scores at 37 days among broiler chickens with sound gait and housed at the farm stocking density (SOUND), broilers with affected gait and housed at farm stocking density (AFFECTED), broilers with mixed gait housed at half of the farm stocking density (MIXED-L), and broilers with mixed gait housed at farm stocking density (MIXED-F).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Odds Ratio Estimate</th>
<th>95% CI Lower Limit</th>
<th>95% CI Upper Limit</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOUND vs AFFECTED</td>
<td>3.33</td>
<td>2.30</td>
<td>4.84</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SOUND vs MIXED-L</td>
<td>1.15</td>
<td>0.73</td>
<td>1.82</td>
<td>0.5330</td>
</tr>
<tr>
<td>SOUND vs MIXED-F</td>
<td>1.10</td>
<td>0.76</td>
<td>1.60</td>
<td>0.6146</td>
</tr>
<tr>
<td>AFFECTED vs MIXED-L</td>
<td>0.35</td>
<td>0.22</td>
<td>0.54</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>AFFECTED vs MIXED-F</td>
<td>0.33</td>
<td>0.23</td>
<td>0.48</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MIXED-L vs MIXED-F</td>
<td>0.95</td>
<td>0.61</td>
<td>1.50</td>
<td>0.8347</td>
</tr>
</tbody>
</table>

*Feather cleanliness*

At 33 days, SOUND broilers had higher odds of having a feather cleanliness score of 0 compared to AFFECTED broilers (OR = 2.45, 95% CI [1.60, 3.75], P < 0.0001). AFFECTED broilers had reduced odds of having a feather cleanliness score of 0 compared to MIXED-F broiler chickens (OR = 0.56, 95% CI [0.37, 0.84], P = 0.0049).

At 37 days, SOUND birds had higher odds of having a feather cleanliness score of 0 compared to AFFECTED birds and MIXED-F birds (Table 2.7). Both SOUND and AFFECTED birds had lower odds of having a feather cleanliness score of 0 than MIXED-L birds. AFFECTED birds had a tendency to have higher odds of having a score of 0 compared to MIXED-F birds. MIXED-L birds had higher odds of having a score of 0 compared to MIXED-F birds.
Table 2.7. Differences in feather cleanliness scores at 37 days among broiler chickens with sound gait and housed at the farm stocking density (SOUND), broilers with affected gait and housed at farm stocking density (AFFECTED), broilers with mixed gait housed at half of the farm stocking density (MIXED-L), and broilers with mixed gait housed at farm stocking density (MIXED-F)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Odds Ratio Estimate</th>
<th>95% CI Lower Limit</th>
<th>95% CI Upper Limit</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOUND vs AFFECTED</td>
<td>1.43</td>
<td>1.01</td>
<td>2.03</td>
<td>0.0467</td>
</tr>
<tr>
<td>SOUND vs MIXED-L</td>
<td>0.41</td>
<td>0.27</td>
<td>0.63</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SOUND vs MIXED-F</td>
<td>2.04</td>
<td>1.42</td>
<td>2.91</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>AFFECTED vs MIXED-L</td>
<td>0.29</td>
<td>0.18</td>
<td>0.44</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>AFFECTED vs MIXED-F</td>
<td>0.26</td>
<td>1.00</td>
<td>2.04</td>
<td>0.0530</td>
</tr>
<tr>
<td>MIXED-L vs MIXED-F</td>
<td>1.13</td>
<td>3.18</td>
<td>7.76</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**Age-related changes in welfare measures**

Broiler chickens’ walking ability decreased in all groups from 33d to 37d. At 37d, 78% of SOUND birds, 17% of AFFECTED birds, 49% of MIXED-F birds, and 29% of MIXED-L birds exhibited decreased walking ability when compared to walking ability at 33 days. Broiler chickens’ scores for gait, footpads, hock, and feather cleanliness at 33 days were all more likely to be a score of 0 compared to 37 days (Table 2.8).

Table 2.8. Odds ratios for the likelihood of broiler chickens having a score of 0 for each welfare measure (gait, footpad dermatitis, and feather cleanliness) at 33 days compared to 37 days of age

<table>
<thead>
<tr>
<th>Welfare measure</th>
<th>Odds Ratio Estimate</th>
<th>95% CI Lower Limit</th>
<th>95% CI Upper Limit</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gait score</td>
<td>3.86</td>
<td>3.33</td>
<td>4.47</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Footpad dermatitis</td>
<td>1.52</td>
<td>1.38</td>
<td>1.68</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Hock burn</td>
<td>2.70</td>
<td>2.40</td>
<td>3.05</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Feather cleanliness</td>
<td>3.13</td>
<td>2.80</td>
<td>3.49</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Age-related differences in welfare measures for each treatment group

**SOUND broilers**

All broiler chickens in the SOUND group had a gait score of 0 or 1 at 33 days, and gait scores at 33 days could not statistically be compared to gait scores at 37 days due to the lack of variability in gait scores. At 33 days, 98.2% of SOUND birds had a score of 1. By 37 days, 21.93% of SOUND birds had a score of 1 and 78.1% had a score of 2 or higher. The odds of SOUND broilers having a score of 0 for footpads, hocks, and feather cleanliness were higher at 33 days than 37 days (Table 2.9).

Table 2.9. Odds ratios for the likelihood of broiler chickens in the SOUND (broiler chickens with sound gait housed at farm stocking density) treatment having a score of 0 for each welfare measure (footpads, hocks, and feather cleanliness) at 33 days compared to 37 days.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Odds Ratio Estimate</th>
<th>95% CI Lower Limit</th>
<th>95% CI Upper Limit</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Footpad 33d vs 37d</td>
<td>1.51</td>
<td>1.26</td>
<td>1.80</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Hock 33d vs 37d</td>
<td>2.30</td>
<td>1.83</td>
<td>2.90</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Feather Cleanliness 33d vs 37d</td>
<td>4.16</td>
<td>3.29</td>
<td>5.26</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**AFFECTED broilers**

The odds of AFFECTED broiler chickens having a score of 0 for gait, footpads, hocks, and feather cleanliness were higher at 33 days than 37 day (Table 2.10).

Table 2.10. Odds ratios for the likelihood of broiler chickens in the AFFECTED (broiler chickens with affected gait housed at farm stocking density) treatment having a score of 0 for each welfare measure (gait, footpads, hocks, and feather cleanliness) at 33 days compared to 37 days.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Odds Ratio Estimate</th>
<th>95% CI Lower Limit</th>
<th>95% CI Upper Limit</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gait 33d vs 37d</td>
<td>1.87</td>
<td>1.42</td>
<td>2.46</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Footpad 33d vs 37d</td>
<td>1.67</td>
<td>1.39</td>
<td>2.01</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Hock 33d vs 37d</td>
<td>3.94</td>
<td>3.13</td>
<td>4.95</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Feather Cleanliness 33d vs 37d</td>
<td>3.04</td>
<td>2.46</td>
<td>3.76</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
**MIXED-L broilers**

The odds of MIXED-L broiler chickens having a score of 0 for gait, footpads, hocks, and feather cleanliness were higher at 33 days than 37 days (Table 2.11).

Table 2.11. Odds ratios for the likelihood of broiler chickens in the MIXED-L (broiler chickens with mixed gait housed at half of the farm stocking density) treatment having a score of 0 for each welfare measure (gait, footpads, hocks, and feather cleanliness) at 33 days compared to 37 days.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Odds Ratio Estimate</th>
<th>95% CI Lower Limit</th>
<th>95% CI Upper Limit</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gait 33d vs 37d</td>
<td>1.91</td>
<td>1.44</td>
<td>2.53</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Footpad 33d vs 37d</td>
<td>1.57</td>
<td>1.19</td>
<td>2.07</td>
<td>0.0016</td>
</tr>
<tr>
<td>Hock 33d vs 37d</td>
<td>3.24</td>
<td>2.27</td>
<td>4.63</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Feather Cleanliness 33d vs 37d</td>
<td>1.98</td>
<td>1.48</td>
<td>2.63</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**MIXED-F broilers**

The odds of MIXED-F broiler chickens having a score of 0 for gait, footpads, hocks, and feather cleanliness were higher at 33 days than 37 days (Table 2.12).
Table 2.12. Odds ratios for the likelihood of broiler chickens in the MIXED-F (broiler chickens with mixed gait housed at farm stocking density) treatment having a score of 0 for each welfare measure (gait, footpads, hocks, and feather cleanliness) at 33 days compared to 37 days.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Odds Ratio Estimate</th>
<th>95% CI Lower Limit</th>
<th>95% CI Upper Limit</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gait 33d vs 37d</td>
<td>5.42</td>
<td>3.79</td>
<td>7.75</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Footpad 33d vs 37d</td>
<td>1.41</td>
<td>1.18</td>
<td>1.69</td>
<td>0.0002</td>
</tr>
<tr>
<td>Hock 33d vs 37d</td>
<td>2.11</td>
<td>1.71</td>
<td>2.61</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Feather Cleanliness 33d vs 37d</td>
<td>4.80</td>
<td>3.80</td>
<td>6.07</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

### 2.4.3 Behavior Results

Running, dust bathing, and threat behaviors were not observed. Data from 33 and 37 days were combined for analysis.

### 2.4.4 Eating

The proportion of birds eating was significantly impacted by the interaction of time of day and treatment ($F_{3, 1063} = 2.68$, $P = 0.0455$, Figure 2.3). A higher proportion of broilers in the SOUND and MIXED-L groups were eating in the morning compared to the evening ($P < 0.0001$). In the morning, a higher proportion of broilers in the MIXED-L treatment group were eating compared to all other treatment groups ($P < 0.0001$).
The average proportion of broiler chickens in each group that were eating (lsmeans ± SE) in the morning and evening averaged across 33 through 37 days of age SOUND: broilers with gait scores of 0-1 (S. Kestin et al., 1992) housed at farm stocking density (29.29 kg/m²), AFFECTED: broilers with gait scores of 2 or higher housed at farm stocking density, MIXED-L: broilers with mixed gait scores (50%: sound, 50%: affected) housed at half of the farm stocking density (14.65 kg/m²), MIXED-F: broilers with mixed gait scores (50%: sound, 50%: affected) housed at farm stocking density. a-d Least squares means without a common superscript differ among treatment groups and time of day (P < 0.05).

2.4.5 Drinking

The average proportion of broiler chickens drinking was significantly impacted by time of day (F₁, 896 = 29.49, P = 0.0109, Figure 2.4) and treatment group (F₃, 896 = 3.74, P < 0.0001, Figure 2.5). A higher proportion of broilers were drinking in the morning than in the evening (P < 0.0001). The proportion of birds in the MIXED-L group that were drinking was higher than that of the MIXED-F group (P = 0.013).
Figure 2.4. The average proportion of broiler chickens that were drinking in the morning and evening averaged across 33 through 37 days of age (lsmeans ± SE). \(^{a-b}\) Least squares means without a common superscript differ due to time of day (P < 0.05).

Figure 2.5. The average proportion of broiler chickens in each treatment group that were drinking (lsmeans ± SE). SOUND: broilers with gait scores of 0-1 (S. Kestin et al., 1992) housed at farm stocking density (29.29 kg/m\(^2\)), AFFECTED: broilers with gait scores of 2 or higher housed at farm stocking density, MIXED-L: broilers with mixed gait scores (50%: sound, 50%: affected) housed at half of the farm stocking density (14.65 kg/m\(^2\)), MIXED-F: broilers with mixed gait scores (50%: sound, 50%: affected) housed at farm stocking density. \(^{a-b}\) Least squares means without a common superscript differ among treatment groups (P < 0.05).
2.4.6 Standing

The average proportion of broiler chickens standing was significantly impacted by time of day ($F_{1,708} = 16.65, P < 0.0001$, Figure 2.6) and treatment group ($F_{3,708} = 5.66, P = 0.0008$, Figure 2.7). The proportion of broilers standing was higher in the morning than in the evening ($P < 0.0001$). The proportion of broilers in the MIXED-L treatment group that were standing was higher than the proportion of broilers standing in AFFECTED ($P = 0.013$) and MIXED-F ($P = 0.0003$) treatment groups.

Figure 2.6. The average proportion of broiler chickens that were standing in the morning and evening averaged across 33 through 37 days of age (lsmeans ± SE). 

\textsuperscript{a-b} Least squares means without a common superscript differ due to time of day ($P < 0.05$).
Figure 2.7. The average proportion of broiler chickens in each treatment group that were standing (lsmeans ± SE). SOUND: broilers with gait scores of 0-1 (S. Kestin et al., 1992) housed at farm stocking density (29.29 kg/m²), AFFECTED: broilers with gait scores of 2 or higher housed at farm stocking density, MIXED-L: broilers with mixed gait scores (50%: sound, 50%: affected) housed at half of the farm stocking density (14.65 kg/m²), MIXED-F: broilers with mixed gait scores (50%: sound, 50%: affected) housed at farm stocking density. a-b Least squares means without a common superscript differ among treatment groups (P < 0.05).

2.4.7 Sitting

The average proportion of broiler chickens sitting was significantly impacted by time of day ($F_{1,1063} = 72.16, P < 0.0001$, Figure 2.8) and treatment group ($F_{3,1063} = 17.40, P < 0.0001$, Figure 2.9). For time of day, the proportion of broiler chickens sitting was higher in the evening than in the morning (P < 0.0001). For treatment groups, there was a significantly higher proportion of birds sitting in the AFFECTED treatment group than all other treatment groups (SOUND: P < 0.0001, MIXED-F: P = 0.0059, MIXED-L: P < 0.0001). There was also a significantly higher proportion of broiler chickens sitting in the SOUND and MIXED-F treatment groups when compared to broilers in the MIXED-L treatment group (SOUND: P = 0.0486, MIXED-F: P = 0.0003).
Figure 2.8. The average proportion of broiler chickens that were sitting in the morning and evening averaged across 33 through 37 days of age (lsmeans ± SE). a-b Least squares means without a common superscript differ due to time of day (P < 0.05).

Figure 2.9. The average proportion of broiler chickens that were sitting in each treatment group (lsmeans ± SE). SOUND: broilers with gait scores of 0-1 (S. Kestin et al., 1992) housed at farm stocking density (29.29 kg/m²), AFFECTED: broilers with gait scores of 2 or higher housed at farm stocking density, MIXED-L: broilers with mixed gait scores (50%: sound, 50%: affected) housed at half of the farm stocking density (14.65 kg/m²), MIXED-F: broilers with mixed gait scores (50%: sound, 50%: affected) housed at farm stocking density. a-c Least squares means without a common superscript differ among treatment groups (P < 0.05).
2.4.8 Walking

The average proportion of broiler chickens walking was significantly impacted by the interaction of treatment group and time of day ($F_{3,575} = 2.89, P = 0.0347$, Figure 2.10). The proportion of broiler chickens walking in the evening was significantly higher in the MIXED-L treatment group than the proportion of broiler chickens in SOUND ($P = 0.0021$) and MIXED-F ($P = 0.024$) treatment groups.

![Figure 2.10](image)

Figure 2.10. The average proportion of broiler chickens that were walking (Ismeans ± SE) in the morning and evening averaged across 33 through 37 days of age. SOUND: broilers with gait scores of 0-1 (S. Kestin et al., 1992) housed at farm stocking density (29.29 kg/m²), AFFECTED: broilers with gait scores of 2 or higher housed at farm stocking density, MIXED-L: broilers with mixed gait scores (50%: sound, 50%: affected) housed at half of the farm stocking density (14.65 kg/m²), MIXED-F: broilers with mixed gait scores (50%: sound, 50%: affected) housed at farm stocking density. a-b Least squares means without a common superscript differ due to treatment group and time of day ($P < 0.05$).

2.4.9 Other

The “other” behavior category included behaviors that were not listed on the ethogram and behaviors that occurred too infrequently for analysis, including preening, environmental pecking, foraging, lateral lying, and leg extension. The prevalence of each of these behaviors, as a percentage of the total number of scans, is presented in Table 2.13. The average proportion of broiler chickens doing other behaviors was significantly impacted by treatment group ($F_{3,589} = 13.84, P < 0.0001$, Figure 2.11). The average proportion of broiler chickens doing “other” behavior
was significantly higher in the MIXED-L treatment group than all other treatments (SOUND: \( P = 0.0001 \), AFFECTED: \( P < 0.0001 \), MIXED-F: \( P < 0.0001 \)), and in the SOUND treatment group compared to the MIXED-F treatment group (\( P = 0.0489 \)).

Table 2.13. Percentage of scans in which behaviors in the “other” category were observed for each treatment group in the morning and evening. SOUND: broilers with gait scores of 0-1 (S. Kestin et al., 1992) housed at farm stocking density (29.29 kg/m\(^2\)), AFFECTED: broilers with gait scores of 2 or higher housed at farm stocking density, MIXED-L: broilers with mixed gait scores (50%: sound, 50%: affected) housed at half of the farm stocking density (14.65 kg/m\(^2\)), MIXED-F: broilers with mixed gait scores (50%: sound, 50%: affected) housed at farm stocking density.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Time of Day</th>
<th>SOUND</th>
<th>AFFECTED</th>
<th>MIXED-L</th>
<th>MIXED-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preening</td>
<td>Morning</td>
<td>1.26%</td>
<td>0.90%</td>
<td>1.07%</td>
<td>0.73%</td>
</tr>
<tr>
<td></td>
<td>Evening</td>
<td>0.84%</td>
<td>0.80%</td>
<td>0.36%</td>
<td>0.91%</td>
</tr>
<tr>
<td>Environmental Pecking</td>
<td>Morning</td>
<td>0.07%</td>
<td>0.57%</td>
<td>0.09%</td>
<td>0.14%</td>
</tr>
<tr>
<td></td>
<td>Evening</td>
<td>0.12%</td>
<td>0.12%</td>
<td>0.07%</td>
<td>0.04%</td>
</tr>
<tr>
<td>Foraging</td>
<td>Morning</td>
<td>1.85%</td>
<td>0.41%</td>
<td>0.92%</td>
<td>1.34%</td>
</tr>
<tr>
<td></td>
<td>Evening</td>
<td>0.14%</td>
<td>0.81%</td>
<td>1.06%</td>
<td>0.98%</td>
</tr>
<tr>
<td>Threat</td>
<td>Morning</td>
<td>0.14%</td>
<td>0.06%</td>
<td>0.17%</td>
<td>0.08%</td>
</tr>
<tr>
<td></td>
<td>Evening</td>
<td>0.07%</td>
<td>0.03%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Lateral Lying</td>
<td>Morning</td>
<td>0.11%</td>
<td>0.15%</td>
<td>0.09%</td>
<td>0.09%</td>
</tr>
<tr>
<td></td>
<td>Evening</td>
<td>0.06%</td>
<td>0.25%</td>
<td>0.15%</td>
<td>0.15%</td>
</tr>
<tr>
<td>Leg Extension</td>
<td>Morning</td>
<td>0.14%</td>
<td>0.11%</td>
<td>0.20%</td>
<td>0.13%</td>
</tr>
<tr>
<td></td>
<td>Evening</td>
<td>0.18%</td>
<td>0.15%</td>
<td>0.04%</td>
<td>0.23%</td>
</tr>
</tbody>
</table>
The average proportion of broiler chickens that were doing “other” (preening, environmental pecking, foraging, lateral lying, and leg extension) behaviors (lsmeans ± SE). SOUND: broilers with gait scores of 0-1 (S. Kestin et al., 1992) housed at farm stocking density (29.29 kg/m²), AFFECTED: broilers with gait scores of 2 or higher housed at farm stocking density, MIXED-L: broilers with mixed gait scores (50%: sound, 50%: affected) housed at half of the farm stocking density (14.65 kg/m²), MIXED-F: broilers with mixed gait scores (50%: sound, 50%: affected) +) housed at farm stocking density. a-c Least squares means without a common superscript differ due to treatment group (P < 0.05).

2.5 Discussion

In this study, we took a unique approach to examine 1) how gait changed for broilers originally scored as having sound gait and broilers scored as having affected gait as they aged, and 2) how stocking density influenced gait and other welfare parameters of broilers housed within a commercial barn. Conducting the study using broilers within a commercial barn provided greater variability in gait scores and higher numbers of broilers than what would be possible in small, experimental facilities.

Overall, our results indicated that broiler chickens that had originally been classified as having sound gait were more likely to have better welfare and a reduced proportion of broilers performing inactive behaviors such as sitting at later ages. Results also indicated that housing broiler chickens at a reduced stocking density may be beneficial to their welfare and lead to a reduced proportion of broiler chickens performing inactive behaviors such as sitting.

The largest decline in walking ability was found for the SOUND group, followed by the MIXED-F, MIXED-L, and AFFECTED groups in that order. These results suggest that broiler
chickens that originally were classified as having sound gait may still experience declines in walking ability later regardless of their initial assessment. This also suggests that through reducing the stocking density at the finisher phase, some of the declines in walking ability may be mitigated.

The results indicated that age impacted all welfare measures, and broiler chickens were less likely to have scores of 0 for all welfare measures as age increased. Walking ability decreased with age, which was expected based on results from previous research (e.g. Bailie et al., 2013; Bassler et al., 2013; Knowles et al., 2008; Rasmussen et al., 2022; Silvera et al., 2017). Other welfare measures also declined with age, which is consistent with the findings from prior research (Hashimoto et al., 2011; Martins et al., 2013). The effects of age, body weight and stocking density cannot be fully separated because body weight, stocking density and age increase concurrently; therefore, the decline in walking ability is also associated with changes in body weight and stocking density, both of which change with age (Kestin et al., 2001; Sanotra et al., 2003; Sørensen et al., 2000).

Results generally supported our hypotheses that broiler chickens originally classified as have sound gait were less likely to experience decreased walking ability, decreased welfare for hocks, footpads, and feather cleanliness, and more likely to have smaller proportion of broiler chickens performing inactive behaviors such as sitting. Results also generally supported our hypotheses that broiler chickens housed at a reduced stocking density would experience better welfare conditions, and an increased proportion of broiler chickens that performed active behaviors such as walking.

At 33 days, there were no differences in gait scores between MIXED-F and MIXED-L groups, but by 37 days, the odds of having sound gait were higher for MIXED-L than MIXED-F birds, and the odds of having sound gait were similar for MIXED-L and SOUND birds, indicating that the lower stocking density may have had a beneficial effect on the walking ability of broilers that started out with mixed gait scores. These findings support the findings of previous research indicating as stocking density increases, gait worsens (Shynkaruk et al., 2023; Sørensen et al., 2000).

At both 33 days and 37 days, there were no differences in footpad scores between MIXED-F and MIXED-L birds, indicating that stocking density may not have an effect on the presence of footpad dermatitis. There was also no difference in footpad scores between SOUND and
AFFECTED birds at both 33 days and 37 days suggesting that earlier gait scores may not have an effect on the presence of footpad dermatitis.

At 33 days, MIXED-L broiler chickens had higher odds of having no signs of hock burn compared to MIXED-F birds, however by 37 days, there was no difference in the presence of hock burn suggesting that reduced stocking density negatively influence the presence of hock burn as there was a difference prior to the implementation of treatment, but not after.

At 33 days, SOUND and MIXED-F broiler chickens had better odds of having cleaner feathers compared to AFFECTED broiler chickens, however by 37 days there were no differences in feather cleanliness between AFFECTED and MIXED-F broilers. By 37 days, MIXED-L broiler chickens had better odds of have clean feathers compared to all other treatment groups, suggesting that reducing stocking density may have had a beneficial effect on the feather cleanliness of broiler chickens. However, a study by Kiani and von Borstel (2019) investigated the relationship between group size and feather cleanliness and found that broilers had higher levels of feather cleanliness in smaller groups. The difference in group size between the stocking density treatment groups may have accounted for the difference in feather cleanliness rather than stocking density and this relationship should be investigated further.

The NOT did not reveal differences in fear levels based on treatment group. However, prior research indicated that stocking density did not have an influence on the results of the NOT (Bailie et al., 2018; van der Eijk et al., 2022).

The results indicated that the original classification of broiler chickens’ gait scores at 33 days of age was associated the proportion of broiler chickens sitting. This is consistent with prior research that suggested as gait scores increased, broilers spent more time sitting (Riber et al., 2021; Weeks et al., 2000). As broiler chickens are sitting more the prevalence of hock burn and FPD is expected to rise due to longer contact time with the litter (De Jong et al., 2014; Groves & Muir, 2016). Prior research has also linked higher (worse) gait scores with an increased prevalence of hock burn and FPD (De Jong et al., 2014; Granquist et al., 2019; Kittelsen et al., 2017; Tullo et al., 2017). While the results of the present study indicate a link between the proportion of broilers sitting, gait scores, and the presence of hock burn, they do not indicate a relationship between gait scores and FPD. This suggests that after broiler chickens’ gait scores worsen, there are more broiler chickens sitting, and then there may be an increase in the presence of hock burn.
There was also a relationship between stocking density and the proportion of broiler chickens sitting, walking, standing, and eating. The proportion of broiler chickens sitting was lower in the MIXED-F treatment group compared to the MIXED-L treatment group which agrees with previous findings that suggested increased stocking density is associated with an increased proportion of broiler chickens sitting (Hongchao et al., 2014). Prior research also reported that as stocking density increased, the proportion of broiler chickens walking decreased (Hongchao et al., 2014; van der Eijk et al., 2022). The results of the current study support the previous findings, however, there was also an interaction with time of day, and the difference in the proportion of broiler chickens walking based on stocking density was only observed in the evening. However, previous studies only observed behavior in the morning and afternoon and still observed a difference based on stocking density (Hongchao et al., 2014; van der Eijk et al., 2022). This suggests that the interaction of stocking density and time of day may have an impact on the proportion of broiler chickens walking, but more research is needed.

The broilers in the lower stocking density treatment also had better gait than the higher stocking broiler chickens which may relate to the differences in the proportion of broiler chickens standing, sitting, and walking. As discussed above, the gait scores of broiler chickens can impact their behavior. Broiler chickens with affected gait spend more time sitting (Riber et al., 2021; Weeks et al., 2000) and therefore less time performing other behaviors such as walking and standing. The gait scores of the broiler chickens in the two stocking densities may be impacting the behavior rather than stocking density and should be investigated further.

The proportion of broiler chickens eating was also different when comparing the treatment groups housed at various stocking densities. While the results indicated that there was a higher proportion of broiler chickens in the reduced stocking density group eating in the morning compared to other groups, this may also be due to feeder space availability. While all pens had access to the amount of feeders recommended in the Ross management guide (Aviagen, 2018), the pens did not have the same amount of feeders available. Prior research has reported inconclusive findings on the impacts of stocking density on eating behaviors where some papers reported no effect on eating behaviors (Buijs et al., 2010, 2011; Ventura et al., 2012) and others have reported that as stocking density increased, eating behavior decreased (Simitzis et al., 2012; van der Eijk et al., 2022). Based on the variable amount of feeders per pen, we cannot conclude that stocking density has an impact on the proportion of broiler chickens eating.
We also found that time of day had a significant impact on the behaviors of broiler chickens, with a higher proportion of broilers drinking and standing in the morning and a higher proportion of broilers sitting in the evening. While there are not many papers focused on the changes in specific behaviors of broiler chickens based on time of day, previous findings suggest that broiler chickens are more active closer to the time of lights-on in the morning than later in the day (Newberry et al., 1988; Norring et al., 2016).

2.5.1 Limitations

There were some limitations within the study design that may have impacted results. This study took place within a curtain-sided barn, and the pens were placed near the openings at the side of each barn in order to include feeders, and drinkers within each pen. During the duration of the study, thunderstorms occurred, and rain got into the barns. This could have potentially influenced the litter quality within the pens which may have been the reason that differences in footpad dermatitis were not observed. Also due to the construction of the pens, the broiler chickens were unable to leave this area of the barn to areas that may have been drier. However, litter quality was not measured, and therefore we are unable to determine if the weather impacted litter quality.

2.6 Conclusions

Overall, the earlier gait scores and stocking density can impact future gait scores, other welfare measures, and behaviors in broiler chickens. Broiler chickens originally classified as having affected gait tend to exhibit worsened scores for other welfare measures such as for hock burn and feather cleanliness. Lower stocking density is also associated with better gait scores, and scores for other welfare measurements as well as differences in behavior compared to higher stocking densities. While some welfare measures were impacted by stocking density, the present study found no link between stocking density and scores for hock burn and FPD. This may be due to the later time of implementation of stocking density changes and should therefore be studied further. However, these findings suggest that implementing reduced stocking density at the finisher phase can mitigate potential decreases in walking ability and can increase feather cleanliness of broiler chickens.
3.1 Abstract

Stocking density and space allowance are often considered one and the same. While they are related, they do not represent the same idea. Stocking density is the weight of broiler chickens per a set area of space measured as kg/m² while space allowance is the amount of space per broiler chicken measured as m²/bird. The stocking density of broiler chickens changes as broiler chickens gain weight while space allowance remains the same throughout the production cycle. While the amount of space per broiler chicken remains the same, the way they use that space may be different as they grow, and their body takes up more of the available space. This leads to a discussion of whether broiler chickens should be provided changes in space allowance as the grow for their welfare and to not limit behavior. The objectives of this study were to examine how the walking ability and active behaviors of broiler chickens change when given access to more space from 28 d onward. Mixed-sex broiler chickens (Ross 708) were randomly assigned to one of two treatment groups. The first treatment group was housed in pens that were 2.44 m x 1.5 m for the entirety of the study (SINGLE). The second treatment group was housed in the same sized SINGLE pens until 28 days of age when the pen size was doubled to 3.05 m x 2.44 m (DOUBLE). Welfare assessments consisting of scoring for gait, feather cleanliness, and for the presence of footpad dermatitis (FPD) and hock burn were conducted on the same ten focal chickens per pen at 22 (Period 1: Before Space Increase) and 28 (Period 2: Time of Space Increase) d of age prior to some of the pens increasing in size, and again at 38 d (Period 3: After Space Increase). Behavior data were collected through focal sampling on two birds in each pen (n=16/treatment) in the morning, afternoon, and evening of Periods 1, 2 and 3 to determine the duration and frequency of behavior (eating, drinking, sitting, standing, walking, running, preening, environmental pecking, aggressive pecking, threat, lateral lying, dust bathing, leg extension, sparring, frolicking, and ground scratching). Durations were averaged at each time point of collection for each individual bird and frequencies were calculated based on the number of times a broiler chicken performed a behavior. Welfare data were analyzed using PROC LOGISTIC (SAS 9.4) to calculate odds ratios. Focal sampling data were analyzed using PROC GLIMMIX. At 38 d, broiler chickens in SINGLE pens...
were less likely to have a score of 0 for FPD (Wald $\chi^2 = 15.45$, $P < 0.0001$), hock burn (Wald $\chi^2 = 7.26$, $P = 0.0071$), and feather cleanliness (Wald $\chi^2 = 11.77$, $P = 0.0006$) than broiler chickens in DOUBLE pens. However, broiler chickens in SINGLE pens were more likely to have a gait score of 0 compared to broiler chickens in DOUBLE pens (Wald $\chi^2 = 11.34$, $P = 0.0008$). Broiler chickens in the SINGLE pens spent more time eating compared to broilers in the DOUBLE pens ($P = 0.0255$). The frequency of sitting was higher in Period 2 than in Periods 1 (P=0.001) and 2 (P = 0.0002) which may be related to the decline in gait scores between periods. In conclusion, broiler chickens in DOUBLE pens had better welfare considering FPD, hock burn, and feather cleanliness but worse walking ability.

### 3.2 Introduction

As broiler chickens age, they rapidly gain weight which impacts their welfare. As broiler chickens approach the finisher phase, their body weight can increase by an average of 90-95 g per day (Aviagen, 2018). This rapid weight gain can lead to decreased activity levels, decreased walking ability, and a rapid decrease in space availability (Bradshaw et al., 2002; Hartcher & Lum, 2020; Knowles et al., 2008; Vaillancourt et al., 1999). Decreased activity levels are also associated with an increase in the presence of leg health issues such as lameness, which, in turn, can be related to an increase in the prevalence of hock burn (Haslam et al., 2007; Tahamtani et al., 2018). The relationships among rapid growth, age, walking ability, space availability, and how these impact activity levels are not fully understood, and are difficult to investigate because they are often interrelated and confounded in experimental studies.

Stocking density impacts the behavior of broiler chickens as increased stocking density is linked with broiler chickens spending more time sitting compared to spending time engaged in active behaviors such as walking (Knowles et al., 2008; Tahamtani et al., 2018). As stocking density increases, broiler chickens perform play (e.g. sparring and frolicking) and comfort (e.g. preening and dustbathing) behaviors less frequently (Baxter et al., 2019; Buijs et al., 2010; Hall, 2001; Hongchao et al., 2014; Liu et al., 2020; van der Eijk et al., 2022; Ventura et al., 2012). This association is thought to be due to there being a decreased amount of space available for broiler chickens to move around freely, therefore decreasing the prevalence of active behaviors (Reiter & Bessei, 2009; Tahamtani et al., 2018). Increased space availability was found to be associated with an increase in some space-consuming behaviors such as jumping and running; increased levels of
active behaviors (Vas et al., 2023). Prior research has not investigated changes in behavior where broilers are given more space in the finisher phase, and increasing the space allowance may elicit more active behaviors.

The relationships among stocking density, space availability, and walking ability are complex. The results from previous studies revealed that increased space improved walking ability and allowed for opportunities to perform more varied behavior (Vas et al., 2023). However, the prior studies on the influence of space have investigated the effects when space was provided at the time of chick placement (Vas et al., 2023); however, the average daily gain of broiler chickens increases more rapidly with age. Currently, there is a lack of information available about whether the provision of space in the finisher phase can be beneficial for walking ability and behavior. The objectives of this study were to examine how the walking ability and active behaviors of broiler chickens change when given access to more space from 28 d onward. We hypothesized that pens that were provided additional space in the finisher phase would have better welfare and would also perform more active behaviors such as playing and walking.

3.3 Methods

3.3.1 Animals and housing

All of the procedures used in this study were approved by the Purdue Animal Care and Use Committee (PACUC 2201002232). A total of 750 mixed-sex broiler chicks were transported to Purdue University’s Poultry Unit (Animal Sciences Research and Education Center (ASREC), West Lafayette, IN, USA) from a commercial hatchery. Once at ASREC, broiler chicks were housed in brood rings in littered (wood shavings) pens (measuring 2.44 m x 1.5 m, 8 ft long x 5 ft wide) for the first 7 days. Thereafter, broilers were randomly assigned to 1 of 16 pens that each housed 46-47 broilers. At 28 days of age, half of the pens doubled in size (DOUBLE, 3.05 m x 2.44 m, 8 ft long x 10 feet wide), while the other half maintained the original dimensions (SINGLE). The DOUBLE pens had an expected stocking density of 15.2 – 15.5 kg/m² (3.11 – 3.17 lbs/ft²) and a space availability of 0.15 to 0.16 m²/ bird while the SINGLE pens had an expected stocking density of 30.4 - 31.1 kg/m² (6.23-6.37 lbs/ft²) and a space availability of 0.07-0.08 m²/bird. Stocking densities were based on the estimations of expected growth provided by Aviagen® (Aviagen, 2022).
All pens had one hanging feeder that provided *ad libitum* standard commercial feed and one bell drinker that provided water *ad libitum*. The photoperiod during the brooding period was 23 h of light and 1 h of darkness. After the brooding period, the photoperiod was changed to 18 h of light and 6 h of darkness for the remainder of the study. In each pen, ten focal birds were randomly selected to be leg banded for individual identification (Plastic bandettes, National Band & Tag Company, Kentucky, USA), and their feathers were color-coded using livestock markers (Ideal®, Lansing, MI). One focal bird in each pen wore an accelerometer (Technosmart, Rome, Italy) on their back, attached via a backpack (accelerometer data will not be presented here).

### 3.3.2 Welfare Assessments

Welfare assessments were conducted on the same ten focal chickens per pen at 22 (Period 1: Before Space Increase) and 28 d of age (Period 2: Time of Space Increase) prior to some of the pens increasing in size, and again at 38 d (Period 3: After Space Increase). Welfare assessments consisted of the scoring of gait (based on the scale by Kestin et al., (1992)) and assessments for footpad dermatitis, hock burn, and feather cleanliness (assessed based on the Welfare Quality Assessment Protocol for Poultry, 2009) by the same trained observers. Prior to the start of data collection, inter- and intra-observer reliability were assessed for gait scoring. Reliability was assessed through a series of videos depicting a range of broiler gait scores where the observer scored the broiler in each video. This assessment was completed a week before the study began and one day before the study began to analyze inter- and intra-observer reliability. In addition, latency to lie and tests of fearfulness (novel object and stationary person tests) were conducted at 22, 28, 30 and 38 days of age; data pertaining to latency to lie and fearfulness will be presented elsewhere.

### 3.3.3 Behavioral observation

**Video recording**

Video was recorded using overhead dome video cameras (Amcrest motorized varifocal 2MP outdoor dome camera – AF-2MVD-VARIB/W, Amcrest Industries LLC, Texas, USA) that were suspended from the ceiling. The single pens had one camera installed at the center of the pen
at a height of approximately 2.4 m (8 ft). The double pens had two cameras suspended from the ceiling with each half of the pen visible on one of the two cameras. Video was recorded onto a DVR (Amcrest 16 channel video security recorder – AMDV108116-H5, Amcrest Industries LLC, Texas, USA) and analyzed for 26 d (Period 1: before space increase), 28 d (Period 2: time of space increase), and 39 d (Period 3: after space increase) to examine the influence of increased space.

Behavior was annotated using focal sampling by a trained observer to determine the duration and frequency of each behavior (Table 3.1). For each day, behavior of two birds in each pen was observed continuously for 10 min at each of: 1) lights-on and 1 h after lights-on (morning); 2) at 7 and 8 h after lights-on, corresponding to the time of day at which pen size doubled in the DOUBLE group, and 1 h after the doubling of space (afternoon); and 3) at 12 and 13 h after lights-on (evening). One of the birds was a marked focal bird wearing an accelerometer. The other bird was unmarked and was randomly selected at each time point. To randomly select the unmarked bird, each pen was divided into six numbered areas of equal size, and a pen location was chosen using a random number generator (RANDOM.org). Based on the number of broiler chickens within the area, another random number was chosen to determine which broiler chicken would be observed. If no broiler chickens were present in that area, another random number was generated, and the process was repeated until a bird was selected. Both marked and unmarked birds were selected for observation to ensure there was no difference in behavior based on coloration.
**Table 3.1. Ethogram of broiler chicken behavior**

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eating</strong></td>
<td>Bird is in front of feeder with head extending into feed (Branco et al., 2020)</td>
</tr>
<tr>
<td><strong>Drinking</strong></td>
<td>Bird is by drinker ingesting water (Branco et al., 2020)</td>
</tr>
<tr>
<td><strong>Sitting</strong></td>
<td>Bird is lying in the litter while the head is resting on ground or erect (Baxter et al., 2019)</td>
</tr>
<tr>
<td><strong>Standing</strong></td>
<td>Bird is standing on both legs with no movement (Shynkaruk et al., 2023)</td>
</tr>
<tr>
<td><strong>Walking</strong></td>
<td>The bird moving at a slow pace (Branco et al., 2020)</td>
</tr>
<tr>
<td><strong>Running</strong></td>
<td>The bird is moving at a fast pace (at least 3 steps quicker than walking) (Branco et al., 2020; Vas et al., 2023)</td>
</tr>
<tr>
<td><strong>Preening</strong></td>
<td>The bird is cleaning and aligning feathers with beak (Baxter et al., 2019; Jacobs et al., 2019)</td>
</tr>
<tr>
<td><strong>Environmental Pecking</strong></td>
<td>The bird is pecking at an object in its environment (Shynkaruk et al., 2023)</td>
</tr>
<tr>
<td><strong>Aggressive Pecking</strong></td>
<td>The bird is aggressively pecking any part of the body of another bird (Bailie et al., 2013)</td>
</tr>
<tr>
<td><strong>Threat</strong></td>
<td>One bird is standing before another, stretching its neck, ruffling its feathers, spreading both wings, and looking at the other bird downwards (Bailie et al., 2013)</td>
</tr>
<tr>
<td><strong>Lateral Lying</strong></td>
<td>The bird is lying laterally with a stretched leg (Branco et al., 2020)</td>
</tr>
<tr>
<td><strong>Dust Bathing</strong></td>
<td>The bird is bathing in the dust with the use of wings, head, neck, and legs; using beak to bring substrate closer to them and then performing head rubbing, vertical wing shakes and leg scratching (Baxter et al., 2019)</td>
</tr>
<tr>
<td><strong>Leg Extension</strong></td>
<td>The bird is stretching one wing and one leg of the same body hemisphere while standing (Pichova et al., 2016)</td>
</tr>
<tr>
<td><strong>Sparring</strong></td>
<td>Bird is simulating fighting behaviors but with no obvious aggression and does not lead to injury (Baxter et al., 2019)</td>
</tr>
<tr>
<td><strong>Frolicking</strong></td>
<td>Bird is spontaneously running, jumping, or wing-flapping with no obvious intention, often with rapid directional changes (Baxter et al., 2019)</td>
</tr>
<tr>
<td><strong>Ground Scratching</strong></td>
<td>Bird is raking substrate with feet, usually kicking motion with alternating feet used (Vas et al., 2023)</td>
</tr>
<tr>
<td><strong>Unknown</strong></td>
<td>Cannot see the birds head</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>Bird is performing a behavior different from those listed above (Bailie et al., 2013; Weeks et al., 2000)</td>
</tr>
</tbody>
</table>

### 3.3.4 Statistical analyses

All data analyses were completed using SAS 9.4 (Cary, N.C.). Welfare assessment data were analyzed using PROC LOGISTIC to calculate odds ratios using methods similar to Dong et al (2021).
For behavior data, the duration of each behavior was averaged across the two hours for each time point so that there was an average duration for each of the morning, afternoon, and evening for each chicken. Duration data were then analyzed with a repeated measures analysis using PROC GLIMMIX. The model included period, pen number, treatment, bird id, time of day, and all interactions among treatment, time of day, and period. If interactions were not significant, they were removed from the model. Random factors included bird id nested within pen. Normality was assessed using studentized residual plots. The durations of sitting, eating, environmental pecking, preening, standing, and walking were not normally distributed and were analyzed using a lognormal distribution and were then back-transformed.

The frequency with which broilers performed each behavior within each 10-min time point was analyzed with a repeated measures analysis using PROC GLIMMIX. The model included period, pen number, treatment, bird id, time of day, time point within time of day (the first hour of the time of day or the second), and all possible interactions between treatment, time of day, and period. Bird id nested within pen was included as a random effect and time point nested within time of day was included as a repeated measure. Random factors were removed for those behaviors where the G matrix was not positive definite (Kiernan et al., 2012). All frequencies of behaviors were analyzed using a Poisson distribution.

Tukey’s test (Tukey, 1949) was used for all post-hoc pairwise comparisons. Only post-hoc analyses of meaningful interaction comparisons are presented here; for example, broiler chickens in the DOUBLE pen in Period 2 will not be compared to broiler chickens in SINGLE pens in Period 3. Whether or not birds wore an accelerometer was included in the model; however, no differences were found between birds wearing an accelerometer and birds without an accelerometer. Therefore, data were pooled across birds.

3.4 Results

3.4.1 Welfare Results

The frequencies of all scores for all welfare measures for each age and treatment group are presented in Table 3.2
Table 3.2. Percentage of broiler chickens with each score for welfare parameters in Period 1: Before Space Increase (22 days of age), Period 2: Time of Space Increase (28 days of age), and Period 3: After Space Increase (38 days of age) in each treatment group (SINGLE: pens that remained 2.44 m x 1.5 m throughout the study and DOUBLE: pens that begun as 2.44 m x 1.5 m and after welfare assessments conducted on 28 d were doubled to 3.05 m x 2.44 m)

<table>
<thead>
<tr>
<th>Welfare Parameter</th>
<th>Treatment Group</th>
<th>Single</th>
<th>Double</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Score</td>
<td>P1</td>
<td>P2</td>
</tr>
<tr>
<td>Gait</td>
<td>0</td>
<td>50.00</td>
<td>8.75</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>50.00</td>
<td>81.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.00</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Footpad</td>
<td>0</td>
<td>97.50</td>
<td>88.75</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.25</td>
<td>7.50</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.25</td>
<td>3.75</td>
</tr>
<tr>
<td>Hock</td>
<td>0</td>
<td>98.75</td>
<td>93.75</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.25</td>
<td>6.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Feather Cleanliness</td>
<td>0</td>
<td>98.75</td>
<td>86.25</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.25</td>
<td>13.75</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Period related differences in welfare measures

Period had a significant impact on gait scores (Wald $\chi^2 = 231.64$, $P < 0.0001$), the presence of footpad dermatitis (Wald $\chi^2 = 29.81$, $P < 0.0001$), hock burn (Wald $\chi^2 = 51.51$, $P < 0.0001$), and feather cleanliness (Wald $\chi^2 = 74.63$, $P < 0.0001$). All comparisons between Periods 1, 2, and 3 were significant. The likelihood of broiler chickens having scores of 0 for gait footpad, hock, and feather cleanliness were higher in Period 1 than Periods 2 and 3 (Table 3.3). Broiler chickens were more likely to have a score of 0 for gait, footpad, hock, and feather cleanliness in Period 2 than Period 3.
Table 3.3. Odds ratios for the likelihood of broiler chickens having a score of 0 for each welfare measure (gait, the presence of FPD, the presence of hock burn, and feather cleanliness) comparing Period 1: Before Space Increase (22 days of age), Period 2: Time of Space Increase (28 days of age), and Period 3: After Space Increase (38 days of age)

<table>
<thead>
<tr>
<th>Welfare Measure</th>
<th>Period Comparison</th>
<th>Odds Ratio Estimate</th>
<th>95% CI Lower Limit</th>
<th>95% CI Upper Limit</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gait</td>
<td>Period 1 vs Period 2</td>
<td>12.90</td>
<td>7.16</td>
<td>23.24</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Period 1 vs Period 3</td>
<td>329.27</td>
<td>155.22</td>
<td>698.46</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Period 2 vs Period 3</td>
<td>25.53</td>
<td>14.35</td>
<td>45.41</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Footpad</td>
<td>Period 1 vs Period 2</td>
<td>8.96</td>
<td>2.017</td>
<td>39.77</td>
<td>0.0039</td>
</tr>
<tr>
<td></td>
<td>Period 1 vs Period 3</td>
<td>29.11</td>
<td>6.87</td>
<td>123.38</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>Period 2 vs Period 3</td>
<td>3.25</td>
<td>1.72</td>
<td>6.16</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Hock</td>
<td>Period 1 vs Period 2</td>
<td>15.30</td>
<td>1.99</td>
<td>117.93</td>
<td>0.0088</td>
</tr>
<tr>
<td></td>
<td>Period 1 vs Period 3</td>
<td>104.21</td>
<td>14.20</td>
<td>764.88</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Period 2 vs Period 3</td>
<td>6.81</td>
<td>3.69</td>
<td>12.89</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Feather Cleanliness</td>
<td>Period 1 vs Period 2</td>
<td>2.16</td>
<td>1.04</td>
<td>4.50</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Period 1 vs Period 3</td>
<td>12.15</td>
<td>6.24</td>
<td>23.66</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Period 2 vs Period 3</td>
<td>5.63</td>
<td>3.30</td>
<td>9.61</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Treatment-related differences in welfare measures

Results from Period 1 were not included in treatment comparisons as there was low variability among scores. In Period 2, treatment group did not have an impact on the odds of having a score of 0 for gait, footpad, hock, and feather cleanliness (Table 3.4). This indicates that prior to beginning the treatments, broilers in the two treatment groups were not significantly different. In Period 3, broiler chickens in SINGLE pens were less likely to have a score of 0 for footpad (Wald $\chi^2 = 15.45$, $P < 0.0001$), hock (Wald $\chi^2 = 7.26$, $P = 0.0071$), and feather cleanliness (Wald $\chi^2 = 11.77$, $P = 0.0006$) than broiler chickens in DOUBLE pens. However, broiler chickens in SINGLE pens were more likely to have a gait score of 0 compared to broiler chickens in DOUBLE pens (Wald $\chi^2 = 11.34$, $P = 0.0008$).
Table 3.4. Odds ratios for the likelihood of broiler chickens having a score of 0 for each welfare measure (gait, the presence of FPD, the presence of hock burn, and feather cleanliness) comparing broilers housed in SINGLE pens (pens that remained 2.44 m x 1.5 m throughout the study) with broilers housed in DOUBLE pens (pens that begun as 2.44 m x 1.5 m and after welfare assessments conducted on 28 d were doubled to 3.05 m x 2.44 m) in Period 2: Time of Space Increase (28 days of age) and Period 3: After Space Increase (38 days of age)

<table>
<thead>
<tr>
<th>Welfare Measure: SINGLE vs. DOUBLE</th>
<th>Period</th>
<th>Odds Ratio Estimate</th>
<th>95% CI Lower Limit</th>
<th>95% CI Upper Limit</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gait</td>
<td>Period 2</td>
<td>1.14</td>
<td>0.79</td>
<td>1.65</td>
<td>0.4921</td>
</tr>
<tr>
<td></td>
<td>Period 3</td>
<td>1.68</td>
<td>1.24</td>
<td>2.28</td>
<td>0.0008</td>
</tr>
<tr>
<td>Footpad</td>
<td>Period 2</td>
<td>0.78</td>
<td>0.28</td>
<td>2.20</td>
<td>0.6340</td>
</tr>
<tr>
<td></td>
<td>Period 3</td>
<td>0.19</td>
<td>0.09</td>
<td>0.44</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Hock</td>
<td>Period 2</td>
<td>1.90</td>
<td>0.61</td>
<td>5.95</td>
<td>0.2694</td>
</tr>
<tr>
<td></td>
<td>Period 3</td>
<td>0.41</td>
<td>0.213</td>
<td>0.78</td>
<td>0.0071</td>
</tr>
<tr>
<td>Feather Cleanliness</td>
<td>Period 2</td>
<td>1.22</td>
<td>0.51</td>
<td>2.91</td>
<td>0.6582</td>
</tr>
<tr>
<td></td>
<td>Period 3</td>
<td>0.34</td>
<td>0.18</td>
<td>0.63</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

3.4.2 Behavior Results

The interaction of treatment and time of day, and the interaction of period, treatment, and time of day did not influence any behavior examined.

Eating

The frequency of broiler chickens eating was impacted by time of day ($F_{2,463} = 16.19$, $P < 0.0001$, Figure 3.1) and the interaction of treatment and period ($F_{2,463} = 16.19$, $P = 0.0078$, Figure 3.2). The frequency of eating was higher in the morning than in both the afternoon and evening ($P < 0.0001$). The frequency of eating was lower in DOUBLE pens in Period 2 than in Period 1 ($P < 0.0001$) and Period 3 ($P = 0.0097$) and SINGLE pens in Period 2 ($P = 0.044$).
Figure 3.1. Frequency of eating at each time of day (lsmeans ± SE). \(^a\)\(^b\) Least square means without a common superscript differ due to time of day (P < 0.05).

Figure 3.2. Frequency of eating (lsmeans ± SE) in each Period based on treatment groups comparing broilers housed in SINGLE pens (pens that remained 2.44 m x 1.5 m throughout the study) with broilers housed in DOUBLE pens (pens that begun as 2.44 m x 1.5 m and after welfare assessments conducted on 28 d were doubled to 3.05 m x 2.44 m). Behaviors were observed in Period 1: Before Space Increase, Period 2: Time of half of the pens being increased in size, Period 3: After Space Increase. \(^a\)\(^c\) Least squares means without a common superscript differ among Periods within the same treatment (P < 0.05).
The duration of eating was impacted by treatment ($F_{1,164} = 6.05, \ P = 0.0149$, Figure 3.3) and the interaction of time of day and Period ($F_{4,164} = 5.14, \ P = 0.0006$, Figure 3.4). The duration of eating was longer in SINGLE pens than in DOUBLE pens ($P = 0.0255$). The duration of eating was longer in the afternoon of Period 2 than in the morning ($P = 0.0002$) and evening of Period 2 ($P = 0.0033$).

![Figure 3.3](image.png)

Figure 3.3. Duration of eating (back transformed means ± SE) based on treatment groups comparing broilers housed in SINGLE pens (pens that remained 2.44 m x 1.5 m throughout the study) with broilers housed in DOUBLE pens (pens that began as 2.44 m x 1.5 m and after welfare assessments conducted on 28 d were doubled to 3.05 m x 2.44 m). a-b Back transformed means without a common superscript differ due to treatment group ($P < 0.05$).
Figure 3.4. Duration of eating (back transformed means ± SE) at each time of day and Period. Behaviors were observed in Period 1: Before Space Increase, Period 2: Time of half of the pens being increased in size, Period 3: After Space Increase. Back transformed means without a common superscript differ among times of days within the same Period (P < 0.05).

Sitting

The frequency of sitting was impacted by Period ($F_{2,212} = 9.46, P = 0.0001$, Figure 3.5) and time of day ($F_{2,212} = 17.81, P < 0.0001$, Figure 3.6). The frequency of sitting was lowest in Period 2 than Periods 1 ($P = 0.001$) and 3 ($P = 0.0002$). The frequency of sitting was higher in the morning than both the afternoon and evening ($P < 0.0001$).
Figure 3.5. Frequency of sitting within each Period (lsmeans ± SE). Behaviors were observed in Period 1: Before Space Increase, Period 2: Time of half of the pens being increased in size, Period 3: After Space Increase. a–b Least squares means without a common superscript differ due to Period (P < 0.05).

Figure 3.6. Frequency of sitting at each time of day (lsmeans ± SE). a–b Least squares means without a common superscript differ due to time of day (P < 0.05).
The duration of sitting was impacted by the interaction of time of day and period ($F_{4,326} = 3.16, \ P = 0.0144$, Figure 3.7). The duration of sitting was shorter in the morning of Period 3 than the morning of Period 2 ($P = 0.0022$) and the evening of Period 3 ($P < 0.0001$).

Figure 3.7. Duration of sitting (back transformed means ± SE) within each time of day and Period. Behaviors were observed in Period 1: Before Space Increase, Period 2: Time of half of the pens being increased in size, Period 3: After Space Increase. Back transformed means without a common superscript differ among times of days within the same Period ($P < 0.05$).

### Walking

The frequency of walking was impacted by time of day ($F_{2,212} = 29.15, \ P < 0.0001$, Figure 3.8) and the interaction of treatment and Period ($F_{2,212} = 3.24, \ P < 0.0001$, Figure 3.9). The frequency of walking was higher in the morning than in both the afternoon and evening. The frequency of walking was higher in DOUBLE pens in Period 1 than in DOUBLE pens in Period 2 ($P = 0.0107$).
Figure 3.8. Frequency of walking at each time of day (lsmeans ± SE). a–b Least squares means without a common superscript differ due to time of day (P < 0.05).

Figure 3.9. Frequency of walking (lsmeans ± SE) within each Period based on treatment groups comparing broilers housed in SINGLE pens (pens that remained 2.44 m x 1.5 m throughout the study) with broilers housed in DOUBLE pens (pens that begun as 2.44 m x 1.5 m and after welfare assessments conducted on 28 d were doubled to 3.05 m x 2.44 m). Behaviors were observed in Period 1: Before Space Increase, Period 2: Time of half of the pens being increased in size, Period 3: After Space Increase. a–b Least squares, means without a common superscript differ among Periods within the same treatment (P < 0.05).
The duration of walking was impacted by the interaction of time of day and Period ($F_{2,274} = 4.19$, $P = 0.0026$, Figure 3.10). The duration of walking is significantly longer in the afternoon of Period 2 than in the morning of Period 2 ($P = 0.017$) and in the afternoon of Period 1 ($P < 0.0001$).

![Figure 3.10](image)

Figure 3.10. Duration of walking (back transformed means ± SE) within each time of day and Period. Behaviors were observed in Period 1: Before Space Increase, Period 2: Time of half of the pens being increased in size, Period 3: After Space Increase. $^a,b,c$ Back transformed means without a common superscript differ among times of days within the same Period ($P < 0.05$).

**Preening**

Time of day had a significant impact of the frequency of preening ($F_{2,212} = 6.04$, $P = 0.0028$, Figure 3.11). The frequency of preening was higher in the morning than in the evening ($P = 0.0018$).
Figure 3.11. Frequency of preening at each time of day (lsmeans ± SE). a–b Least squares means without a common superscript differ due to time of day (P < 0.05).

The duration of preening was significantly influenced by time of day ($F_{2,217} = 4.61$, $P = 0.0109$, Figure 3.12) and the interaction of treatment and Period ($F_{2,217} = 4.09$, $P = 0.0181$, Figure 3.13). The duration of preening was longer in the afternoon ($P < 0.0001$) and evening ($P < 0.0001$) than in the morning. The duration of preening was longer in DOUBLE pens in Period 3 than in Period 1 ($P < 0.0001$) and Period 2 ($P < 0.0001$).
Figure 3.12. Duration of preening (back transformed means ± SE) occurring at each time of day. \(^{a,b}\) Back transformed means without a common superscript differ due to time of day (P < 0.05).

Figure 3.13. Duration of preening (back transformed means ± SE) within each Period and treatment group comparing broilers housed in SINGLE pens (pens that remained 2.44 m x 1.5 m throughout the study) with broilers housed in DOUBLE pens (pens that begun as 2.44 m x 1.5 m and after welfare assessments conducted on 28 d were doubled to 3.05 m x 2.44 m). Behaviors were observed in Period 1: Before Space Increase, Period 2: Time of half of the pens being increased in size, Period 3: After Space Increase. \(^{a,b}\) Back transformed means without a common superscript differ due among Periods within the same treatment (P < 0.05).
*Leg Extensions*

The frequency of leg extensions was influenced by treatment ($F_{1,212} = 4.01$, $P = 0.0465$, Figure 3.14), which were higher in DOUBLE pens than in SINGLE pens ($P = 0.0465$). The interaction between time of day and Period also influenced the frequency of leg extensions ($F_{4,212} = 3.32$, $P = 0.0115$, Figure 3.15). The frequency of leg extensions was higher in the evening of Period 1 than in the morning of Period 1 ($P = 0.0460$).

![Figure 3.14](image)

Figure 3.14. Frequency of leg extension (lsmeans ± SE) of broilers housed in SINGLE pens (pens that remained 2.44 m x 1.5 m throughout the study) and broilers housed in DOUBLE pens (pens that began as 2.44 m x 1.5 m and were doubled to 3.05 m x 2.44 m at 28 d). "a"b Least squares means without a common superscript differ due to treatment group ($P < 0.05$).
Figure 3.15. Frequency of leg extension at each time of day and Period (lsmeans ± SE). Behaviors were observed in Period 1: Before Space Increase, Period 2: Time of half of the pens being increased in size, Period 3: After Space Increase. a-b Least Square means without a common superscript differ among times of day within the same Period (P < 0.05).

**Environmental Pecking**

Period significantly impacted the duration of environmental pecking (F$_{2,74}$ = 4.12, P = 0.02, Figure 3.16) with more broiler chickens environmental pecking in Period 2 when compared to Period 1 (P < 0.001).
Figure 3.16. Duration of environmental pecking within each Period (back transformed means ± SE). Behaviors were observed in Period 1: Before Space Increase, Period 2: Time of half of the pens being increased in size, Period 3: After Space Increase. Back transformed means without a common superscript differ due to Period (P < 0.05).

Standing

The duration of standing was significantly impacted by time of day ($F_{2,44} = 3.79$, $P = 0.0304$, Figure 3.17). The duration of standing was longer in the afternoon than in the evening ($P < 0.0001$).

Figure 3.17. Duration of standing (back transformed means ± SE) occurring at each time of day. Back transformed means without a common superscript differ due to time of day ($P < 0.05$).
3.5 Discussion

In this study, we increased the space available for broiler chickens to examine 1) how welfare measures changed for broilers housed in pens that doubled in size during the finisher phase and broilers that were housed in pens that remained the same size throughout, and 2) how differences in space availability influenced the behavior of broiler chickens. Overall, our results indicated that broiler chickens housed in pens that doubled in size during the finisher phase had worse gait but were more likely to have improved footpad, hock burn and feather cleanliness scores.

We hypothesized that broiler chickens that were provided increased space in the finisher phase would have better welfare and would perform more active behaviors than those that remained in the same size pens. These results support some aspects of our hypothesis as broiler chickens that were provided additional space in the finisher phase did have better welfare when considering scores for footpads, hock, and feather cleanliness. However, broiler chickens provided additional space did not have better walking ability. There was also no difference between treatment groups in the duration or frequency of broiler chickens sitting or performing active behaviors such as walking.

3.5.1 Welfare

In Period 2, there were no differences in the gait scores of broiler chickens due to treatment; however, by Period 3, the odds of having a gait score of 0 were higher for broiler chickens housed in SINGLE pens than broilers housed in DOUBLE pens. This indicates that increasing the space in the finisher phase may have had a negative effect on the walking ability of broiler chickens. It is also possible that the additional space influenced the observations of gait such that birds in the DOUBLE pens were scored differently than birds in the SINGLE pens due to the limited space available for observation in the SINGLE pens. This is an important consideration that should be investigated in future studies.

The scores for footpads, hocks, and feather cleanliness in Period 2 were not significantly different based on treatment; however, by Period 3 broiler chickens housed in DOUBLE pens were more likely to have a score of 0 than broilers housed in SINGLE pens. This indicates that providing additional space in the finisher phase may have beneficial effects for the welfare measurements of
footpads, hocks, and feather cleanliness. However, prior research has found a relationship between gait and the presence of hock burn where the prevalence of hock burn was higher for broilers with worse gait, which may have been due to broilers with worse gait spending more time sitting and therefore having more contact with the litter (Haslam et al., 2007; Tahamtani et al., 2018). The results from the current study are contradictory to the findings of previous studies as broiler chickens in the DOUBLE pen were more likely to have better scores for hock, but also more likely to have reduced gait. This may be due to litter quality as the new space provided to the DOUBLE pens included new litter while SINGLE pens did not receive new litter. The results could also be due to there being more space available to score gait in DOUBLE pens compared to SINGLE pens resulting in potential differences in gait scoring among observers (all observers were trained to score gait and had a reliability of 0.56 for scoring gait).

3.5.2 Behavior

Broiler chickens housed in the pens that had the provision of space in the finisher phase were eating for shorter durations than those housed in pens that remained the same size. Prior research has not analyzed the relationship between space availability and the duration of eating; however, the results from the current study indicate that increased space availability is associated with a decrease in the duration of eating. The duration of eating may be associated with the gait scores of broiler chickens within each treatment group rather than solely the difference in space. Prior research found that broiler chickens with affected gait were more likely to perform abnormal eating behaviors, such as eating while sitting (Riber et al., 2021; Weeks et al., 2000). The feeders in the pens for this study were not low enough to the ground for broiler chickens to be sitting while feeding, and broilers were even observed to sit between bouts of eating in the current study. As the gait scores were worse for broilers housed in pens that had the provision of space, and broilers were unable to eat while sitting, this may be why we observed reduced durations for eating in broilers with increased space allowance.

The frequency of eating was impacted by the interaction between period and treatment. The frequency of eating was higher in SINGLE pens in Period 2, but there were no differences between treatments at other periods. The relationship may be due to the timing of the observations as this was the Period where the additional space was provided to some pens. It is possible that
broiler chickens in the pens that were provided with additional space were making less frequent trips to the feeder to eat due to there being a novel environment.

Period had an impact on the frequency and duration of sitting with the frequency of sitting being lowest in Period 2 where the duration was the highest. Period 2 occurred at 28 days of age which is when broiler chicken gait has previously been observed to begin to decline based on preliminary data. The decline in walking ability that occurred between Period 1 and Period 2 welfare assessments could also be related to the increased duration of sitting as prior research has reported increased durations of sitting as gait worsens (Riber et al., 2021; Weeks et al., 2000). However, these results are not consistent with the findings when comparing Periods 2 and 3. While gait continued to decline between Periods 2 and 3, the duration of sitting also declined. These results indicate that the changes in duration of sitting based on the decline of gait may reflect only the initial decline in gait scores. It is possible that as broiler chickens continue to habituate to the worsened gait, their behavior continues to change and this should be investigated further.

The interaction of period and treatment affected the frequency of walking. The frequency of walking was higher in DOUBLE pens in Period 1 than Period 2 which may be related to the decline in gait between Period 1 and 2. Broiler chickens with affected gait are more likely to spend longer amounts of time sitting that broilers with sound gait (Riber et al., 2021) and are therefore less likely to perform other behaviors such as walking.

The duration of environmental pecking was impacted by Period, where duration increased between Periods 1 and 2. The increase in environmental pecking in Period 2 could be related to the provision of space for broiler chickens. While the broiler chickens in DOUBLE pens were provided with a new area to explore, SINGLE pens were also exposed to new things. The SINGLE pens may have had new birds in the adjacent pen resulting in the increase of environmental pecking as it is an exploratory behavior (Shynkaruk et al., 2022). This may also explain why there is not difference between Period 3 and the other periods, as the provided spaces are no longer novel.

The duration of preening was impacted by the interaction of Period and treatment where the highest duration of preening was for DOUBLE pens in Period 3 when feather cleanliness was at its lowest. These results indicate that as broiler chickens’ feather cleanliness decreases, they spend more time preening.
3.5.3 Limitations

There were limitations with the design of the study that may have impacted the results. When the additional space was provided to the selected pens, new litter was also available in the new areas. However, new litter was not provided to the pens that did not receive additional space. It is possible that the availability of unused litter may account for the differences in welfare measures as litter quality is associated with the prevalence of hock burn and footpad dermatitis (Haslam et al., 2006; Shepherd & Fairchild, 2010). Another limitation is that the weight and feed intake of broiler chickens were not measured in this study. The broiler chickens in the DOUBLE pens may have been consuming more feed leading to increased body weight. Increased body weight is associated with worse gait, and therefore a difference in feed intake and body weight gain may be the reason for worse gait in the DOUBLE pens.

3.6 Conclusions

Overall, the results indicated that the provision of space in finisher phase may be beneficial for footpads, hocks, and feather cleanliness. However, results also indicate that the provision of space may be detrimental to the walking ability of broiler chickens. Broiler chickens provided with additional space performed leg extensions more often and ate for shorter durations which may be related to their decreased walking ability.
CHAPTER 4. CONCLUSIONS

The overarching goal of this dissertation was to examine the interactive effects of space during the finisher phase on the behavior and welfare measures of commercial broiler chickens. Specific objectives included investigating the consistency of gait scores as broilers age and investigating how stocking density influences broiler chicken welfare and behavior through manipulating space availability in the finisher phase.

In Chapter 2, welfare assessments consisting of an assessment of gait, footpad condition, hock condition, and feather cleanliness were evaluated and compared based on gait scores at the initial welfare assessment (33 days of age) and stocking density. Broilers initially classified as having affected gait exhibited worse scores for all other welfare measures by 37 days of age, indicating that affected gait contributes to the development of other welfare concerns such as footpad dermatitis (FPD) and hock burn. There was a higher proportion of broiler chickens with affected gait sitting than those with sound gait, indicating that as broiler chicken walking ability decreases, the proportion of broilers performing inactive behaviors increases.

Also in Chapter 2, broiler chickens with mixed gait housed at different stocking densities were compared based on welfare assessments. Broiler chickens housed at the reduced stocking density had better scores for gait and other welfare measures, indicating that implementing changes in stocking density at a later age may have a beneficial effect on welfare measures. It is possible that implementing a reduced stocking density at the end of the broiler chicken production cycle may mitigate the potential concerns with the welfare of broiler chickens. The reduced stocking density treatment group also had a higher proportion of broiler chickens standing, which may be related to the differences in walking ability among treatments.

In Chapter 3, broiler chickens were provided with an increase in space during the finisher phase. The results for this study suggest that the provision of space in the finisher phase for broiler chickens may be beneficial for footpad condition, hock condition, and feather cleanliness; however, there may be detrimental effects to the walking ability of broiler chickens. The provision of space also led to a decrease in eating duration and an increase in the frequency of leg extensions which may be related to the reduced walking ability in pens that were provided with increased space. This indicates that the provision of space may lead to decreases in walking ability that will lead to changes in behavior as well overall reducing welfare.
Further research is still needed to fully understand the impacts of the provision of space during the finisher phase. While the first study indicated that having more space available per broiler chicken led to better walking ability, the second study showed the opposite to be true as those with more space had reduced walking ability. Between the two studies, there are differences that may have led to the difference in results. While the broiler chickens in Chapter 2 were given more space per broiler chicken, overall, they had a smaller area to move around in once they were placed in the pens. As this study took place in a commercial barn where broilers were free to move throughout the whole barn prior to the implementation of pens, the pens limited their options on how far they could travel. Whereas Chapter 3 took place in an experimental facility where pen sizes were more restrictive from the beginning. The broiler chickens in the commercial facility (Chapter 2) may have been accustomed to traveling greater distances making the change in space availability not as intense as it was for those in Chapter 3.

Another possible explanation to the differences in results is that there may have been differences in growth rates between studies. The weight of the broiler chickens was not investigated in either study, and it is possible that growth rates were different between studies. This is a limitation in study design as if there were differences in growth rate based on stocking density in either study, that may be the reason for the differences in walking ability and not the provision of space. While both studies resulted in other welfare measures being better with changes in stocking density and space availability, there is still more research to be conducted and it is important that future research include growth rate measures to ensure differences are due to stocking density. Prior to any implementation of space changes in commercial facilities, there should be a better understanding of the effects of the provision of space on broiler chicken welfare such as how much space should be given to ensure there is room for movement, but not enough for detrimental impacts on welfare.
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