ABSTRACT

Title of Thesis: **EFFECTS OF BARRIER PERCHES AND STOCKING DENSITY ON THE BEHAVIOR, SPACE USE, AND LEG HEALTH OF THE DOMESTIC FOWL (*GALLUS GALLUS DOMESTICUS*)**

Beth Ventura, Master of Science, 2009

Thesis Directed By: **Professor, Dr. Inmaculada Estevez, Department of Animal and Avian Sciences**

The objective of this study was to discern whether providing enrichment in the form of barrier perches across a range of densities might improve leg and foot health and promote behavioral expression and more even use of space in broilers. To investigate this, 2,088 day-old broiler chicks were randomly assigned to one of three barrier treatments at one of three densities. Effects on behavior, space use, foot and hock health, tibia fluctuating asymmetry, fear and production were subsequently assessed. Higher densities appeared to compromise broiler welfare, seen by increased tibia length asymmetry, poorer foot and hock health, suppression of activity, increased disturbances, and decreased use of space. Conversely, barrier perches – particularly simple barriers – appeared to improve footpad quality, promote increased perching and activity, decrease aggression and disturbances, and improve use of the central pen space, all without negatively impacting production traits.

EFFECTS OF BARRIER PERCHES AND STOCKING DENSITY ON THE BEHAVIOR, SPACE USE, AND LEG HEALTH OF THE DOMESTIC FOWL (*GALLUS GALLUS DOMESTICUS*)

By

Beth Ann Ventura

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2009

Advisory Committee:
Dr. Inmaculada Estevez, Chair
Dr. Frank Siewerdt
Dr. W. Ray Stricklin

ACKNOWLEDGEMENTS

Dr. Inma Estevez, thank you for taking me on as your student, for your sage guidance and mentoring, and for your endless efforts to help me discover my potential.

My parents, thank you for your unconditional faith in me. Your love and support have always been major factors in my success.

Adam, thank you for being by my side, through everything.

Dr. Frank Siewerdt, thank you for everything you've done as my committee member, and for your endless assistance in all things statistical. You are a wonderful teacher.

Dr W.R. Stricklin, thank you for serving on my committee and for sharing your boundless knowledge of ethology. Most of all, thank you for always appreciating that welfare is the heart of the matter.

Mark Spicknall, thank you for *always* seeing to everything I needed at the farm. It was a pleasure to work with you. I literally could not have done it without you.

Taylor Callicrate, the amount of aid you lent me should be sufficient to make this thesis as much yours as it is mine. I'm deeply grateful for everything you have done for me.

Kat Baczynski, thank you for all your help at the farm and in the lab, and most especially for your friendship and your humor. You always made my days brighter.

Erin Leone, goddess of the knowledge universe, you always knew the answers to my questions. Thank you for being an amazing mentor and friend.

Avanti Mallapur, thank you for your company and for all your invaluable help at the farm. Your unflagging cheer and enthusiasm are such an inspiration.

Kelly Brannan, it is a rare person who, under no obligation to do so, would willingly lend a hand to restrain, measure, and perform various other chicken-wrangling duties during the last week of the project. Thank you for making the job so much easier!

Charlotte Sanford-Crane, Vaneesha Bradford, and Dana Cannon, thank you so much for being my right hands during the project. It wasn't always pleasant and it certainly was never pleasantly fragrant, which makes me that much more grateful for all your hard work.

And finally, this thesis is dedicated to my birds (yes, I know): it was for them and because of them that I did this at all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Broiler production has soared since the 1940's as average weight gain per bird has accelerated and production companies have become increasingly vertically integrated (NASS, 2002). In 1945, 366 million broilers were produced annually in the United States; in 60 years that number has increased to 8.88 *billion* (NASS, 2007). In 2000, over 20 billion broiler chickens were estimated to be involved in production worldwide (SCAHAW, 2000). Such expansion of the industry has heightened public and producer awareness of animal welfare issues and their relevance to the large number of birds involved in production. Bird welfare has been undeniably affected by such exponential growth in the broiler industry. Issues arising from intensive confinement and genetic selection for production traits are of particular interest. Specifically, lameness, stocking density, and space use are some of the prime topics that have been the focus of recent attention by scholars, industry professionals, and welfare advocates.

Environmental enrichment, defined as a modification to the environment that improves an animal's biological functioning (Newberry, 1995), has the potential to address certain welfare issues. Enrichment can alter the captive physical space, enabling animals to interact more often and effectively with their surroundings. Adding relevant objects may also stimulate behaviors deemed beneficial or natural to the animal. Given the potential for improvement, there has recently been a concerted effort to design appropriate enrichment programs for species in agricultural production, including the broiler chicken. However, with a few exceptions, there has been little progress in developing successful environmental enrichment for broiler chickens.

1.2 Topics in Broiler Welfare

1.2.1 Structural lameness

Lameness is widely considered to be one of the most serious welfare problems facing the broiler industry (Reiter and Bessei, 1998a,b; Koene et al., 1999; SCAHAW, 2000). Broilers may be afflicted by a range of lameness issues, including tibial dyschondroplasia (Lynch et al., 1992), leg deformities such as twisted legs (Haye and Simons, 1978), and general leg weakness (Kestin et al., 1992). It is generally perceived that much of this can be attributed to selection for rapid growth (SCAHAW, 2000), which has contributed to an increase in structural abnormalities and bone and muscle strain (Lilburn, 1994). For instance, Reiter and Kutritz (2001) found that, compared to strains bred for low growth, birds selected for high growth are less active and able to walk. The connection between lameness and activity is important, as broilers spend most of their time lying (Murphy and Preston, 1988; Bizeray et al., 2000; Weeks et al., 2000; Cornetto and Estevez, 2001a). Even when reared at very low densities where declining competition should theoretically allow for fulfillment of spatial preferences, broilers still spent a considerable amount of time lying (Arnould and Faure, 2004). Such inactivity is thought to aggravate problems associated with lameness, in part because increasing activity level has been shown to improve leg condition in laying hens. For example, exercise machines decrease the amount of broken bones in caged layers (Meyer and Sunde, 1974) and layers with access to perches, who move seven times more than caged layers, have stronger tibias (McLean et al., 1986). However, success in improving broiler leg condition by provision of exercising opportunities has been more limited (Bizeray et al., 2002b) and other studies have not found any benefits (Tablante et al., 2003).

1.2.2 Footpad and hock dermatitis

Apart from the lameness attributable to bone and muscular weakness, foot and hock burns are a common problem that contribute to broiler lameness. Burns on the feet and hocks are forms of contact dermatitis (described in Greene et al., 1985), an ulcerative skin condition thought to be caused primarily by prolonged contact with poor quality litter (Martland, 1985; Haslam et al., 2007). Specifically, it appears that the high moisture content and chemical irritation caused by urea-containing excreta produce the lesions typical of the condition (Haslam et al., 2007). Footpad dermatitis, known also as plantar pododermatitis, is characterized by lesioning on the ventral footpads of poultry (Martland, 1985; Berg, 1998). Footpad dermatitis affects the plantar surface of the feet and is characterized by skin discoloration, hyperkeratosis, and inflammatory ulcerations as the condition worsens. Hock burns occur as the skin of the hocks becomes discolored; the skin becomes increasingly scabby as the condition progresses in severity (Kjaer et al., 2006).

Given that footpad dermatitis can occur if birds are exposed to prolonged contact with wet litter, it would be expected to correlate positively with increased stocking density since more birds per unit space produce a greater volume of excreta. However, this issue is somewhat controversial. Some studies have found an adverse effect of increased stocking density on dermatitis incidence (Arnould and Faure, 2004; Haslam et al., 2007), whereas others have not (Haslam et al., 2006; at least not up to 14 birds/m^2, Sirri et al., 2007). In cases where a relationship between stocking density and contact lesions was not detected, one explanation may be that conditions denser than the experimental concentrations used are required for the condition to manifest itself. Given these observed complexities and after conducting a large-scale study of major broiler companies in the United Kingdom, Dawkins et al. (2004) suggested that stocking density *per se* is not the issue; rather, overall environmental conditions and ventilation quality, which directly govern litter quality, are much more influential.

Regardless of causation, footpad dermatitis and hock burn are problematic from a health and welfare standpoint because the resulting deterioration of the skin likely causes a certain amount of pain and discomfort depending on the severity of the condition. Evidence from Campo et al. (2005) also suggests that footpad dermatitis causes increased fear in cocks. It is therefore relevant to explore avenues that reduce the incidence of these conditions, such as providing birds the opportunity to move away from poor quality litter.

1.2.3 Stocking density

As mentioned previously, the issue of stocking density and its effects on broiler welfare is complex. Abundant studies have shown that increases in stocking density are accompanied with decreases in health and performance. These include reduced growth, body weight, carcass quality, and immune status, as well as increased breast blisters, ammonia burns, and heat stress mortality (Proudfoot et al., 1979; Cravener et al., 1992; Heckert et al., 2002; Pettit-Riley et al., 2002; Dozier et al., 2006). The literature has consistently shown that space allowances below a certain point (usually around 14-16 birds/m^2) compromise production and welfare (Estevez, 2007). However, there exists a great deal of variability in response to different densities depending on the study, with either conflicting evidence or a lack of negative effects observed. For example, although Dozier et al. (2006) observed an adverse effect of increased density on body weight gain, feed consumption, feed conversion, and footpad lesions, they did not distinguish any effect on physiological stress parameters such as plasma corticosteroids, glucose, or cholesterol, in contrast to the results observed by Thaxton et al. (2006). The variability in response to different densities, both within and across studies, is likely due to confounding factors like genetics and particularly the differences in environmental conditions determined by type of facility, ventilation and air quality, and general management practices (Dawkins et al., 2004; Estevez, 2007).

1.2.4 Differential use of space

Chickens do not usually distribute themselves randomly in an enclosure (Hughes et al., 1974). Rather, they tend to favor specific areas of a pen, clustering particularly around walls and other objects (Newberry and Hall, 1990; Cornetto and Estevez, 2001b), even when raised at very low stocking densities (Arnould and Faure, 2004). This clustering effect likely occurs because areas like pen walls are considered to provide birds with a sense of cover and protection (Cornetto and Estevez, 2001b). However, much of commercial broiler production involves environments that are generally barren. The resulting lack of complexity is problematic from a space use standpoint because birds seeking cover are relegated to the pen periphery, leaving the remaining central pen space largely unused. Unoccupied central regions provide prime sites for aggressive interactions between broilers (Cornetto et al., 2002). Furthermore, since conspecifics bump into and walk over each other to join the areas near the walls, birds in these areas tend to experience a higher level of disturbances, which interrupt resting time (Cornetto et al., 2002). Research on broiler time budgeting has demonstrated that such disturbances can terminate up to 21% of lying bouts (Murphy and Preston, 1988), a problem that is intensified with increased stocking densities (Hall, 2001). In addition, since birds walking over other birds inflict scratches on their pen mates' backs, disturbances may also be linked to scabby hip syndrome (Frankenhuis et al., 1991). Finally, the diminished flow of air within these aggregations (Estevez, 1999) may exacerbate the risk of heat stress in the summer months. Dozier et al. (2005) showed that birds subjected to a still-air environment display decreased growth, indicating that a deterioration of airflow is an issue for production as well as for welfare.

An increase in complexity by placing biologically relevant objects in the pen should result in a greater, more even use of space, as demonstrated by past studies of enrichment for domestic fowl. For example, laying hens with access to perches use more of the available space than do hens in battery cages (McLean et al., 1986). In broilers, artificial cover in the form of vertical panels has proven successful in promoting a more uniform distribution by attracting birds away from the periphery toward central core areas (Cornetto and Estevez, 2001b). In broiler breeders, vertical panels have also been shown to increase male home ranges in addition to their beneficial effects on reproductive performance (Leone and Estevez, 2008a).

1.2.5 Fear

Fear can be "a powerful and potentially damaging stressor" in intensive commercial farming situations when birds cannot fully escape fearful stimuli (Jones, 1996). Fear is challenging in such situations for many reasons. Strong fear can induce violent escape and panic reactions, which can potentially injure or kill birds.

Furthermore, highly fearful birds are less able to adapt to changes in their environment, putting them at a disadvantage in interactions with conspecifics or use of resources. Prolonged fear can also decrease production by impacting feed conversion and growth (Jones, 1996). Since high fear levels negatively impact bird welfare and performance, it is in the best interest of all involved to seek a reduction of fear in poultry. Environmental modification – along with human interaction, dietary supplementation, and selective breeding – has been cited as one of the most promising approaches toward achieving this goal (Jones, 1996). For example, broiler breeder pullets provided with perches display an attenuated fear response as they age (Brake et al., 1994) and layers exposed to short-term, enriched housing show more exploratory behavior and vocalize less in a novel Y-maze than do layers without enriched housing (Krause et al., 2006).

1.3 Environmental Enrichment

1.3.1 Overview

Often used inconsistently in the literature (Newberry, 1995), the concept of environmental enrichment is vague partly because effective enrichment depends on so many factors, including age, gender, prior experience of the animal and reason (production, zoological, or companion) for its captive state (Meehan and Mench, 2007). The crux of the issue regarding enrichment is finding practical ways to increase the complexity of the captive environment in a way that is biologically relevant to the animal, will improve physical health, decrease stress, and stimulate natural behavior, all without creating new or exacerbating current problems (Newberry, 1995).

Environmental enrichment can be categorized according to function and purpose. Most commonly, enrichment can alter the quality of the physical space where an animal lives. Increasing the biological relevance of the captive space is commonly done by adding objects or substrates with which animals have a natural penchant to interact, such as giving perches and dust baths to fowl (Newberry, 1995). Related to the practice of adding objects, dividing enclosures into different functional areas can also improve the captive environment. Division of space into discrete sections can be effective in reducing conspecific aggression, either by reducing visual communication (Chamove, 1989) or by providing places for animals to retreat from their aggressors. Dividing the space can also reduce the amount of empty areas which in broilers provide prime sites for aggression (Cornetto et al., 2002). Partitioning the environment may also require animals to cover more distance during their daily routines, thus increasing their activity (Sandusky and Heath, 1988; Chamove, 1989). Presenting food in a way that stimulates foraging behavior or increasing the variety of food offered are other common forms of environmental enrichment (Newberry, 1995).

Finally, as the concept of animal welfare has moved beyond just a reduction of suffering, providing opportunities for animals to experience positive affective states has become an increasingly important goal of enrichment strategies (Meehan and Mench, 2007). Enrichment programs that offer problem-solving opportunities, as long as they are designed appropriately (e.g., mastery of the problem is within the animal's capabilities), allow animals to master causal relationships. Animals therefore become active participants in their environments because they are able to influence their surroundings through their behavior (for more detail, see Meehan and Mench, 2007).

1.3.2 Constraints and limitations of environmental enrichment

A common concern over providing environmental enrichment is disease transmission, which can be complicated by social housing and greater difficulty in cleaning enriched areas and objects (Newberry, 1995). However, adding enrichment does not unequivocally diminish sanitation and can in some cases even improve environmental hygiene. For example, lower bacterial load was reported when woodchips were added to primate enclosures (Chamove, 1989). Nonetheless, care does need to be taken to ensure that increasing environmental complexity does not create more problems that it solves. Meticulous design and evaluation can go a long way in avoiding such issues. Aside from maintaining proper hygiene, additions to the environment must be practical and cost-effective if they are to prove attractive to animal managers and caretakers (Newberry, 1995).

Furthermore, one of the most important considerations when implementing new enrichment is whether it is actually relevant and has functional significance to the animal. Tossing a few toys into an enclosure, for example, may satisfy humans but fail to truly benefit the target animal. Mere investigation of an object cannot be interpreted as success of that enrichment device; rather, prolonged interest and interaction with enrichment, along with continued evaluation of the effects on the animal, are better indicators of a successful enrichment program (Newberry, 1995). Perches, for example, are used frequently by laying hens and thus provide an excellent example of relevant enrichment for those birds (Appleby et al., 1992; Newberry et al., 2001). On the contrary, though broilers have exhibited higher perching rates in the past (up to 27% of birds perching at eight weeks of age, Hughes and Elson, 1977), recent studies have demonstrated that average broiler use of perches during rearing is quite low (between 1.0-2.6%, Le Van et al., 2000; Su et al., 2000; Pettit-Riley and Estevez, 2001). Though perch use in these studies was low, it was not necessarily because perching has ceased to be a motivated and natural behavior for broilers. Rather, it could be traced to a failure of appropriate perch design in accordance with broilers' biological needs, or to their varying genetic backgrounds. This illustrates the range of issues that must be considered when designing environmental enrichment programs.

1.3.3 Use of barrier perches as enrichment for broilers

Provision of traditional perches to broilers, though well-intentioned, may create additional problems if the birds fail to use them. Since perches occupy space, a further reduction in available floor space will occur if they remain unused, thus increasing the risk of heightened stress (Heckert et al., 2002). The problem may become particularly evident as stocking density increases. Therefore, it is important to discern why broilers are not using perches in order to develop alternate enrichment strategies. It is probable that modern broilers fail to use traditional perches because they are simply too high for young birds to access (I. Estevez, personal communication). Furthermore, the effort required to jump up to the perch may exceed ability or motivation to do so, especially as the increased weights of the birds surpass what their legs can handle (Le Van et al., 2000). Approaching perch design differently should therefore improve bird propensity to perch.

Work by Bizeray et al. (2002a,b) has shown that barrier perches may be a more suitable form of enrichment for modern broilers, as birds were observed perching on the barriers at greater than expected rates (9.7% on average) as compared with previous studies (Le Van et al., 2000; Pettit-Riley and Estevez, 2001). Specifically, wooden barriers placed at floor level may serve as functional, accessible perches because their low height can facilitate easy access early in life. Providing access to young chicks may be particularly important because early experience with perches may be critical in allowing perching to be incorporated into the behavioral repertoire later in life (Newberry, 1995). It is also thought that early access to perches may help develop bone strength, particularly because bone growth and mineralization is high during this period (Rose et al., 1996). Bizeray et al. (2002b) have shown that barriers increase the diameter of the tibia, which could potentially underlie leg strength. At that time the authors suggested that perching on and navigating over barriers exercised muscles and joints differently than did the action of walking and thus potentially contributed to differences in bone morphology. Finally, barrier perches may serve as effective enrichment because the increased width of the barrier surface, as opposed to traditional perches, should also be more suited to heavier birds.

The potential benefits of providing barrier perches between important resources are far-reaching. They include: 1) Increased complexity of the environment, which may allow for more opportunities for social interactions and natural behaviors (Newberry, 1995). 2) Because birds have to negotiate around barriers to gain access to food and water, barrier perches should stimulate bird movement (Reiter and Bessei, 1998a,b). They may thus encourage more and different types of activity, which may ultimately influence leg strength (Sandusky and Heath, 1988). 3) Perching on barrier perches should minimize contact with poor quality litter, thus reducing the incidence of footpad and hock dermatitis, especially at higher stocking densities (Haslam et al., 2007). 4) Provision of barrier perches may create an effect similar to that observed with the use of cover panels (Cornetto and Estevez, 2001a,b), therefore reducing the proportion of birds aggregating near the periphery and increasing their use of the central space. 5) Finally, by providing broilers with more complexity and behavioral choices, barrier perches may serve to moderate fear in broilers, as has been shown with providing perches to broiler breeders (Brake et al., 1994).

Although barrier perches seem to have the potential to improve bird health and welfare, their effects at industry-relevant stocking densities have not been investigated. It is also unknown how barriers affect other factors that can impact welfare, including space use and non-structural lameness issues like foot and hock burn. Furthermore, it remains unclear if the impact of barrier perches on parameters measured in previous studies – including behavioral repertoire, leg health, fear, and production – changes as stocking density increases.

Therefore, the objective of this study was to explore the effects of providing barrier perches at varying densities on broiler chicken behavior and health. This was accomplished by pairing three barrier treatments (simple barrier, complex barrier, and no barrier) with stocking densities below, near, and above U.S. industry standards in order to elucidate the effects of density on barrier usage. Effects on behavioral repertoire, space use, footpad and hock dermatitis, fluctuating asymmetry, tonic immobility and production were subsequently assessed.

CHAPTER 2: GENERAL MATERIALS AND METHODS

2.1 Animals and Management

2474 day-old, straight run broiler chicks (Ross 308) were obtained from a commercial hatchery. The study originally required 2424 chicks with an additional 50 (2%) to be used as replacements during the first four days in case of early mortality. The chicks were transported to the University of Maryland's Applied Poultry Research Facility in Upper Marlboro, MD, where they were housed in 36 pens located in two identical rooms in the same building. Birds were kept for seven weeks before going to market. Each pen had an area of 4.46 m^2 and was bedded with approximately 5 cm of wood shavings. Because of damp weather and humid environmental conditions, litter quality was such that litter in all pens was replaced on day 32 to avoid compromising bird welfare unduly.

Due to an unusual 7% mortality rate during Week 1 (attributed to poor chick quality), new lower stocking densities were calculated and birds were redistributed on day 7 (details of the original and revised densities are discussed further in *Section 2.2: Experimental Design*). Some pens had exceedingly high mortality rates to the extent that existing bird counts did not meet even the new lower density requirements. In these cases, care was taken to ensure that the birds added to meet the required number had come from pens with the same treatment. Birds that were removed from existing pens and not needed elsewhere were transferred to separate 'spare' pens. Birds in these 'spare' pens were not used in the experiment.

Feed and water were provided *ad libitum* throughout the experiment. Water was provided via nipple drinkers positioned along one side of the pen (seven nipples per pen). A large tubular hopper (circumference 209 cm) was filled with feed and placed in each pen before chicks were placed, where it remained for the rest of the experiment. Feed was also provided in two shallow feed trays per pen to facilitate access for young chicks during the first two weeks. The feeding program consisted of a 3-phase commercial diet. Starter crumble with the coccidiostat Coban was fed for the first 16 days (19% CP, 2800.00 kcal/kg ME), followed by pelleted grower with Coban from day 16 to 32 (17% CP, 2801.70 kcal/kg ME) and pelleted finisher (no Coban) for the remainder of the experiment (19% CP, 3251.70 kcal/kg ME).

In order to maintain a constant available feeder space of approximately 2.3 cm per bird across the different density treatments, proportions of the tubular feeder were originally blocked in certain pens: feeders in high density pens remained unblocked, 25% of the feeder was blocked in the moderate density pens, and half of the feeder was blocked in the low density pens. After readjustment of bird numbers for the new densities, feeder blocks were not adjusted; available feeder space per bird became approximately 2.61, 2.68, and 2.90 cm for the high, moderate and low density pens, respectively. Similarly, proportions of the drinker nipples were originally blocked in order to preserve equal access (approximately 13 birds/nipple) to drinkers across treatments. All seven nipples remained open in the high density pens, five nipples remained unblocked in the medium density pens, and four nipples were left open in the low density pens. After redistributing birds to account for the new densities, access became 11.4, 11.6, and 9 birds/nipple for the high, moderate and low density pens, respectively.

The lighting program used was designed to promote activity and reduce the incidence of leg problems (24L:0D from day 0-2 and 14L:10D for the remainder of the experiment). Temperature regulation followed standard commercial practice (See Table 2-1 for details). Supplementary heat was provided to each pen during the brooding period via electric brooding lamps with red bulbs suspended over the pens. Brooders were removed on day 16. Ventilation was provided via temperature-sensitive curtains, a central air tube, and ceiling fans. Curtains were automatically lowered when in-house temperatures exceeded set limits.

Birds were inspected twice per day. Health status was determined by general physical appearance, including comb and feather condition and demeanor. Leg condition was also monitored by assessing ability to ambulate without difficulty. At the conclusion of the experiment all birds, including spares, were sold back to the company that provided the birds and harvested according to commercial standards. This protocol (R-08-01) was approved by the Institutional Animal Care and Use Committee at the University of Maryland.

Table 2-1 Temperature schedule during rearing

Age (Days)	Temperature (C°)
1-3	34-32
4-5	32-31
6-7	31-31
8-9	31-30
10-11	29-29
12-13	29-28
14-15	28-28
16-17	27-27
18-19	26-26
20-21	26-25
22-23	25-24
24-25	24-23
26-28	23-22
29-49	22-22

2.2 Experimental Design

2424 birds were randomly divided into 36 groups, equally divided among treatments arranged in a 3 by 3 factorial. Birds were randomly assigned to one of three barrier treatments at one of three stocking densities. The barrier treatments included: 'Simple barrier,' 'Complex barrier,' and 'Control' (no barriers). Original stocking densities were: 1) 'Low,' corresponding to 10 birds/m^2 or 45 birds/pen, 2) 'Moderate,' corresponding to 15 birds/m^2 or 67 birds/pen and 3) 'High,' corresponding to 20 birds/m^2 or 90 birds/pen. However, due to high mortality during the first week, these densities were reduced on day 7 to 8, 13, and 18 birds/m^2, which required 36, 58, and 80 birds/pen, respectively. Each combination of barrier and density was replicated four times.

On day 1, 10 birds per pen (360 birds total) were randomly designated as focal birds and tagged on the neck using the Swiftack Poultry Identification System (Heartland Animal Health, Inc.). Tags were placed on both sides of the neck using a needle gun to attach the tags with a plastic fastener. The laminated paper tags measured 2.5 cm in diameter. This tagging protocol allows for swift and permanent identification of birds without negatively affecting behavior or welfare.

2.3 Barrier Perch Design

Simple barrier treatment pens contained three wooden barriers measuring 100 cm x 15 cm x 4 cm (length x height x width) with flat brackets added to the base for stability. Since pens contained approximately 5 cm of wood shavings, the effective height of simple barriers was 10 cm. Complex barrier treatment pens also contained three barriers of the same dimensions as those used in the simple barrier treatment, but two of the barriers consisted of the same wooden block used for the simple barrier with three additional ‘arms’ attached to one side of the barrier, creating an ‘E’ shape when viewed from above. Each arm measured 20 cm x 15 cm x 4 cm (length x height x width). Complex barriers were also bracketed for stability. Once placed in the pens containing 5 cm of wood shavings, the effective height of complex barriers was also 10 cm. All barriers were placed in a staggered setup in two rows between the food and water sources. See Figure 2-1 for a schematic layout of the experimental pens.

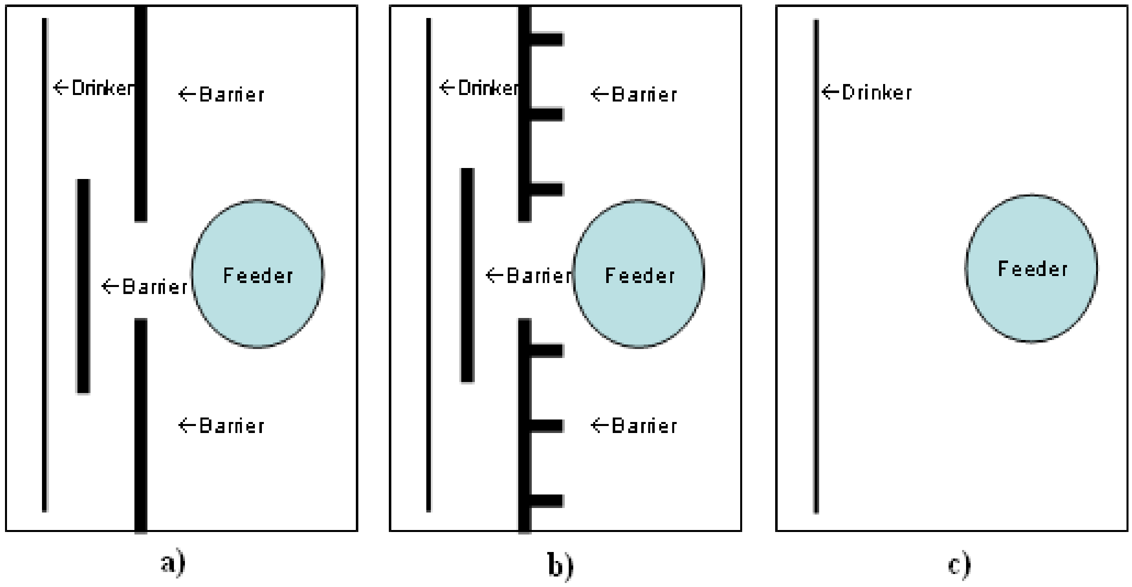


Figure 2-1 Schematics of experimental pen layouts
a) Simple barrier b) Complex barrier and c) Control.

CHAPTER 3: EFFECTS OF BARRIER PERCHES AND DENSITY ON LEG HEALTH, FEAR AND PRODUCTION

3.1 Abstract

Deterioration of leg and foot health is a main welfare concern facing modern broiler production, particularly when birds are kept at high densities. It has been argued that environmental enrichment may help address these issues. We hypothesized that access to barrier perches across a range of densities may improve leg and foot health by providing perching opportunities. To assess this, 2,088 day-old broiler chicks were randomly assigned to one of the following barrier and density treatment combinations over four replications: simple barrier, complex barrier, or control (no barrier) and low (8 birds/m^2), moderate (13 birds/m^2), or high (18 birds/m^2) density. At the end of rearing, data were collected on average tibia width (TW) and length (TL), fluctuating asymmetry (FA) of TW and TL, footpad and hock lesions, tonic immobility (TI), feed conversion, mortalities, and final body weight. TI attempts and footpad and hock lesion scores were analyzed using chi-square analysis. A permutation test was used to analyze duration of TI. All other data were analyzed with a mixed model ANOVA. Significance was set at $\alpha=0.05$.

Results revealed that high density increased the severity of footpad ($P<0.0001$) and hock lesions ($P<0.0001$). Tibias were also longer ($P<0.0001$) and less symmetric in length at higher densities ($P<0.05$). Birds raised in complex barrier pens showed more symmetry in tibia length compared to controls ($P<0.05$). While barrier treatment had no effect on hock burn, there was a trend for simple barriers to reduce the severity of footpad lesions compared to the control treatment ($P=0.0886$). Though TI duration was

unaffected by treatment, birds raised in simple barrier pens were more susceptible to TI induction ($P<0.05$). Final body weight, feed conversion, and mortalities were not affected by any treatment combination. As observed in previous studies, high densities had a negative impact on leg health. Though they did not appear to reduce fearfulness, the improvement in foot health suggests that simple barriers may provide key welfare benefits to broiler chickens.

3.2 Introduction

3.2.1 Structural lameness

Lameness attributable to leg disorders is a significant cause of decreased broiler welfare in commercial production (SCAHAW, 2000). A wide range of disorders is found in modern broilers, including tibial dyschondroplasia, intertarsal varus and valgus deformities and general bone and muscle weakness (Kestin et al., 1992; Lynch et al., 1992; SCAHAW, 2000). It is generally perceived that much of this can be attributed to selection for rapid growth (SCAHAW, 2000), which has contributed to an increase in structural abnormalities and a reduction in bone and muscle strain (Lilburn, 1994). Bird activity level appears to have an important relationship with lameness, as high levels of inactivity are thought to aggravate lameness (Arnould and Faure, 2004; Meyer and Sunde, 1974).

Though gross dissection of the legs provides a more comprehensive assessment of leg disorders, measurement of fluctuating asymmetry (FA) of the tibias can provide a noninvasive snapshot of one facet of leg health. The use of FA as a measure of welfare arises from the upholding of bilateral symmetry as the ideal phenotypic form, where any random or minor fluctuation from the norm is thought to occur as a result of environmental stressors (Palmer and Strobeck, 1986). The degree of asymmetry is interpreted as an indication of the animal's ability to maintain developmental stability in the presence of "random perturbations of cellular processes" (Palmer and Strobeck, 1986; Van Poucke et al., 2007). FA thus represents "the ability of the individual to cope with the sum of challenges that it faces during development" (Van Poucke et al., 2007).

According to Moller (1999) and Moller et al. (1999), FA can be objectively measured and has been found to correlate with fear levels, growth, survival, and fecundity. They have also noted that FA is positively related to the severity of tibial dyschondroplasia as well as walking performance and thus may provide a relatively robust indication of lameness. However, others have questioned the strength and validity (or failed to find strong evidence) of the relationship between FA and various fitness characteristics, including leg problems (Clarke, 1998; Sanotra et al., 2001; as reviewed in Knierim et al., 2007).

3.2.2 Footpad and hock dermatitis

Maintaining proper footpad and hock health is not an insignificant problem for the poultry industry. Over 18% of British broiler flocks (based on an unweighted overall estimate of 190 flocks at slaughter) have been found to exhibit footpad lesions in recent years (Pagazaurtundua and Warriss, 2006). A study conducted on Swedish broilers found that up to 32% of broilers had at least mild footpad lesions, with 6% afflicted with severe lesions (Ekstrand et al., 1997). Along with bone and muscle strength, health of the footpads and hocks is directly relevant to bird welfare because lesions in those areas can cause pain and therefore constitute a welfare problem (Berg, 1998). Footpad dermatitis can also have adverse effects on production: for example, affected broilers take longer to gain weight, perhaps due to a pain-induced depression in appetite (Martland, 1985).

Provided basic dietary needs are met[1], as is the case in commercial production, the lesions in the condition are caused primarily from high ammonia content in wet litter and are hence commonly referred to as ‘ammonia burns’ (Martland, 1985; Berg, 1998;

[1] Early research into causative factors of footpad dermatitis has implicated biotin and riboflavin levels as well (Harms et al., 1977; McGinnis and Carver, 1947).

Dawkins et al., 2004). While lesions may heal if litter quality is improved (Martland, 1985), this is rare in production settings (Berg, 1998). Much research suggests that increasing the number of birds per unit area has a detrimental effect on the incidence of footpad dermatitis (Berg, 1998; Arnould and Faure, 2004; Haslam et al., 2007). Therefore, foot health is important to consider when evaluating the effects of density on broiler welfare.

Footpad and hock lesions are difficult to assess from afar because lesions are generally distributed equally on both feet, creating birds that do not limp and are less likely to move (Berg, 1998). Macroscopic examination of the affected surfaces has become the traditionally accepted method of evaluation (Berg, 1998; Pagazaurtundua and Warriss, 2006) and since assessment is noninvasive, it is relatively straightforward to conduct assessment on live birds. Lesion status is typically recorded according to various classification systems, which rank the subject's footpad against an *a priori* scale. A good example is the detailed 6-step scale designed by Ekstrand and colleagues, which ranges from 1) no visible lesions: smooth epidermis, no discoloration to 6) severe ulcerations and papillae: discoloration, hyperkeratosis, ulcers and signs of inflammatory reactions (Ekstrand et al., 1997). A similar scale based on four categories has been developed by Pagazaurtundua and Warriss (2006).

3.2.3 Fear

Fear in broilers can reduce adaptability to the environment, compromise feed conversion and growth, and induce strong escape responses, which can lead to injury and death (Jones 1986, 1996). High fear levels are hence undesirable from a production as well as from a welfare standpoint. Fear levels in poultry are traditionally assessed by studying the tonic immobility (TI) response. Characterized by a "catatonic-like state of reduced responsiveness to external stimulation," TI is an unlearned response that can be elicited after brief periods of physical restraint (Jones, 1986). Assessment of TI is widely used to estimate fearfulness and is considered to be positively correlated with fear (Jones, 1986, 1996; Forkman et al., 2007). For example, it has been indicated that birds exhibiting long TI durations are likely to show high levels of fear in other fearful situations (Jones and Mills, 1983) and that aversive handling prior to TI increases the duration of the response (Jones, 1992; Marin et al., 2001). Assessment of the response is consequently considered to be a reliable measure of fearfulness (Jones, 1986; Forkman et al., 2007).

3.2.4 Potential of barrier perches to improve welfare

Environmental enrichment can help alleviate the health and welfare issues mentioned previously. Wooden barrier perches may be a particularly suitable form of enrichment for modern broiler chickens, as birds provided with barriers have shown increased activity levels in the form of perching (Bizeray et al., 2002a). Barriers have also been shown to increase the width of the tibia diaphysis, indicating a potential change in bone morphology that could underlie improved leg strength (Bizeray et al., 2002b). Strategies that increase activity levels in broilers – like barrier perches – increase distances between important resources and thus may force birds out of the inactivity that has been implicated in some structural lameness problems (Sandusky and Heath, 1988; Reiter and Bessei, 1998a,b). Furthermore, perching on barriers should allow birds to get off of abrasive, poor quality litter, which may then decrease the incidence of footpad and hock lesions or at least facilitate healing of old lesions at high densities, though this has not been previously studied. Finally, since environmental modification has been cited as one of the most promising approaches toward attenuating high fear levels (Jones, 1996), perches may also serve to decrease fear in broilers.

Therefore, the objective of this study was to explore the effects of providing barrier perches to broiler chickens raised at varying densities. This was accomplished by employing three barrier treatments (simple barrier, complex barrier, and no barrier) at stocking densities below, near, and above U.S. industry standards in order to elucidate the effects of density on barrier usage. Effects on incidence and severity of footpad and hock dermatitis, fluctuating asymmetry of the tibias, tonic immobility, and production traits were subsequently assessed. It was hypothesized that barrier perches would improve foot and hock health, decrease fluctuating asymmetry, and moderate fear in broilers without compromising production, particularly at higher densities.

3.3 Methods

3.3.1 Leg measurements and fluctuating asymmetry

Fluctuating asymmetry (FA) and relative fluctuating asymmetry (relative FA) of tibia width and length were quantified for half (180) of the focal birds at the end of rearing (during week 7). FA was defined as the absolute difference between the right and left legs. Relative FA was obtained by dividing FA by the mean of the right and left legs (Moller et al. 1999). One replication of all treatment combinations (45 birds, or 9 pens) was randomly assessed per day on each of four days. Measurements were taken on the length of the tibia and on diameter (subsequently referred to as width), which was recorded at the spur point on the mid-diaphysis with a digital caliper to the nearest 0.01

mm (modified from Moller et al., 1999). Length and width measurements were each taken twice on both the right and left leg in order to reduce the effects of measurement error and obtain a more robust estimate. A mean width and length for each leg was calculated for analyses.

3.3.2 Foot and hock health

Birds removed from their home pens for FA measurement were also examined relative to foot and hock health at that time in order to reduce stress from repeated handling. Incidence and severity of footpad dermatitis was quantified using the scale described in Pagazaurtundua and Warriss (2006). Lesions were scored on a four-point scale as follows: 0-no lesions, 1-mild lesion affecting a very small area of skin, 2-severe lesion, 3-grossly affected region with lesion covering most of the footpad area. The advantage of this system is that it was based on fewer categories and therefore had a manageable yet acceptable level of detail. Furthermore, the authors' inclusion of photographs of each stage discouraged subjectivity and confusion as to what category a lesion should be assigned. See *Appendix 6.1* for a photographic representation of this scale. Half scores were also recorded to consider lesions that were intermediate between scores. Right and left feet were scored separately as different feet often displayed lesions of varying severity. These scores were later averaged to attain one score per bird for analysis.

Hock burns were scored on a three-point scale based on that used by Kjaer et al. (2006). Unaffected hocks were assigned a score of 0, hocks displaying minor discoloration or lesions resulted in a score of 1, and severe scabbing and lesions were assigned a score of 2. Right and left hocks were scored separately and later averaged in the same method used for footpad scoring.

3.3.3 Fear

The tonic immobility (TI) response was also assessed on the same 180 focal birds directly prior to measurement of FA and foot and hock health. Assessment was conducted in a quiet room separate from the rooms containing the home pens. The same experimenter, who was the only individual in the room during testing, induced all birds. Each bird was gently restrained on its back on a table with the experimenter exerting firm but gentle pressure on the sternum for 10 seconds while holding the feet to prevent struggling. This process is referred to as induction. A timer was started with the release of pressure; the experimenter then stood quietly until the bird struggled or made an attempt to right itself. If the bird struggled immediately upon release of pressure, the experimenter attempted the induction process again. If the bird remained immobile for a period of more than a few seconds, it was assumed to be in tonic immobility and its duration in TI was recorded. The number of inductions necessary to induce TI, as well as the duration of the TI response, was noted. Maximum number of induction attempts was three; after this point the bird was deemed unsusceptible and an induction score of 3 was recorded in conjunction with a duration score of 0. Duration of TI was recorded up to 300 seconds (Mills and Faure, 1991).

3.3.4 Production

Data were collected on feed conversion, total percent mortality, and mean individual body weights at days 16 and 43 and analyzed on a per-pen basis. Although the original intent was to record feed consumption and calculate feed conversion ratios for the entire rearing period, the early mortality rate and subsequent readjustment for new densities compromised measurements taken during the starter feed period. Measurement resumed when starter feed was removed and replaced with grower feed on day 16. Therefore, feed conversion per pen was calculated for day 16 to day 43. All birds in each pen were weighed as a group on day 16 for feed conversion calculations; from this information, day 16 mean individual weight per pen was estimated (referred to as 'starter weight'). On day 43, five focal birds per pen were weighed individually and the weights averaged to obtain a 'final' individual body weight per pen. Feed conversion per pen was calculated by dividing the total amount consumed during this period by the 'collective weight' of the birds, where collective weight = summed weights of dead birds + (mean weight gained by focal birds during this period*number of birds in the pen on day 43) (modified from Bizeray et al., 2002b).

Mortalities and culls were recorded per pen on a daily basis. Total percent mortality was calculated per pen from weeks 2-7 (data from week 1 were omitted due to the complications previously discussed).

3.3.5 Statistical analysis

Data that met analysis of variance (ANOVA) assumptions were analyzed using a mixed model ANOVA with pen as the experimental unit. Barrier and density treatments were treated as fixed factors in a 3 by 3 factorial arrangement. Means and standard errors from data that required transformation for analysis have been back-transformed for reporting. *P* values are reported as analyzed. Analyses were performed using Statistical Analysis System v 9.1 (SAS Institute Inc.) and significance was set at α=0.05 for all analyses. P values above this threshold indicate non-significance and are not reported here.

Leg condition

In order to rule out directional and anti-symmetry, a one-sample t-test with pen as the experimental unit was first used to test whether the signed right-minus-left character values deviated significantly from normal distributions with a mean of zero (Moller et al., 1999). Six variables were then analyzed: FA of tibia width, FA of tibia length, relative FA of tibia width, relative FA of tibia length, average tibia width, and average tibia length. FA and relative FA variables were square-root transformed prior to analysis. Means comparisons were performed when applicable using a Tukey test (Steel and Torrie, 1960) in order to control Type I error.

Footpad and hock scores

Data were reclassified into three categories and a chi-square test was performed. Categories were as follows: 'good' for scores less than or equal to 1, 'fair' for scores greater than 1 and less than or equal to 2, and 'poor' for scores greater than 2. All categories were included in the analysis for effect of density treatment. Because frequency of individuals falling into the 'good' classification was nearly equal among the barrier treatments, this category was dropped from the analysis for effect of barrier treatment in order to discern any effect of barrier treatment on the remaining variable categories of 'fair' and 'poor.'

Hock score data were reclassified into two categories and a chi-square test was performed. Categories were 'good' for scores less than or equal to 1 and 'poor' for scores greater than 1. Treatment comparisons were performed for foot and hock data when applicable by conducting individual pairwise chi-square analyses in order to evaluate significant pairwise differences.

Tonic immobility

Data for the categorical variable *induction attempts* were reclassified into three categories and a chi-square test was performed. Categories included: ‘immediate’ for birds induced after 1 attempt, ‘delayed’ for birds induced after 2 or 3 attempts (which represents birds that were induced into TI, just not immediately) and ‘unresponsive’ for birds that failed to enter TI after 3 inductions (and for which a duration score of ‘0’ was recorded). Because no difference in frequency of ‘unresponsive’ individuals was found among the barrier treatments, this category was dropped from analysis for effect of barrier treatment in order to detect any barrier treatment effect on the remaining variable categories. Treatment comparisons were performed when applicable by conducting individual pairwise chi-square analyses in order to evaluate significant pairwise differences.

The ANOVA assumption of residual normality was not met for the TI duration data due to a high number of 300s. Therefore a permutation test was performed on this data, for which P values were generated based on 100,000 permutations.

Production

Model residuals for mean individual body weight at days 16 and 43, feed conversion, and total percent mortality met ANOVA assumptions, so these variables were analyzed using a mixed model ANOVA with pen as the experimental unit.

3.4 Results

3.4.1 Leg measurements and fluctuating asymmetry

Average tibia width, FA of tibia width, and relative FA of tibia width were not affected by density or barrier treatment. However, average tibia length was affected by density ($F_{2,27}$=6.24, *P*=0.006; Fig. 3-1), with longer tibias found at higher densities. Tibias were 4.143 ± 1.198 mm longer when birds were raised at a density of 18 birds/m^2 compared to 8 birds/m^2 (*P*=0.005).

Relative FA of tibia length was not significantly affected by density. However, increases in stocking density produced a significant increase in FA of tibia length ($F_{2,27}$=3.66, *P*=0.039; Fig. 3-2) such that tibia length was more asymmetrical at 18 birds/m^2 compared to 8 birds/m^2 (*P*=0.050). The effect of barrier approached significance for both relative FA and FA of tibia length ($F_{2,27}$=3.17, *P*=0.058 and $F_{2,27}$=3.21, *P*=0.056, respectively), with higher levels of both types of asymmetry in control pens compared to the complex barrier treatment. Means comparison revealed a significant difference between FA of tibia length in the complex barrier treatment compared to the control (*P*=0.046), with birds in the complex barrier treatment exhibiting less asymmetry than birds reared without barriers (Fig. 3-3). See Table 3-1 for a summary of treatment effects on FA and relative FA.

Table 3-1 Summary statistics for fluctuating asymmetry (FA) and relative FA of tibia width and length (mm)

Variable	Simple Barrier	Complex Barrier	Control	F-value	*P*-value
		Barrier treatment		*Treatment effect*	
Tibia width					
Average size	13.43 ± 0.15	13.42 ± 0.15	13.32 ± 0.15	0.17	0.845
FA	0.28 ± 0.02	0.29 ± 0.02	0.26 ± 0.02	0.20	0.823
Relative FA	0.021 ± 0.001	0.022 ± 0.001	0.019 ± 0.001	0.17	0.841
Tibia length					
Average size	84.38 ± 0.84	84.25 ± 0.84	84.61 ± 0.85	0.05	0.954
FA	1.48[ab] ± 0.09	1.24[b] ± 0.08	1.90[a] ± 0.10	3.21	0.056
Relative FA	0.018[ab] ± 0.001	0.015[b] ± 0.001	0.023[a] ± 0.001	3.17	0.058
		Density treatment			
	8 birds/m^2	13 birds/m^2	18 birds/m^2		
Tibia width					
Average size	13.41 ± 0.15	13.22 ± 0.15	13.54 ± 0.15	1.13	0.339
FA	0.35 ± 0.02	0.23 ± 0.02	0.25 ± 0.02	2.75	0.082
Relative FA	0.026 ± 0.002	0.018 ± 0.001	0.018 ± 0.001	2.72	0.084
Tibia length					
Average size	82.59[b] ± 0.85	83.93[ab] ± 0.84	86.73[a] ± 0.84	6.24	0.006
FA	1.15[b] ± 0.08	1.70[ab] ± 0.10	1.78[a] ± 0.10	3.66	0.039
Relative FA	0.014 ± 0.001	0.020 ± 0.001	0.021 ± 0.001	2.94	0.070

Values are LSM ± SEM.

Treatment means with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

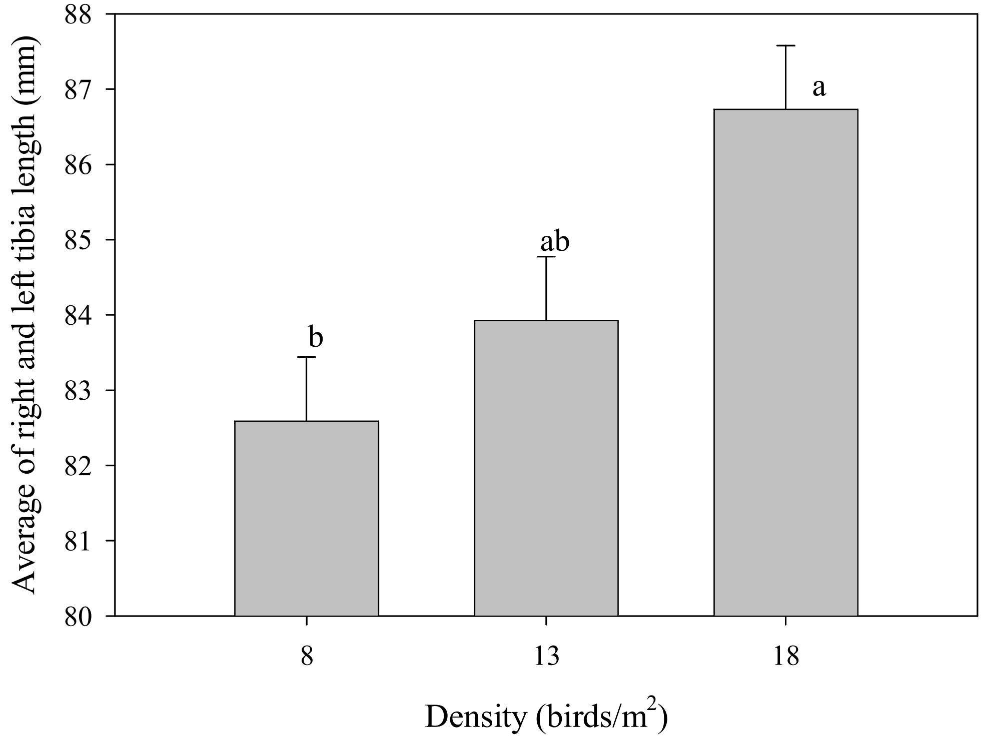


Figure 3-1 Density effect on average tibia length (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

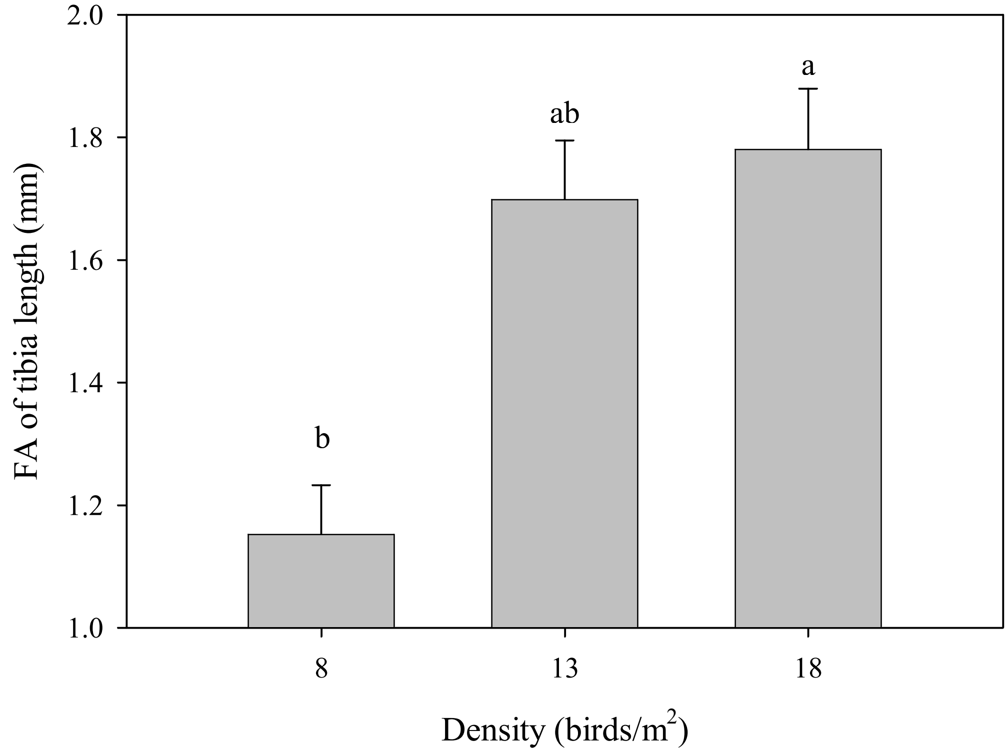


Figure 3-2 Density effect on FA of tibia length (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

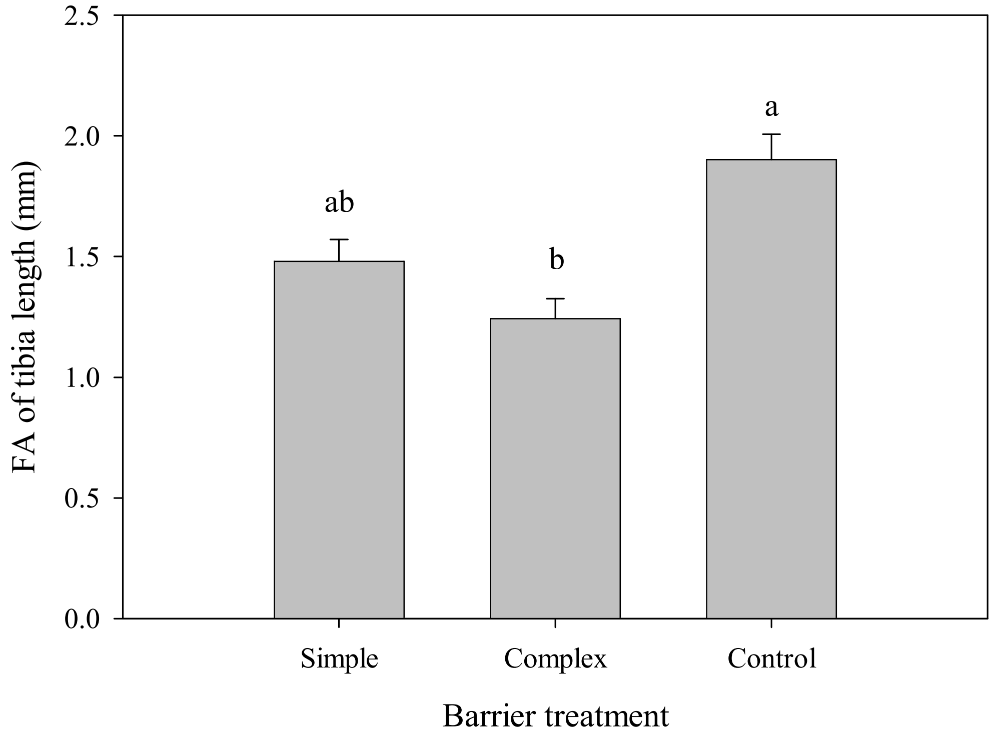


Figure 3-3 Barrier treatment effect on FA of tibia length (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

3.4.2 Foot and hock health

Density treatment significantly affected average footpad lesion score (χ^2=29.699, 4 df, *P*<0.0001; Fig. 3-4) such that 'good' lesion scores were more frequent in the lower densities and 'poor' scores were more frequent in the higher densities. A significant (χ^2=7.214, 2 df, *P*=0.027; Fig. 3-5) barrier effect on footpad score was detected after 'good' scores were omitted from analysis. Simple barrier pens contained more birds with 'fair' and fewer with 'poor' footpad lesion scores than the complex barrier pens (χ^2=7.272, 1 df, *P*=0.007). This contrast also approached significance between the simple barrier and control treatments, with the simple barrier pens again containing more birds with 'fair' and fewer with 'poor' scores than those raised in the control treatment pens (χ^2=2.900, 1 df, *P*=0.0886).

Similarly, hock scores were negatively affected by increased density (χ^2=26.946, 2 df, *P*<0.0001; Fig. 3-6), with 'good' scores decreasing and 'poor' scores increasing in frequency as density increased. There was no significant barrier effect on hock lesion score.

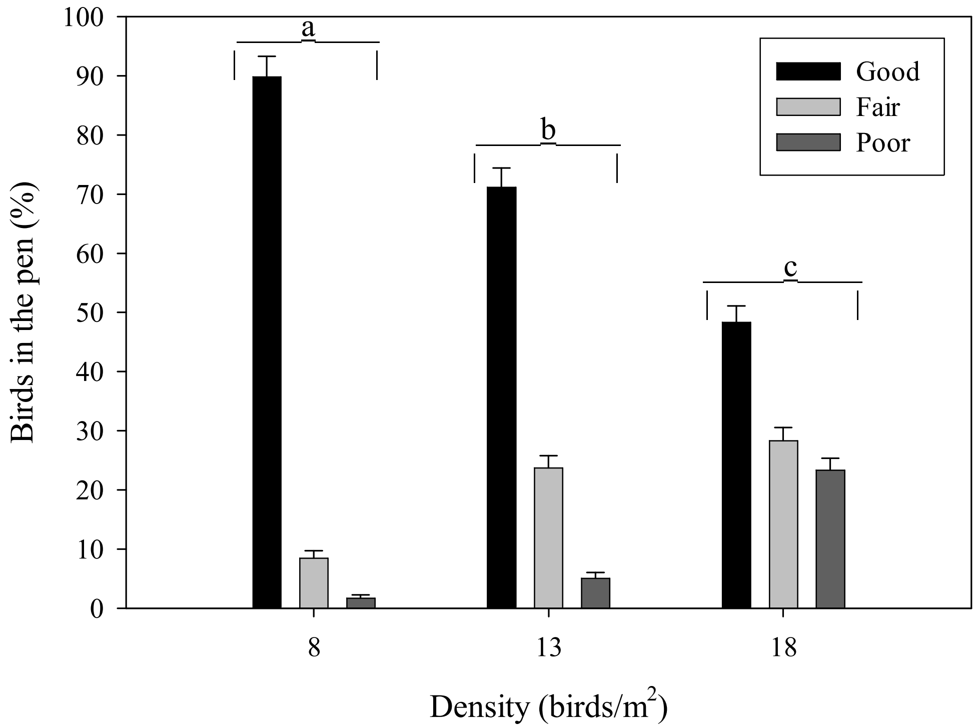


Figure 3-4 Density effect on footpad lesion score (± SE)
Treatments with different letters are significantly different, $p<0.05$, after pairwise comparison with χ^2 test.

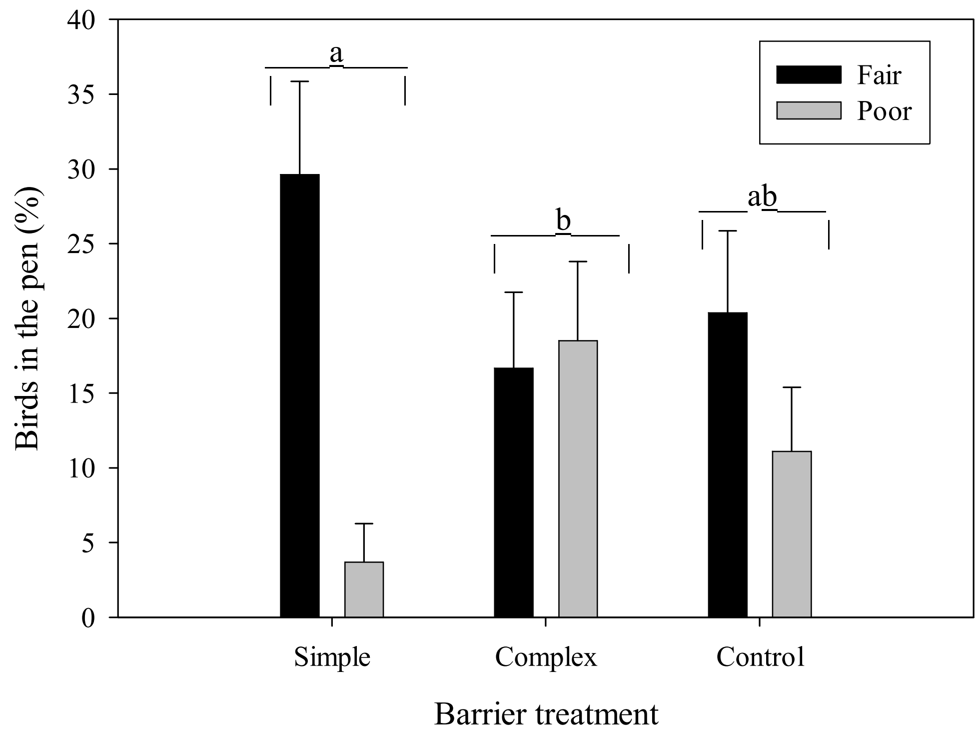


Figure 3-5 Barrier treatment effect on footpad lesion score (± SE)
Treatments with different letters are significantly different, $p<0.05$, after pairwise comparison with χ^2 test.

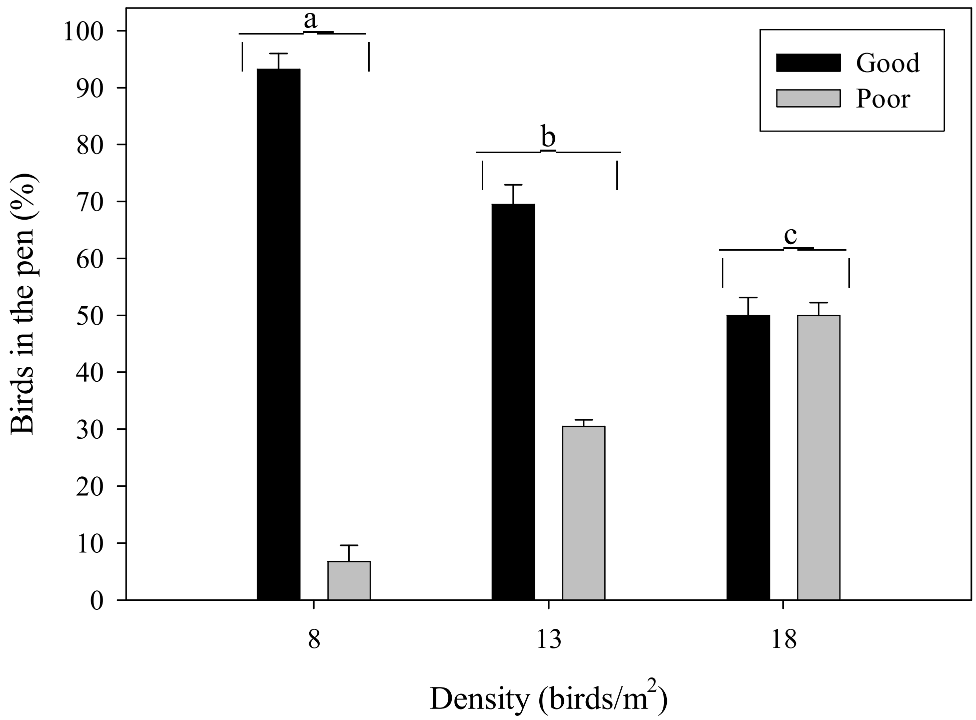


Figure 3-6 Density effect on hock lesion score (± SE)
Treatments with different letters are significantly different, $p<0.05$, after pairwise comparison with χ^2 test.

3.4.3 Fear

While density did not have a significant effect on the number of attempts required to induce TI, barrier treatment was found to be significant (χ^2=6.720, 2 df, *P*=0.035; Fig. 3-7). Over 90% of individuals in the simple barrier treatment were induced after the first attempt, which was significantly greater than that percentage for birds raised in complex barrier or control pens (χ^2=5.901, 1 df, *P*=0.015; χ^2=5.314, 1 df, *P*=0.021, respectively). Conversely, more birds in both the complex barrier and control treatments were induced after the 2nd or 3rd attempt compared to birds raised with simple barriers. Neither density nor barrier treatment exerted significant effects on the duration that birds remained in TI.

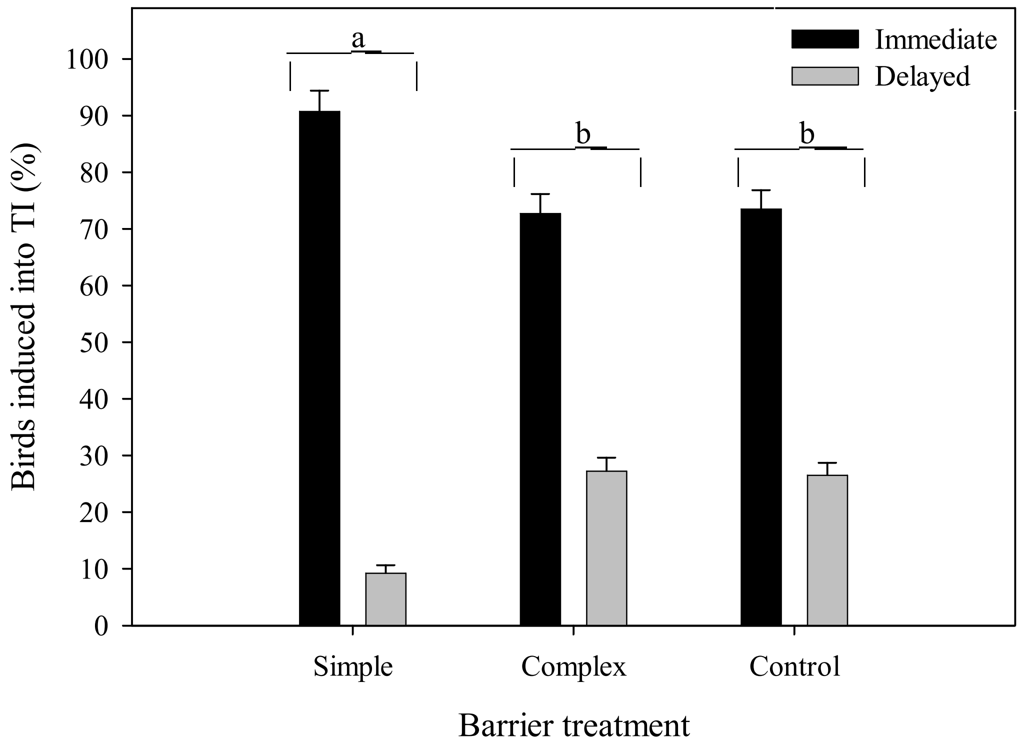


Figure 3-7 Barrier treatment effect on proportion of immediate or delayed attempts to induce TI (± SE)
Birds induced 'immediately' were induced on the 1st attempt, whereas 'delayed' birds required 2-3 attempts. Treatments with different letters are significantly different, $p<0.05$, after pairwise comparison with χ^2 test.

3.4.4 Production

Mean individual body weight at day 16 was significantly affected by barrier treatment ($F_{2,26}$=5.79, *P*=0.008) but not by density (Table 3-2) such that birds in control pens were heavier at day 16 than birds in either of the barrier treatments (*P*<0.05). There was no significant difference in individual body weights at day 43, total percent mortality, or feed conversion among any of the experimental treatments.

Table 3-2 Barrier treatment and density effects on production (LSM ± SEM)

	Barrier treatment			*Treatment effect*	
Variable	Simple Barrier	Complex Barrier	Control	F-value	*P*-value
Mortality %	2.598 ± 0.008	2.276 ± 0.008	2.327 ± 0.008	0.05	0.950
Feed conversion	1.783 ± 0.028	1.806 ± 0.028	1.793 ± 0.028	0.16	0.850
Body weight (g)					
Day 16	500[a] ± 0.005	498[a] ± 0.006	515[b] ± 0.005	5.79	0.008
Day 43	2661 ± 0.046	2623 ± 0.051	2690 ± 0.046	0.68	0.515

Means with different letters are significantly different after Tukey's adjustment.

	Density treatment			*Treatment effect*	
Variable	Low Density	Moderate Density	High Density	F-value	*P*-value
Mortality%	2.546 ± 0.008	2.155 ± 0.008	2.500 ± 0.008	0.08	0.924
Feed conversion	1.803 ± 0.028	1.788 ± 0.028	1.792 ± 0.028	0.07	0.928
Body weight (g)					
Day 16	509 ± 0.005	501 ± 0.005	503 ± 0.005	0.96	0.395
Day 43	2677 ± 0.046	2650 ± 0.049	2647 ± 0.048	0.16	0.850

3.5 Discussion

3.5.1 Leg measurements and fluctuating asymmetry

Neither barrier nor density treatment significantly affected average tibia width, a finding that is in contrast to Bizeray et al. (2002b), who reported that barriers increased tibia width and interpreted that result as promising for improving leg condition (Bizeray et al., 2002b). However, the present results show that tibias were longer for birds maintained at higher densities. The effects of varying stocking densities in the present study, combined with multiple barrier treatments, probably accounts for the reported differences between the present study and Bizeray et al.'s report (2002b).

Regarding the effects of barrier treatment and density on fluctuating asymmetry (FA), the complex barriers used in the current study appear to have reduced FA of tibia length to a certain degree, suggesting that they may aid broilers in maintaining developmental stability, at least for this parameter. Significant barrier treatment effects on FA have not been observed in the past (Bizeray et al., 2002b).

Increasing stocking density had a negative effect on FA, with higher asymmetry in tibia length for birds reared at higher densities. It has previously been reported that increases in density are accompanied by increases in FA of tibia length (Van Poucke et al., 2007). Given the breadth of work on the detrimental effects of increasing stocking density beyond a certain point (Proudfoot et al., 1979; Cravener et al., 1992; Heckert et al., 2002; Pettit-Riley et al., 2002; Dozier et al., 2006; Estevez, 2007), such results may be expected. It is plausible that increases in stocking density create high stress during development, which is ultimately manifested in greater asymmetry between the right and left sides of the body. Alternatively, there may have been a greater chance of observing increased FA as density increased since mean length of the tibias also increased with density.

3.5.2 Foot and hock health

In this study simple barrier perches appeared to have a beneficial impact on footpad health, evidenced by the trend for a lower proportion of birds with 'poor' footpad scores in the simple barrier treatment compared to birds in the control treatment. A possible underlying mechanism for this is that simple barriers allowed birds to minimize time spent on wet litter. In contrast, it seems as though complex barriers had a negative impact, as lesion rates were highest and most severe in this treatment. It is possible that as birds grew, the complex barriers occupied increasingly precious space and perhaps hindered bird movement, thus increasing the effects of relative density and interfering with footpad health. The negative effect of complex barriers on footpad health was unanticipated. This result underscores the fact that small variations in enrichment design can have a major impact, and therefore serves as recommendation for comprehensive evaluation of the impact of enrichment items before they are implemented into commercial systems (Newberry, 1995).

Contrary to expectations, we did not detect any benefit of using barriers to improve hock condition, as hock lesion scores were unaffected by barrier treatment. This may be related to the fact that the experimental density used, rather than barrier treatment, more directly affects litter quality, which is the primary factor affecting hock health. Since footpads have more continuous and direct contact with litter than the hocks, this may explain why barriers, in addition to density, did have an effect on footpad health.

Regarding the effect of density on lesion scores, pens with lower densities contained a higher proportion of birds with healthier feet and fewer with severe lesions as compared with higher densities. These findings corroborate prior results of declining footpad health with increased stocking density (Berg, 1998; Haslam et al., 2007). In the present study, birds housed at a density of 8 birds/m^2 were less afflicted with footpad dermatitis than those kept at 13 birds/m^2. The detrimental impact of increasing density was especially pronounced at the increase to 18 birds/m^2. For example, the percentage of birds with 'poor' footpad scores jumped from around 5 to 23% as density increased from 13 to 18 birds/m^2. Increased density also negatively affected hock health, as 50% of the birds raised in the 18 birds/m^2 pens showed 'poor' hock scores compared to approximately 30% in the 13 birds/m^2 pens. This serves as additional evidence that increasing stocking density compromises bird welfare, since individuals in the 8 birds/m^2 pens were least afflicted by hock lesions. Though birds in the 13 birds/m^2 pens did experience a greater incidence and severity of lesions on the footpads and hocks compared to birds in the lower density, these rates remained below those of birds housed at this study's highest experimental density. These results seem to be in accordance with previous studies (Martland, 1985; Berg, 1998; Dozier et al., 2006), which suggested that deterioration of litter quality plays a key role, as quality of litter was visibly much poorer in the more crowded pens (Ventura, personal observation).

3.5.3 Fear

Neither barriers nor stocking density affected the duration of time that birds remained in TI, indicating that these factors were not influential on broiler fear levels. Since TI duration is widely considered to be a robust measure of fearfulness in domestic fowl (Jones, 1996), and because previous studies have shown that perches can reduce fear in chicks (Brake et al., 1994), we expected to detect a similar reduction in fear with the addition of enrichment in the form of barrier perches. The present results, however, are in line with the absence of enrichment effect described by Bizeray et al. (2002b). However, in contrast to Bizeray et al.'s (2002b) results, barriers significantly affected the number of TI inductions in the present study. Fewer inductions were required to elicit the TI response from birds raised in simple barrier pens, suggesting that they were more susceptible to entering TI and hence more inherently fearful. This is in direct contrast with our expectations and with previous work which has hinted at the fear-alleviating nature of enrichment (Jones and Waddington, 1992; Brake et al., 1994; Jones, 1996). At this time it is not clear why birds raised in simple barrier pens would have been significantly more susceptible to TI induction, particularly when birds raised in the complex barrier pens showed no such response and since no effects on TI duration were observed. Since it has been suggested that duration of TI, rather than induction attempts, seems to be a more sensitive measure of fear for poultry (Forkman et al., 2007), and since there were no observed effects of barrier treatment on TI duration, it does not appear at this time that the barrier perches used in the current study substantially affect fear levels in broilers.

3.5.4 Production

Although body weight at day 16 was significantly greater in the control pens, this may have been connected to the high mortality rates during the initial week of the study. Regardless, the current study clearly indicates that by the end of rearing neither density nor barrier treatment had any significant effect on final body weight, feed conversion, or total percent mortality. This evidence suggests that while barrier perches may not improve production, neither do they pose any negative consequences for bird performance, and consequently, for economic profit for production companies.

CHAPTER 4: EFFECTS OF BARRIER PERCHES AND DENSITY ON BEHAVIOR AND SPACE USE

4.1 Abstract

Restriction of opportunities for natural behavior and uneven use of space are considerable welfare concerns in modern broiler production, particularly when birds are kept at high densities. Environmental enrichment can help address some of these issues. It was hypothesized that increased environmental complexity by provision of barrier perches would encourage perching and a more even use of the pen space across a range of densities. In this experiment, 2,088 day-old broiler chicks were randomly assigned to one of the following barrier and density treatment combinations over four replications: simple barrier, complex barrier, or control (no barrier) and low (8 birds/m^2), moderate (13 birds/m^2), or high (18 birds/m^2) density. Data on behavior and use of space were collected on focal birds via instantaneous scan sampling from 2 to 6 weeks of age. Mean estimates per pen for the percent of observations seen performing each behavior, as well as percent of observations in the pen periphery vs. core, were quantified and analyzed with a mixed model ANOVA with week as the repeated measure. Significance was set at α=0.05.

Results showed that the behavioral time budget of broilers was affected by barrier perches, stocking density, and age. Both simple and complex barrier perches effectively stimulated high perching rates; a mean perching rate of 24.15% was observed in the 8 birds/m^2 treatment during the 4th week of age. Aggression and disturbances were lower in both barrier perch treatments compared to control pens ($P<0.05$). Birds fed less often in complex barrier pens compared to control pens ($P<0.001$), and birds housed with simple

barriers drank less often compared to those in control pens ($P<0.01$). Increased density generally had a suppressive effect on activity levels, with lower foraging ($P<0.005$), decreased perching ($P<0.0001$) and increased sitting ($P=0.001$) earlier in the rearing period when birds were housed at a density of 18 birds/m^2 compared to the lower densities. Increases in stocking density were also accompanied by an increase in disturbances ($P<0.05$). Use of the central pen space was significantly higher in simple barrier pens compared to controls ($P<0.001$), while increasing density above 8 birds/m^2 suppressed use of the central space ($P<0.05$). Overall, the results demonstrate that increasing density compromises bird welfare by promoting inactivity, increasing disturbances, and limiting bird distribution. We suggest that barrier perches have the potential to improve broiler welfare by encouraging activity (notably by providing accessible opportunities to perch), decreasing aggression and disturbances, and promoting more even distribution of birds throughout the pen space.

4.2 Introduction

4.2.1 Behavior

The opportunity for an animal to engage in natural behavior is recognized as a crucial component of good welfare and has been designated as one of the Five Freedoms (Brambell, 1965). However, broiler chickens are typically reared in two-dimensional environments of low complexity, where required travel and foraging effort is generally low and bird movement and activity is restricted (Newberry and Hall, 1990; Estevez et al., 1997; Estevez and Christman, 2006). Restriction of natural behavior is most likely to occur when birds are least active (SCAHAW, 2000). Though consensus on the ultimate causative factor of inactivity has yet to be reached, it is likely that a combination of factors has an impact. Lameness caused by increased growth rate and body weight (Kestin et al., 1992) and restricted opportunity for movement due to high stocking density levels (Estevez et al., 1997; SCAHAW, 2000) have been suggested as two possible factors.

In general the effects of high stocking density are reflected in a reduction of broiler behavioral expression. Locomotion and distance traveled have consistently been reported to decline as density increases (Lewis and Hurnik, 1990; Estevez et al., 1997; Hall, 2001; Leone and Estevez, 2008c), perhaps because an increased number of birds per unit area creates a barrier effect by hampering bird dispersion throughout the pen (Newberry and Hall, 1990; as suggested by Estevez, 2007). Birds also spend significantly less time resting at higher densities due to the resultant increase in disturbances (Murphy and Preston, 1988; Lewis and Hurnik, 1990; Hall, 2001; Cornetto et al., 2002). Increases in stocking density also appear to have a suppressive effect on behaviors like scratching and walking, though this may be due more to indirect effects of increasing density – like a decline in litter quality – rather than to a lack of space (Reiter and Bessei, 2000).

These issues can be addressed, at least partially, through implementation of environmental enrichment. Increasing the complexity of the environment by adding enrichment can have a substantial influence on the behavior of domestic fowl (Cornetto and Estevez, 2001a; Bizeray et al., 2002a; Leone and Estevez, 2008a) in part because enrichment offers increased opportunities for a wider range of behavioral expression, thereby improving the animal's biological functioning (Newberry, 1995).

For modern broilers, perching is a good example of a behavior with little opportunity for expression in traditional production facilities. Past attempts to stimulate perching in broilers by offering perches have generally failed (Le Van et al., 2000; Su et al., 2000; Pettit-Riley and Estevez, 2001). However, since higher perching rates have been observed with application of wooden barriers (Bizeray et al., 2002a), it is likely that perching is still a motivated behavior in broilers. Providing barrier perches, which are lower to the ground and thus more adapted to the current nature of broiler chickens, may therefore serve to increase environmental complexity and provide enhanced opportunities to express a wider variety of natural behaviors (Newberry, 1995).

4.2.2 Use of space

Animals possess "physiological and behavioral needs that can only be met through certain spatial relationships" (Stricklin, 1995); they thus require adequate space to express their natural behaviors. Use of space is well documented for the domestic fowl, and has been shown to be influenced by the distribution of resources, conspecifics, predators, and cover and roosting sites (Cornetto and Estevez, 2001b; Arnould and Faure, 2004; Leone and Estevez, 2008b). Use of space is also dependent on density and particularly enclosure size, with dispersion and distance traveled increasing as space becomes more available (Leone and Estevez, 2008c). Generally, individuals within the flock do not utilize the entire portion of space available to them in commercial facilities (Leone and Estevez, 2008a), nor do they distribute themselves randomly throughout the captive space (Hughes et al., 1974; Leone and Estevez, 2008b). Specifically, poultry tend to cluster around the periphery or around objects in the pen in order to maintain a sense of concealment and protection (Newberry and Hall, 1990; Cornetto and Estevez, 2001b**;** Arnould and Faure, 2004).

Such uneven use of the pen can create a variety of welfare issues. Aggregations around the periphery of the pen can render the central space largely unoccupied, thus providing prime space for agonistic interactions (Cornetto et al., 2002; Pettit-Riley et al., 2002). Individuals entering or leaving congregations of birds disrupt the resting intervals of birds already in the group (Cornetto et al., 2002). Furthermore, airflow may be diminished in these aggregations, which can be especially problematic at high temperatures because birds can become more exposed to heat stress (Estevez, 1999; Dozier et al., 2005).

Compared to research in free-ranging wildlife, traditional techniques for analyzing space use in captive species have generally been fairly simple (according to Estevez and Christman, 2006; Leone et al., 2007). Notably, past research has considered the proportion of animals located in newly accessible areas (Newberry, 1999) or in areas containing resources such as food and water or attractive objects (Cornetto and Estevez, 2001b; Arnould and Faure, 2004). These techniques involve a type of quadrant analysis, whereby the enclosed space is divided into sections so that counts of the number of animals appearing in each quadrant can be compared (Cornetto and Estevez, 2001b; Leone et al., 2007). Specifically, because poultry tend to congregate around pen walls (Newberry and Hall, 1990), quadrant analyzes of use of the central vs. peripheral areas is a simple way to estimate bird spatial distribution.

Given the issues of uneven use of space and behavioral restriction, we assert the importance of seeking avenues to promote maximal use of space and an increased range of behavioral expression. As has been documented with cover panels (Cornetto and Estevez, 2001b), objects like barrier perches may serve to attract birds away from the periphery of the pen, though they could also restrict bird movement. It is hypothesized that maximum bird dispersion can be achieved by placing barrier perches throughout the pen and between important resources. It is also anticipated that by offering additional behavioral opportunities, strategically located barrier perches should influence the behavioral repertoire of broilers.

Therefore, the objective of this part of the study was to explore the effects of providing barrier perches at varying stocking densities on behavior and use of space in the broiler chicken. Effects on behavioral repertoire and time spent in the periphery vs. pen center were subsequently assessed.

4.3 Methods

4.3.1 Observations and data collection

Behavioral and spatial data were collected on focal birds via instantaneous scan sampling (Altmann, 1974). Recorded behaviors included: feeding, drinking, foraging, aggression, disturbances, standing, sitting, walking, running, perching, preening, dust bathing, flapping, and other (See Section *4.3.2 Behavioral Definitions* for the full ethogram). Data concerning the birds' distribution in the pen were recorded simultaneously. Observations were conducted in the following manner: the observer stood quietly outside each pen and recorded the instantaneous location and behavior of as many of the 10 focal birds in the pen as could be located. Each of the 36 pens was visited in a random order as one cycle; this cycle was repeated twice per day, producing 72 pen scans per day, four times per week from weeks 2 to 6 (week 1 observations were omitted due to the early high mortality rate). Data collection was performed between 8am and 1pm to reduce variability attributable to diurnal behavior patterns (Mankovich and Banks, 1982). Behavior and space use data were recorded on a tablet PC (Toshiba, Inc.) using the Chickitizer program[2]. This software allows each bird's location to be digitized into an XY coordinate system and recorded in conjunction with the behavior the bird was performing at that point in time (See *Appendix 6.2* for a screenshot of the program). Data collection was facilitated with the use of a numbered grid coordinate system that was marked on all pen walls. Pens were divided into 70 rectangles measuring approximately 26.14 x 24.40 cm each.

---

[2] Sanchez, C. and Estevez, I., 1998. The chickitizer, v.4, University of Maryland, College Park, MD, USA.

Use of space was quantified by calculating the proportion of observations in the central vs. peripheral areas of the pen. The periphery was defined as the area within 25 cm of the pen walls and the remaining area was considered the central area. Relative areas of the center and periphery were accounted for by dividing the percent observations in either zone by the area of that zone before proceeding with analysis. The percentage of observed locations in the center vs. periphery was calculated for each focal bird and then averaged across focals within each pen to obtain weekly percent means per pen for analysis. Because percent of observations in the periphery vs. center is complementary, only percent of observations in the central area is reported here. Analysis of minimum convex polygons (MCPs) to gauge estimates of individual home range was not pursued because Leone et al. (2007) have noted that MCP may have limited relevance in confined settings. This is because observations of animals near the periphery will cause a polygon to approach the actual size of the enclosure after a large number of observations, which is not useful in measuring true home range (Leone et al., 2007).

4.3.2 Behavioral definitions

Appetitive Behaviors

Feeding	Bird is located next to feeder and has its beak inside the feeder.
Drinking	Bird’s head is raised toward nipple drinkers, and is either attempting to or is currently contacting its beak with the drinker.
Foraging	Bird is pecking or scratching at the ground.

Aggression and Disturbances

Aggression — Any behavior in the following categories was recorded under this label:

Chase	One bird runs at least three steps after another bird.
Fight	Two birds are standing facing each other with heads and necks raised to the same level. One bird delivers more than two vigorous kicks at opponent. Pecks may or may not be observed.
Leap	Two birds face each other; one or both jump without extending legs toward other bird.
Peck	Face-to-face encounter in which one bird raises its head and directs vigorous pecks toward another bird (usually the head).
Standoff	Two birds stand facing one another with heads at the same level for more than two seconds.
Threat	Bird stands with raised feathers and erect neck while opponent holds its head at a lowered level.

Disturbances — Another bird makes physical contact with resting focal bird, causing it to readjust itself or stand.

Activity Behaviors

Sitting	Bird has ceased locomotion and its breast is in contact with the ground. Bird may or may not have its eyes closed.
Standing	Bird maintains upright position on motionless, extended legs.
Walking	Relatively low-speed displacement of bird on the ground in which the propulsive force is derived from the action of the legs.
Running	Higher speed displacement of bird on the ground in which the propulsive force is derived from the action of the legs.
Perching	Bird's feet are grasping the barrier and bird is not locomoting. Breast of bird may or may not be in contact with barrier (sitting on and standing on barrier both considered perching).

Dust bathing	Bird is lying on the ground and tossing dirt onto its back/wings by ruffling and shaking its feathers.
Flapping	Bird is in an upright position and extends its wings repeatedly.
Preening	Bird is using its beak to peck, stroke, or comb plumage.
Other	Any behavior not belonging to the previous categories was recorded under this label.

4.3.3 Statistical analysis

Data that met analysis of variance (ANOVA) assumptions were analyzed using a mixed model ANOVA unless otherwise stated. Barrier and density were treated as fixed factors in a 3 by 3 factorial arrangement. Analyses were performed using Statistical Analysis System v9.1 (SAS Institute Inc.) and significance was set at $\alpha=0.05$. P values above this threshold indicate non-significance and are not reported here.

Behavioral scans

The proportion of observations that birds were seen performing each behavior each day was determined for each focal bird and averaged across focal birds and days to obtain weekly pen means for analysis. Residuals for the behaviors *feed, drink, sit, stand, walk,* and *preen* met ANOVA assumptions of normality so these variables were analyzed with a mixed model ANOVA with week as the repeated measure and pen designated as the experimental unit. The behavior *perch* was analyzed under the same model after control pens were omitted from analysis since it was not possible to observe perching in the control treatment. Data on the variables *forage, aggress, disturb,* and *run* were averaged across weeks to obtain overall pen means for the entire study period, which were then analyzed under a similar model except that repeated measures were not included. The *flapping*, *dust bathing*, and *othe*r behaviors occurred so infrequently that they were not included in the analysis. Tukey HSD was used for all means comparison (Steel and Torrie, 1960).

Space use

Mean percent of observations in the central area was analyzed under a mixed model ANOVA with week as the repeated measure.

4.4 Results

Interactions between factors were not significant unless otherwise stated.

4.4.1 Behavior

Appetitive behaviors

Stocking density did not significantly affect the proportion of time birds were observed feeding. However, both barrier ($F_{2,27}$=9.83, $P<0.001$, Fig. 4-1) and age ($F_{4,108}$=21.55, $P<0.0001$, Fig. 4-2) exerted significant effects, with broilers in the complex barrier treatment feeding less often than those in the control treatment ($P<0.001$). Feeding was highest during week 2 and mostly declined thereafter ($P<0.0001$).

The proportion of observations that birds were observed drinking was also affected significantly by barrier treatment and by age ($F_{2,27}$=8.89, $P=0.001$, Fig. 4-3; $F_{4,108}$=8.23, $P<0.0001$, Fig. 4-4, respectively) but not by density. Drinking was less frequently observed in the simple barrier treatment compared to the control ($P<0.001$) and was lowest during the second week of age compared to subsequent weeks (means comparison between week 2 and each of the subsequent weeks yielded at least $P<0.05$).

In contrast, the proportion of observations of foraging behavior was not affected by barrier treatment but was affected by density ($F_{2,26}$=8.90, P=0.001; Fig. 4-5) such that the proportion of time that broilers spent foraging was lowest at 18 birds/m^2 compared to the lower experimental densities (P<0.0001 for both comparisons).

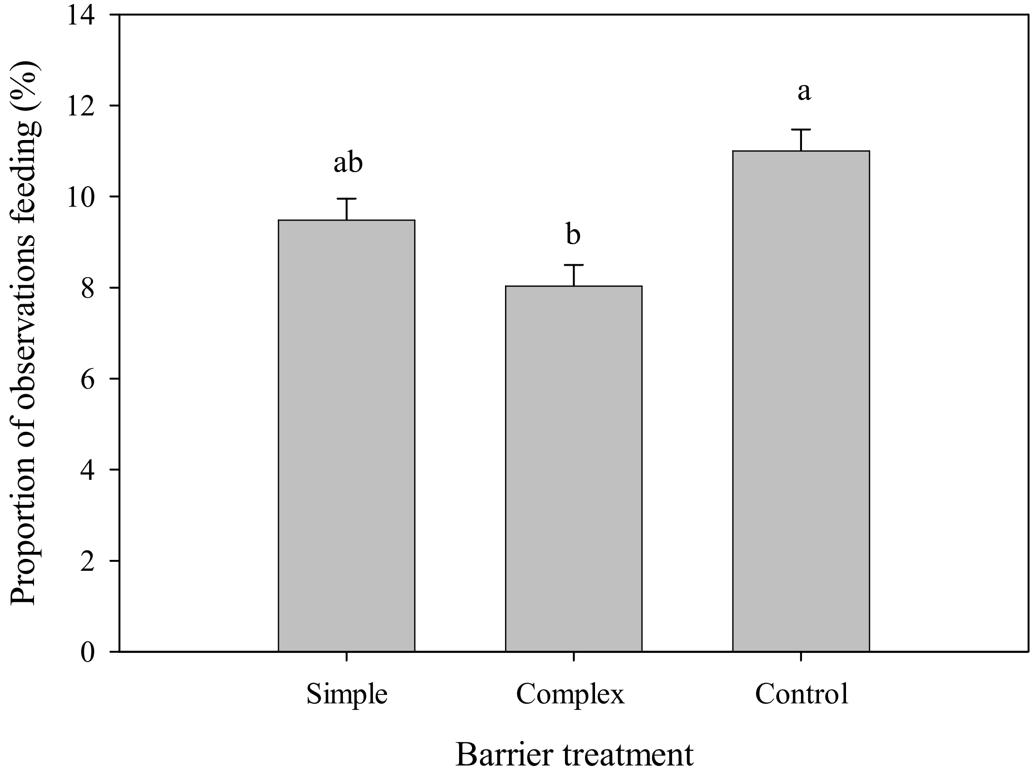


Figure 4-1 Barrier treatment effect on mean percent feeding (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

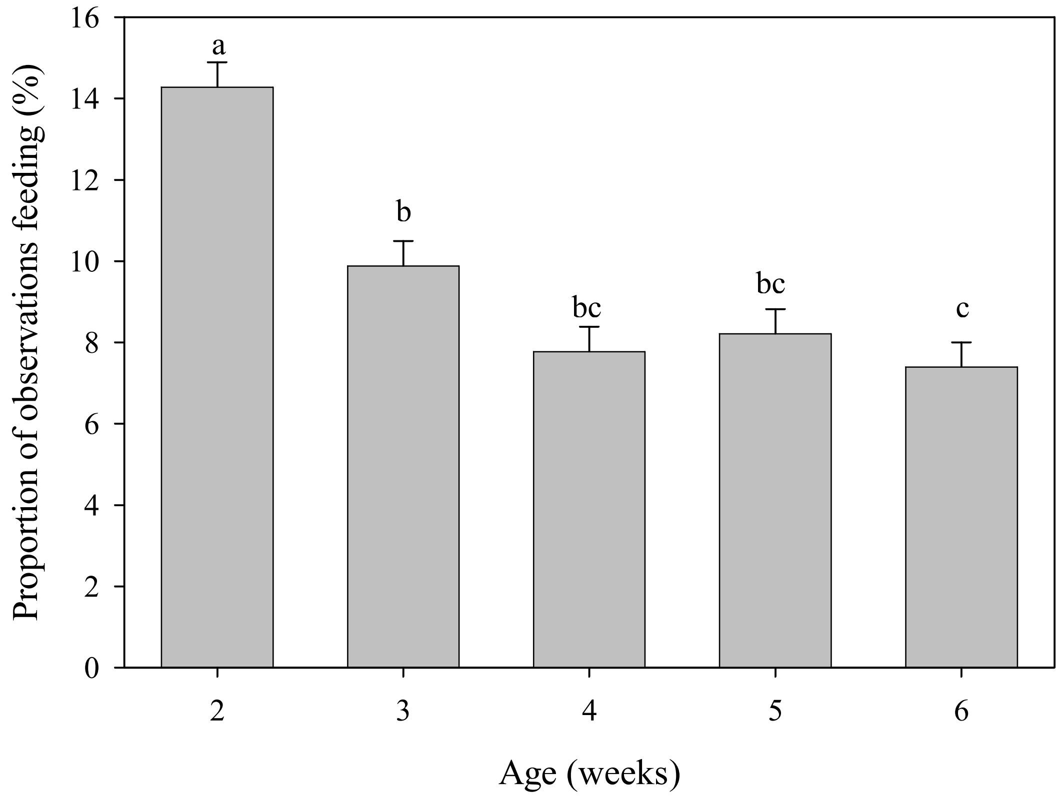


Figure 4-2 Age effect on mean percent feeding (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

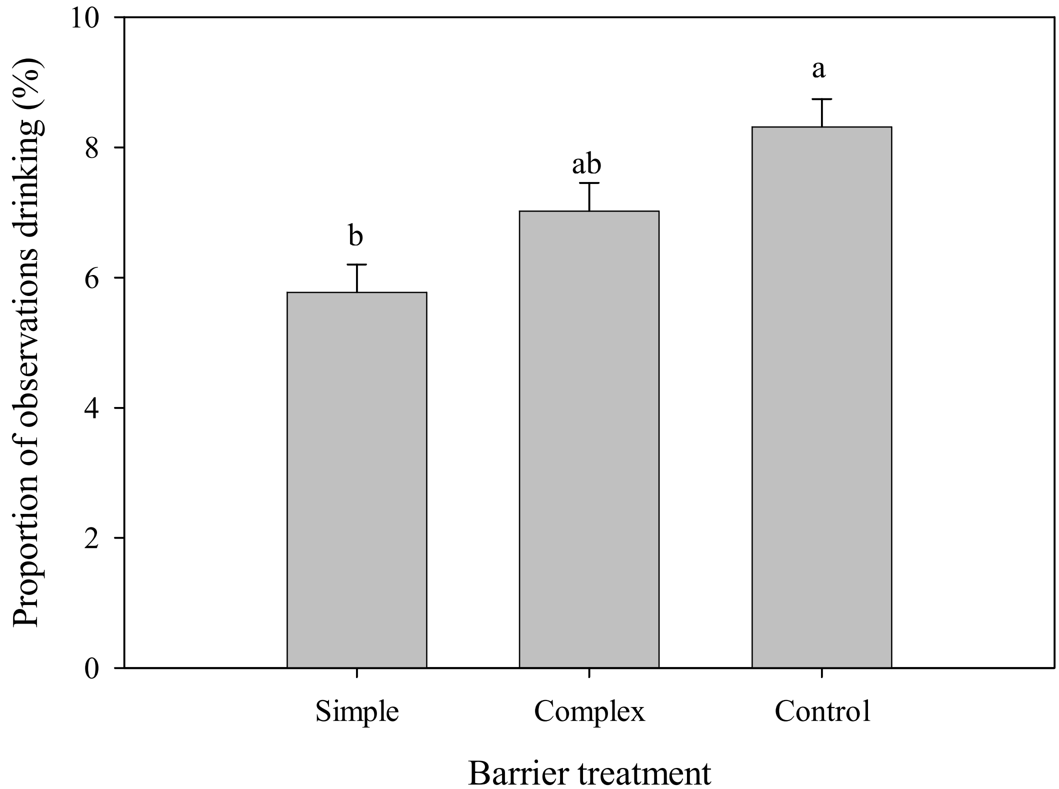


Figure 4-3 Barrier treatment effect on mean percent drinking (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

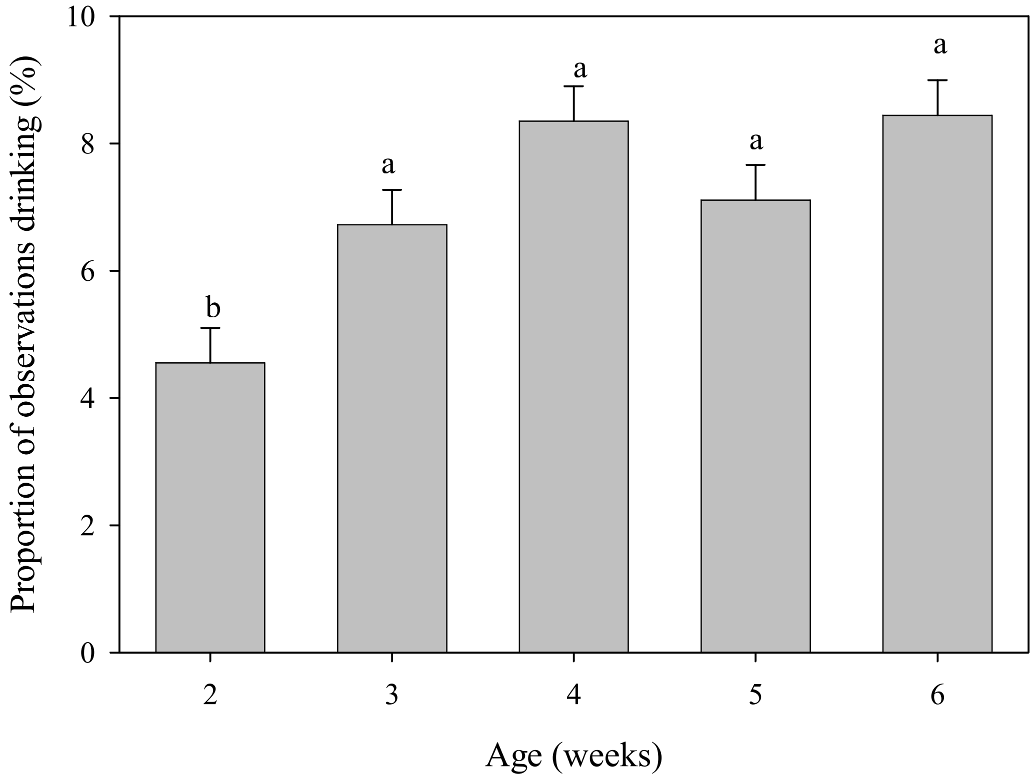


Figure 4-4 Age effect on mean percent drinking (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

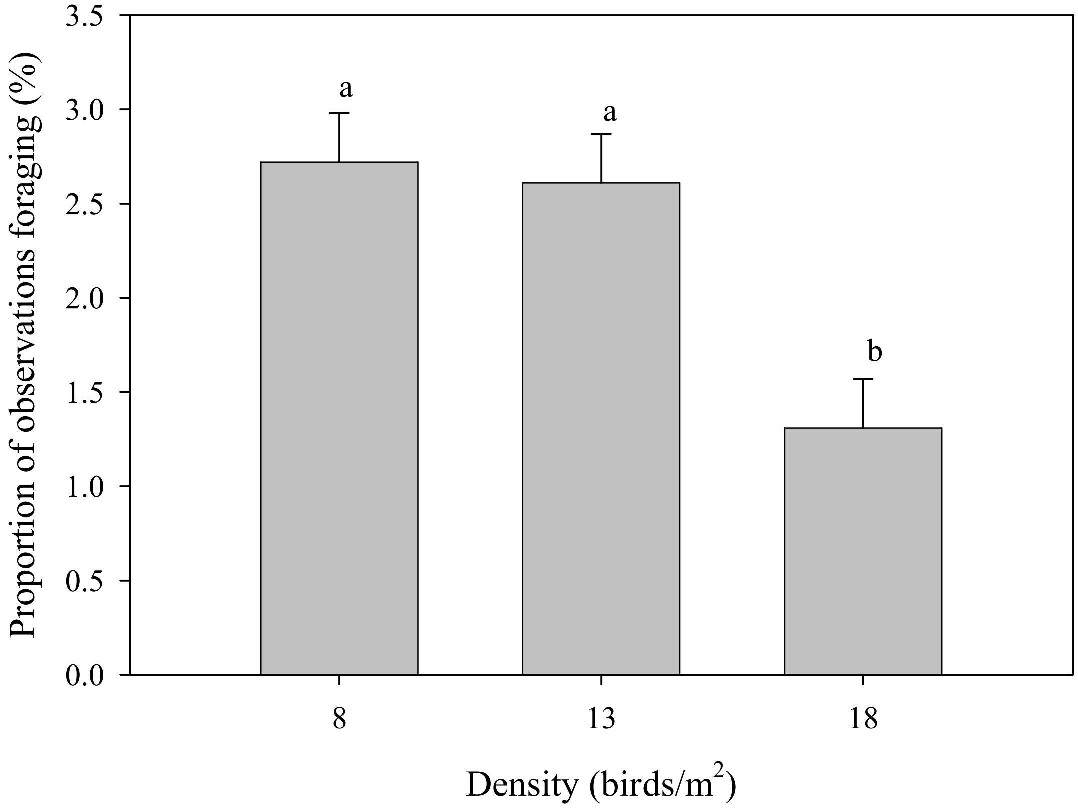


Figure 4-5 Density effect on mean percent foraging (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

Activity

Barrier treatment did not affect the proportion of time that birds were observed sitting. However, there was a significant interaction between density and age ($F_{8,108}$=3.57, *P*=0.001; Fig. 4-6), mostly due to a high proportion of sitting during week 5 in the 18 birds/m^2 pens compared to the 8 birds/m^2 pens (*P*=0.015).

In the low density pens, sitting declined from 2 to 4 weeks of age (*P*=0.014) and fluctuations in sitting from week to week were mildest in this density treatment. In contrast, in the moderate density treatment sitting declined from week 2 to weeks 3 and 4 (P<0.0001 for both comparisons) and then rose again by week 6 (*P*<0.0001 for week 3 vs. 4 and 4 vs. 6 comparisons). The same general pattern was also evident in the high density pens: sitting was significantly higher during the 2^{nd}, 5^{th}, and 6^{th} weeks compared to weeks 3 and 4 (*P* at least <0.05 for all comparisons).

Though the proportion of observations of standing was not significantly affected by density, barrier treatment ($F_{2,27}$=15.68, *P*<0.0001; Fig. 4-7) and age ($F_{4,108}$=26.19, *P*<0.0001; Fig. 4-8) were both significant. Broilers in control pens were observed standing significantly more than individuals in simple and complex barrier pens (*P*=0.0001 for both comparisons). Regarding age effects, birds stood significantly less during week 2 as compared with all subsequent weeks, with the highest frequency of standing occurring during week 4 (*P* at least <0.005 for all comparisons).

Proportion of observations of walking was affected by density ($F_{2,27}$=6.45, *P*=0.005; Fig. 4-9) and age ($F_{4,108}$=8.23, *P*<0.0001*;* Fig. 4-10) but not by barrier treatment. Broilers were observed walking more often in the low density treatment as compared to either moderate (*P*=0.021) or high density (*P*=0.007) pens. Regarding age effect, walking peaked during week 4 and then dropped significantly by weeks 5 ($P<0.0001$) and 6 ($P=0.0001$). The decline in walking by the last two weeks of observations was such that week 5 and 6 rates were also significantly lower than walking during the first week of collection ($P=0.018$, $P=0.020$).

The proportion of time that birds were observed running was not significantly affected by density. However, barrier treatment had an effect ($F_{2,26}=4.17$, $P=0.027$; Fig. 4-11) such that birds in the simple barrier treatment were observed running less often than birds in the control treatment ($P=0.022$).

On the other hand, perching frequency was not affected by barrier type, though a clear density by age interaction effect was detected ($F_{8,72}=5.09$, $P<0.0001$; Fig. 4-12). The general trend for all density treatments was for perching to rise during the first few weeks of observation, peak at week 4, and decline thereafter. Perching peaked for all densities at week 4 and ranged from 19.39 to 24.15 ± 1.52% of the time, depending on density treatment. Perching in the highest density treatment declined significantly earlier (between 4 and 5 weeks of age, $P=0.041$) than it did in the lowest density treatment (between 5 and 6 weeks of age, $P<0.0001$).

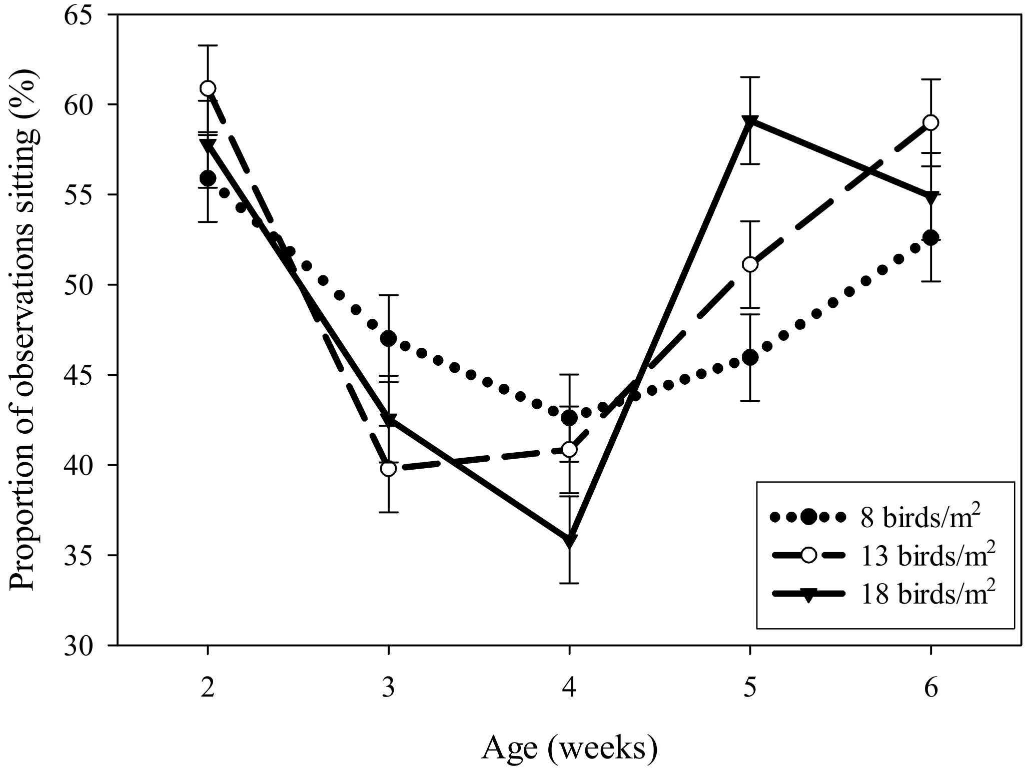


Figure 4-6 Density by age interaction effect on mean percent sitting (LSM ± SEM)

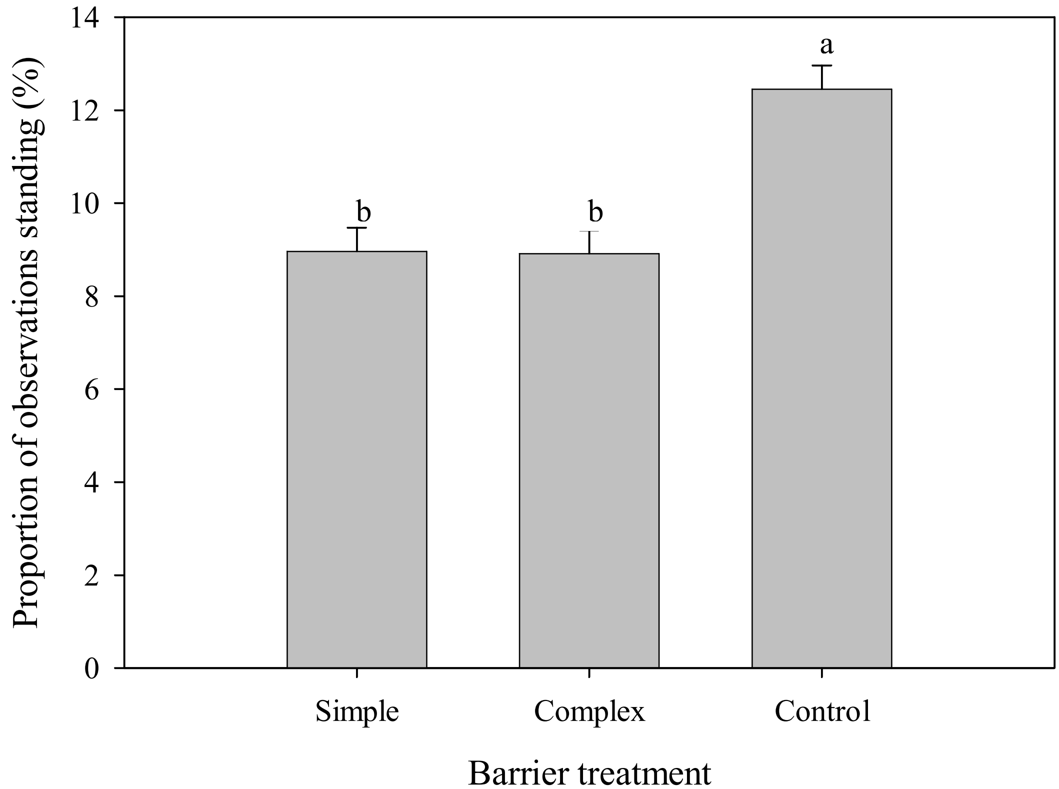


Figure 4-7 Barrier treatment effect on mean percent standing (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

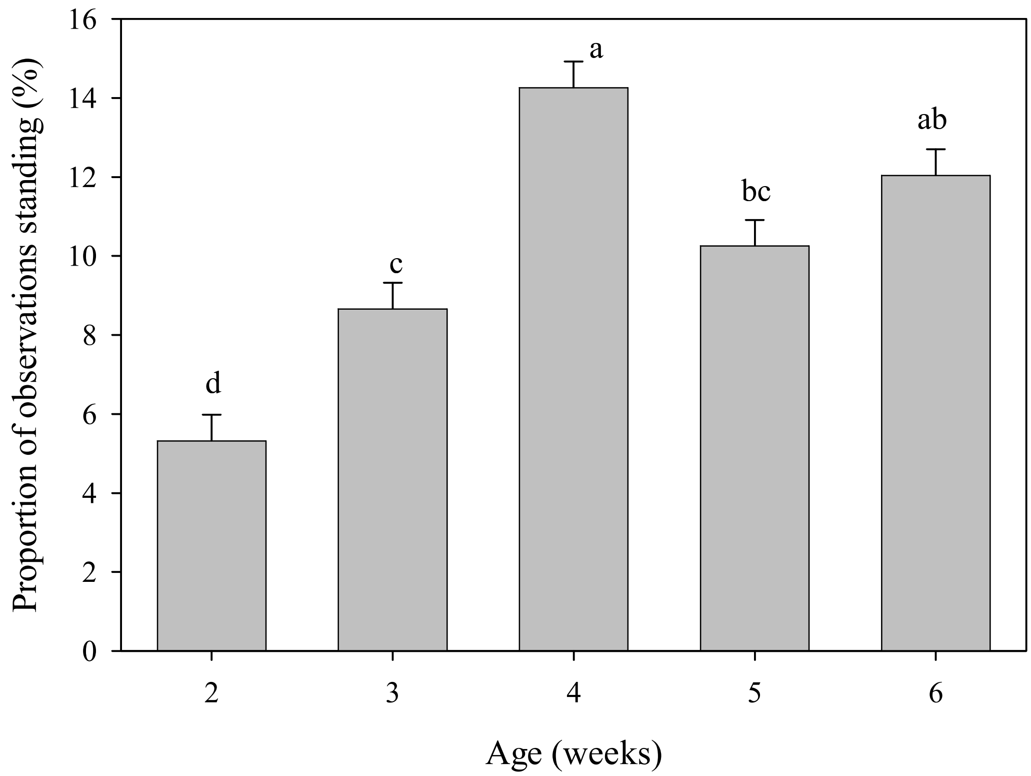


Figure 4-8 Age effect on mean percent standing (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

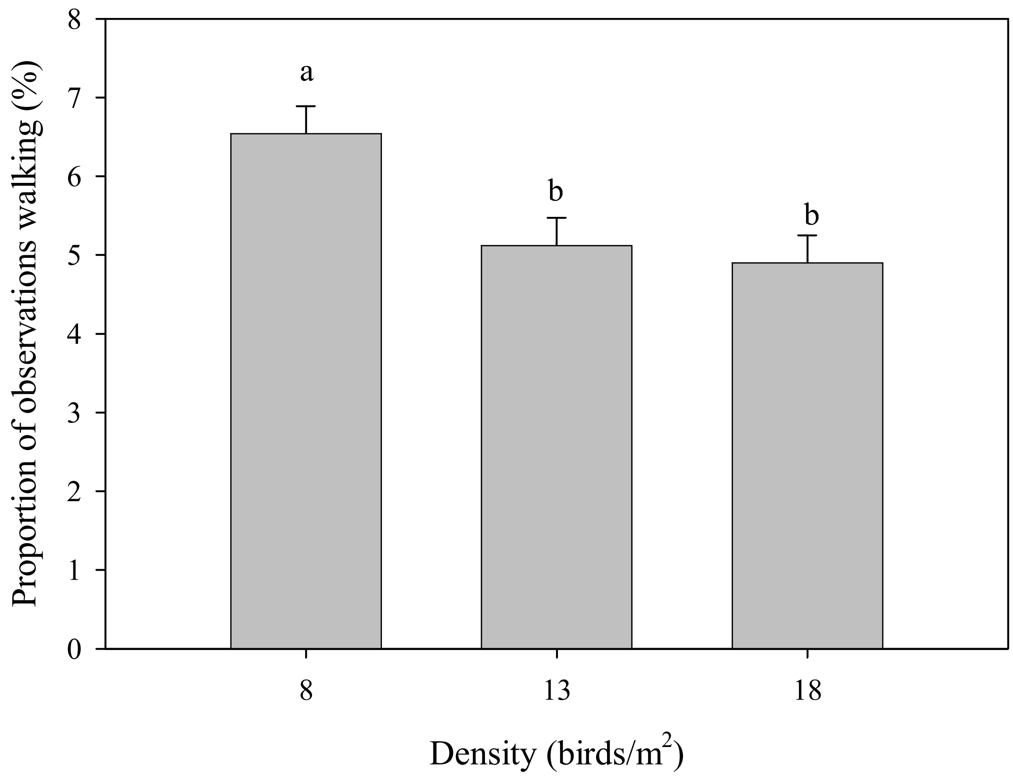


Figure 4-9 Density effect on mean percent walking (LSM ± SEM)
Bars with different letters are significantly different, p<0.05, after Tukey's adjustment.

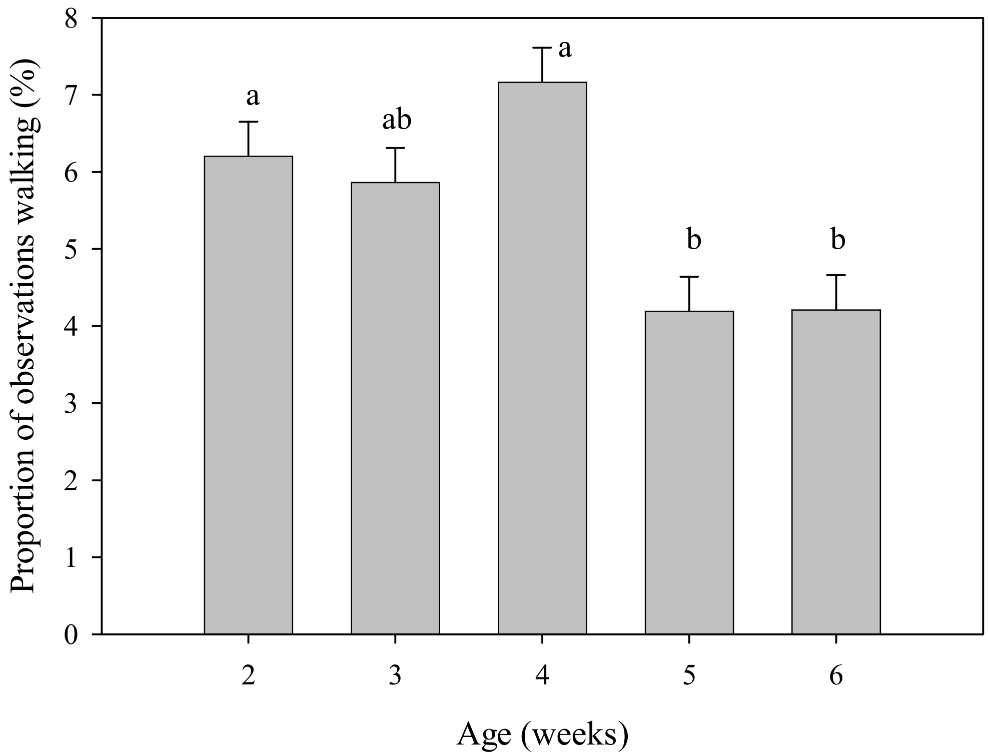


Figure 4-10 Age effect on mean percent walking (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

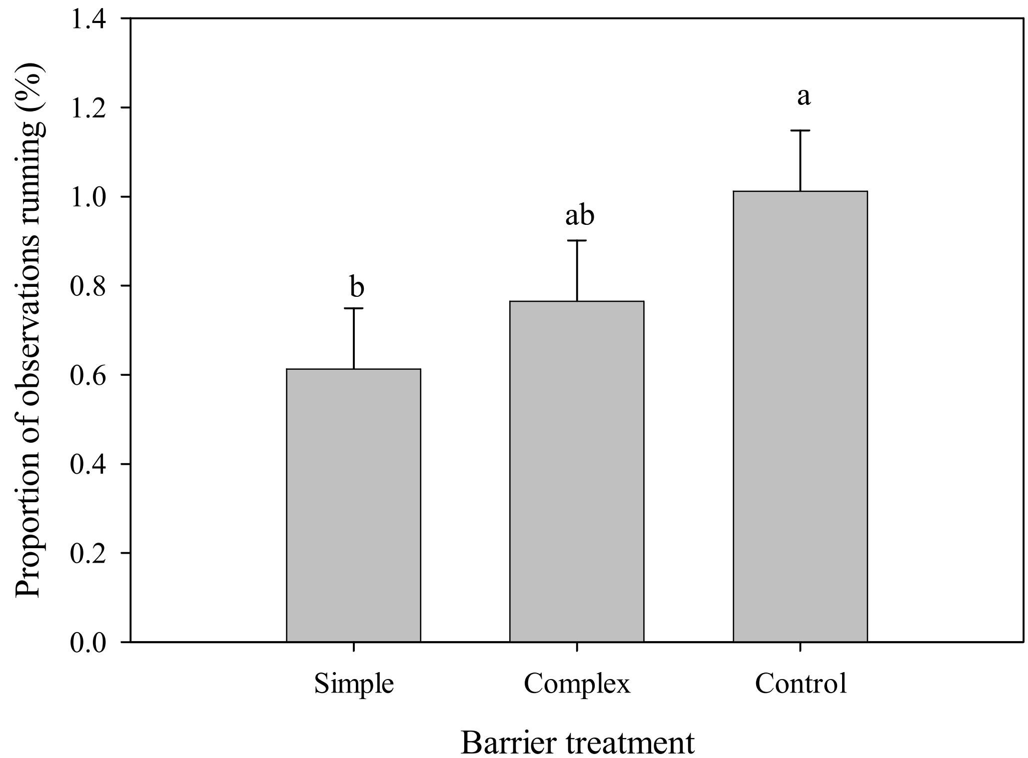


Figure 4-11 Barrier treatment effect on mean percent running (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

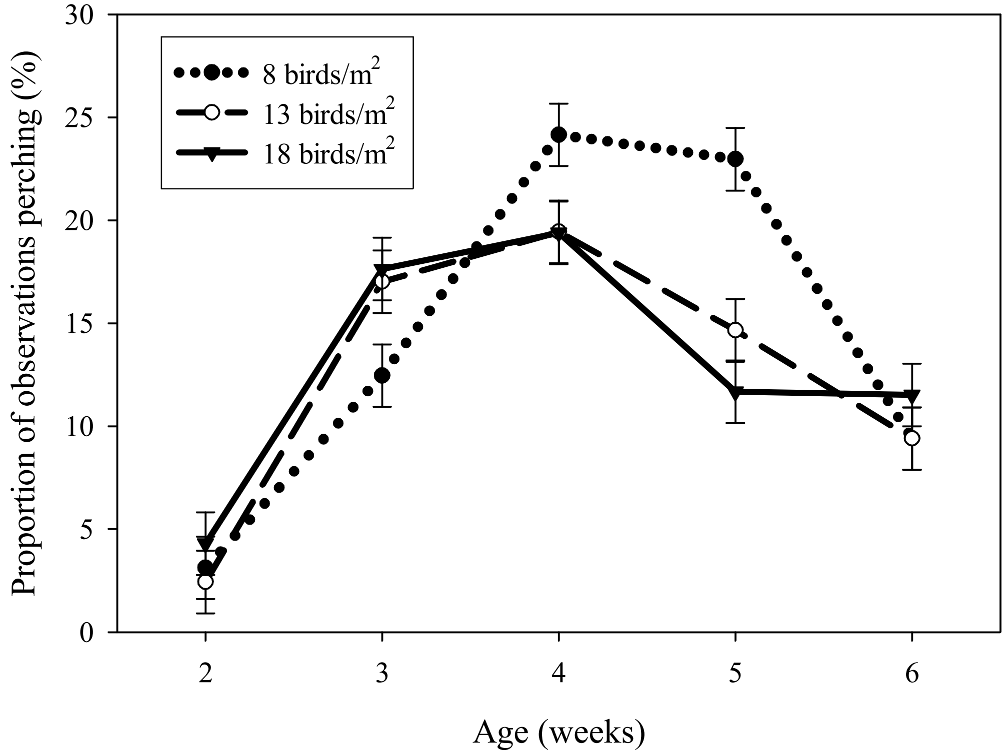


Figure 4-12 Density by age interaction effect on mean percent perching (LSM ± SEM)

Aggression and Disturbances

The proportion of observed aggressive interactions was unaffected by density. However, barrier treatment had a clear effect ($F_{2,26}$=12.30, *P*<0.001; Fig. 4-13), with birds in the control treatment observed engaging more frequently in aggression compared to individuals in either the simple or complex barrier treatment (*P*=0.016 and *P*<0.001, respectively).

Rate of disturbances did not depend on barrier type *per se*, though a significant barrier treatment by density interaction effect was observed on the proportion of observations that birds were seen being disturbed by a conspecific ($F_{4,26}$=3.28, *P*=0.027; Fig. 4-14). Disturbance frequency was highest for the control treatment and generally increased at higher densities. The rise in disturbances as density increased was most pronounced in the control pens, particularly from 13 to 18 birds/m^2.

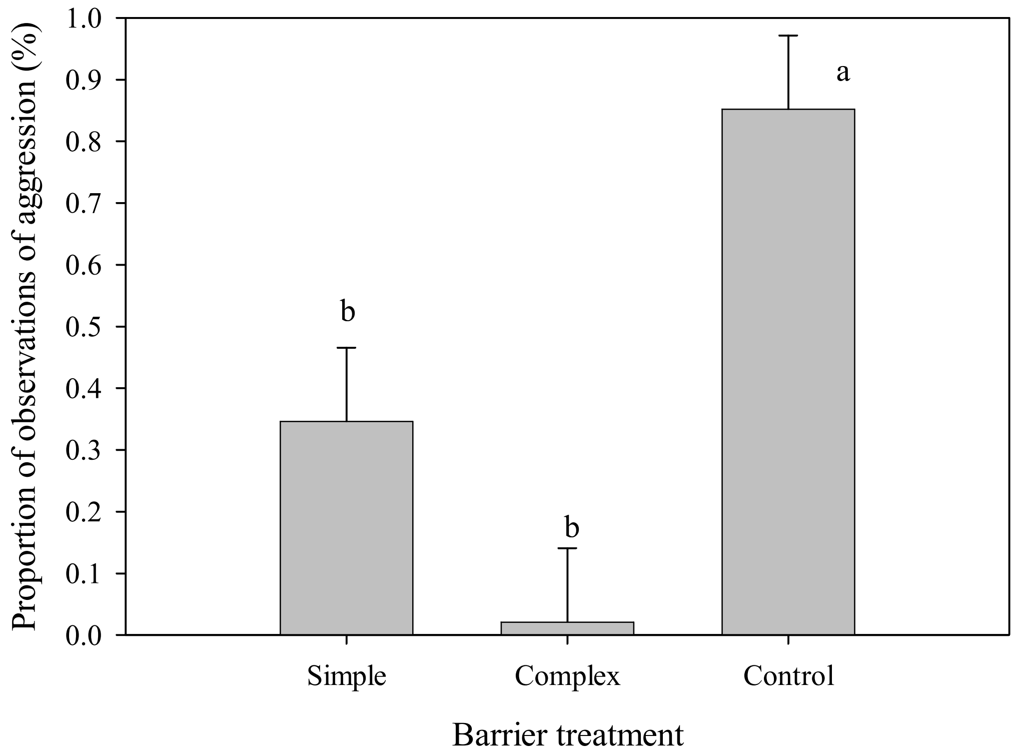


Figure 4-13 Barrier treatment effect on mean percent aggression (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

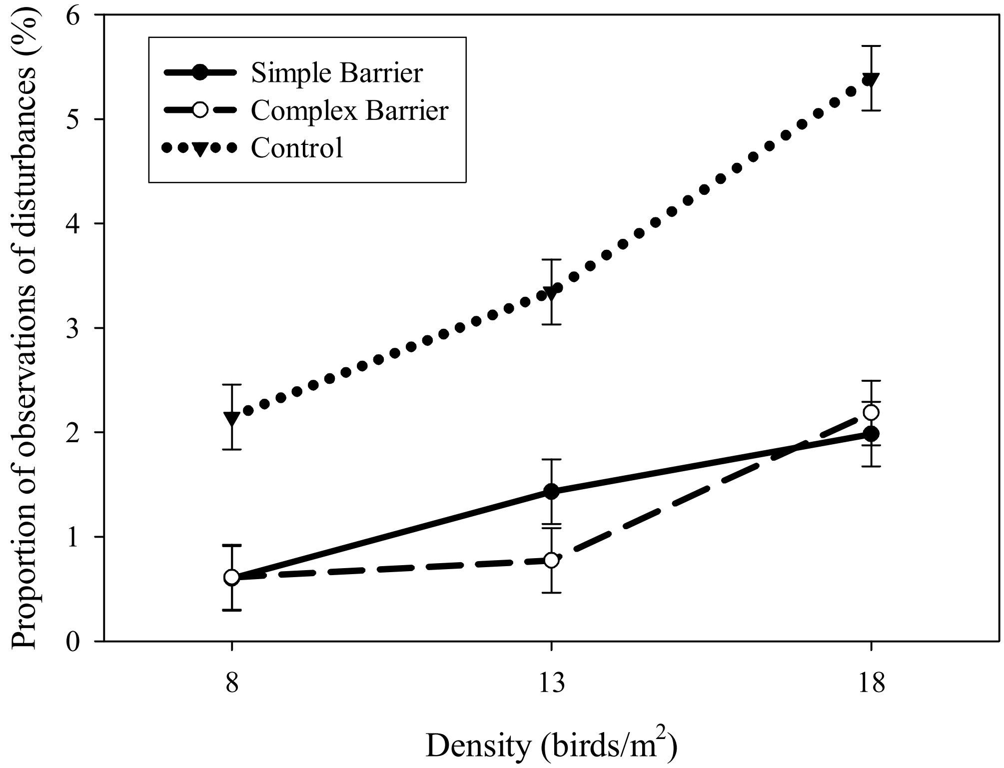


Figure 4-14 Barrier treatment by density interaction effect on mean percent disturbances (LSM ± SEM)

Preening

The proportion of observed preening was not influenced by either barrier or density treatment but was significantly affected by age ($F_{4,108}$=13.44, *P*<0.0001*;* Fig. 4-15), with a peak in preening observed at 3 weeks of age (*P*<0.0001 for comparison with weeks 2, 5, and 6; *P*<0.001 for comparison with week 4).

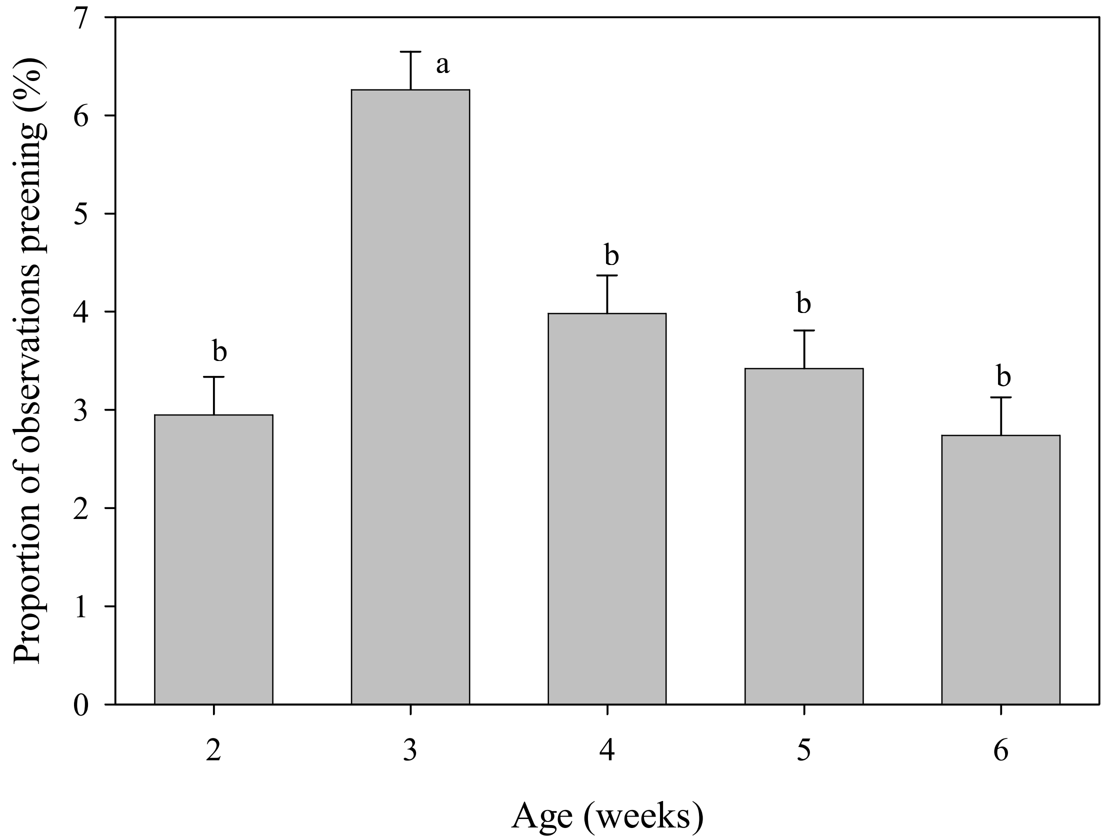


Figure 4-15 Age effect on mean percent preening (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

4.4.2 *Use of space*

Bird distribution in space was significantly affected by the presence of barriers in the pen ($F_{2,27}$ = 8.46, *P*=0.001; Fig. 4-16). Proportion of observations in the central area was significantly increased by addition of the simple barriers compared to control treatment pens (*P*<0.001). Time spent in the central area was also higher in the complex barrier pens compared to the control treatment pens, though this difference was not significant.

Space use was also affected by density ($F_{2,27}$=5.70, *P*=0.009; Fig. 4-17). Use of the central area was greatest in the 8 birds/m^2 treatment compared to the 13 birds/m^2 (*P*=0.016) as well as the 18 birds/m^2 treatment (*P*=0.021). Time spent in the pen center was not significantly different between the 13 and 18 birds/m^2 treatments.

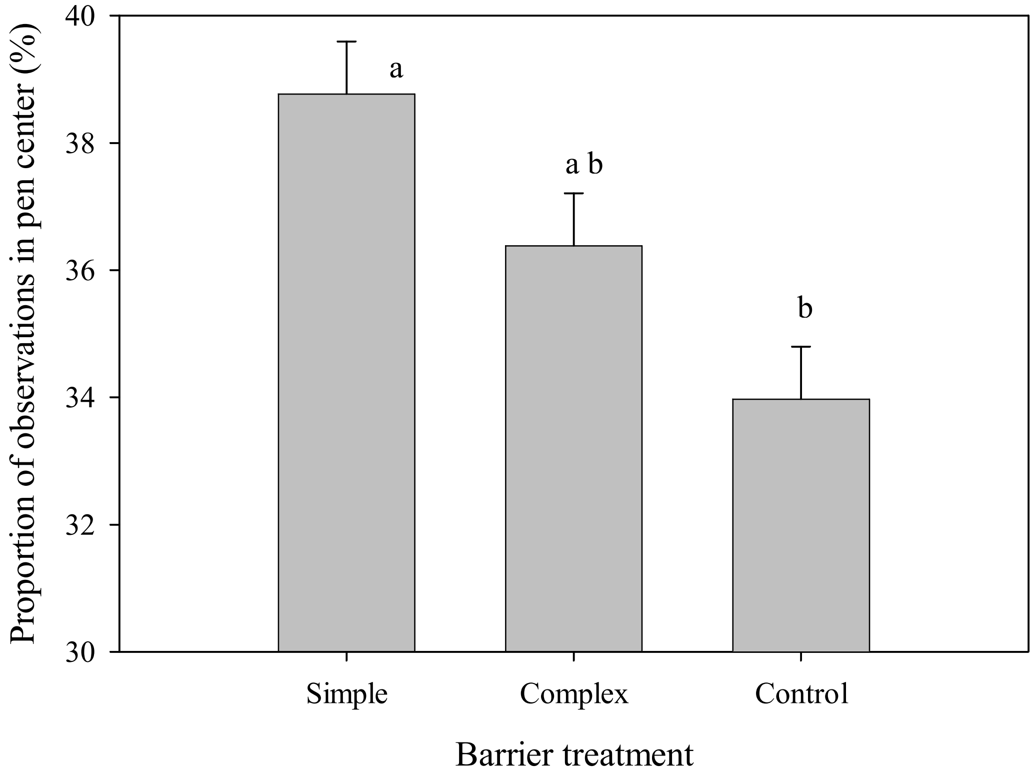


Figure 4-16 Barrier treatment effect on mean proportion of observations in pen center (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

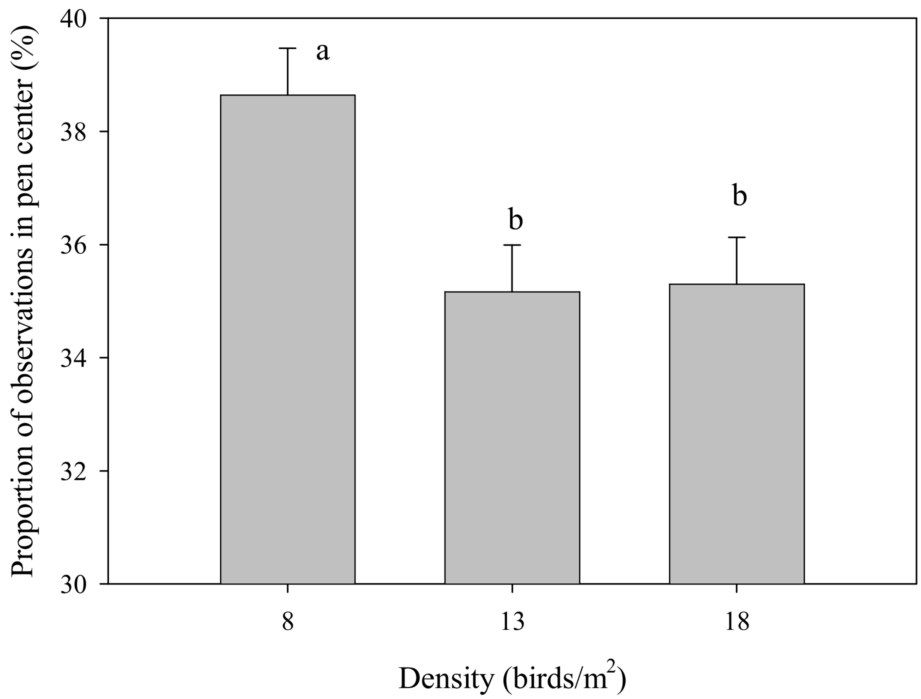


Figure 4-17 Density effect on mean proportion of observations in pen center (LSM ± SEM)
Bars with different letters are significantly different, $p<0.05$, after Tukey's adjustment.

4.5 Discussion

4.5.1 Behavior

Increasing the complexity of the environment by adding barrier perches had a clear effect on the behavioral repertoire of broilers in this study, most notably by providing broilers an opportunity to perch, which was not possible in control pens. Foraging, sitting and preening were not affected by the presence of barrier perches, but appetitive and activity behaviors, along with behaviors related to aggression and disturbances, were substantially affected. For example, birds fed more often in control pens than did those raised in complex barrier pens, in contrast to the lack of difference between barrier and control treatments reported by Bizeray et al. (2002a). A potential explanation for the difference between these two studies is that access to the feeder was better facilitated in the control pens as opposed to the complex barrier pens. Since the simple barrier perches (which were adapted from the barriers used by Bizeray et al., 2002a) did not impact feeding behavior differently from the control pens, it is possible that the extra arms of the complex barriers may have impeded flow, preventing birds from visiting the feeder more often. However, since complex barriers did not have a negative impact on feed conversion or final body weights, it appears that birds in these pens eventually compensated for visiting the feeder less often, perhaps by becoming more efficient in their intake while at the feeder. Regarding drinking behavior, the present results confirm Bizeray et al.'s (2002a) findings that birds in control pens drank more often than those raised with simple barrier perches. It is possible that simple barriers impeded access to drinkers, though if this were true we might have expected to observe a similar or even more pronounced decline in drinking in the complex barrier pens, which was not the case.

In this study birds raised in control pens spent more time standing than did birds in either of the barrier perch treatments, results that are in contrast to Bizeray et al.'s study (2002a), which found no effect on standing. The reasons for this discrepancy are unclear, though the present results could be related to the varying experimental densities used. Nevertheless, the general reduction in standing in the enriched pens compared to control pens was probably related to the additional behavioral opportunities afforded by the barrier perches, therefore reducing time spent standing.

Notably, the presence of barrier perches also had a strong impact on the rate in which birds engaged in aggressive interactions and disturbances. Both aggression and disturbances occurred more frequently in the control pens than in either type of barrier treatment. These results are in line with findings from Cornetto and Estevez (2002), who reported that provision of enrichment in the form of cover panels as enrichment was an effective strategy to reduce disturbances and potentially also aggression, though in that study the effect of cover panels on aggression was not significant. It has been indicated that aggressive interactions occur mainly in open areas of the enclosed environment (Cornetto et al., 2002; Pettit-Riley et al., 2002). It is probable that the presence of barrier perches reduced the occurrence of these behaviors by breaking up the open space that provides a prime location for aggression. This idea is supported by the finding that the proportion of observed aggressive interactions was lowest in the complex barrier pens, which technically leave the least amount of open space due to the presence of the extra arms of the complex barrier. Alternatively, barrier perches may have provided an effective refuge for birds to escape aggression. A similar effect of open space availability may also explain the differences found between control and simple barrier treatments for the occurrence of running in this study, as running sometimes occurred prior to the initiation or after the resolution of aggressive interactions (Ventura, personal observation). On the other hand, running may have been more facilitated in the control treatment where birds were not required to navigate around barriers.

Although much attention has focused on the occurrence of aggressive behavior, disturbances are also highly relevant to bird welfare as well as to production because disturbances interrupt resting time (Murphy and Preston, 1988; Cornetto et al., 2002) and have a negative effect on carcass quality (Frankenhuis et al., 1991). Birds walking over the backs of other individuals may inflict scratches and subsequent infection, thus explaining the decrease in carcass quality (Proudfoot et al., 1979; Cravener et al., 1992; Estevez, 2007). It is evident from this study that the occurrence of disturbances was directly related to density, with effects being particularly clear in control pens as density increased from 13 to 18 birds/m^2. These results seem to provide further evidence that rearing densities beyond 14 to 16 birds/m^2 may compromise broiler welfare (Estevez, 2007). Interestingly, the impact of density on the occurrence of disturbances was diminished in enriched pens compared to controls. Disturbances occur more frequently as density increases (Lewis and Hurnik, 1990; Cornetto et al., 2002) as chickens look for areas near the walls to rest (Newberry and Hall, 1990; Cornetto et al., 2002). It is likely that barrier perches have a similar effect of 'adding' additional wall space, reducing potential competition for prime resting locations and therefore attenuating the negative repercussions of high density on disturbances in the enriched pens. Alternatively, the effect of barrier perches may also be related to a more homogeneous bird distribution in enriched pens.

In addition to these combined effects with barrier treatment, density also had a direct impact on the occurrence of foraging and walking. However, no effects of density were detected on behaviors like feeding, drinking, standing and preening, a finding which largely agrees with past work (Cornetto and Estevez, 2001a; Arnould and Faure, 2004; Estevez 2007). Likewise, aggression was unaffected by high density, which is similar to findings by Estevez et al. (1997). A suppressive effect of density on foraging was detected at 18 birds/m^2, whereas the reduction in walking frequency appeared for densities at and above 13 birds/m^2. The reduction in foraging at higher densities supports past work by Hall (2001) and may relate to a reduction in floor space and subsequent opportunity to walk and forage, as density clearly constrains how well animals can use the available space (Leone and Estevez, 2008c). Alternatively, foraging may be discouraged in high density environments where litter quality declines most severely and rapidly (Berg, 1998), as poor quality litter may not lend itself as an attractive rooting substrate.

Age, and particularly the combined effects of age with density or barrier treatment, had clear effects on broiler behavior. In general, appetitive behaviors and activity were strongly affected by age or by the interaction of age with barrier treatment or density. Birds in the present study fed most frequently during the second week of age, while drinking was lowest during this period. The pattern in feeding may be related to the availability of additional pan feeders during that time, which meant that birds encountered feeding opportunities more often. The lower drinking frequency during that week may relate to the fact that birds were still learning to navigate the pen space and find their way to the drinker, or it may be due to the demands of increased body size as birds aged.

Age is well known to affect activity levels in broilers (Bizeray et al., 2000; Cornetto and Estevez, 2001a; Pettit-Riley and Estevez, 2001; Bizeray et al., 2002a). When birds become more active they necessarily allocate proportionately less of their time toward more inactive behaviors like sitting. In this study the reduction in sitting during weeks 3 and 4 thus translated into peaks in activity during this period, which has also been reported in other studies (Estevez et al., 1997; Bizeray et al., 2002a). In the current study, as with past research (Le Van et al., 2000; Bizeray et al., 2002a), activity peaks resulted from high levels of perching and walking, especially during week 4 compared to later weeks. The observed rise in sitting and decline in walking and perching during the final weeks is likely explainable by rapid broiler growth rates. Previous authors have repeatedly linked the disproportionately high growth rate of broilers with declining activity levels (Faure and Jones, 1982; SCAHAW, 2000; Reiter and Kutritz, 2001). It is a feasible assumption that the decrease in activity after the fourth week is a symptom of this trend toward inactivity as birds age.

However, it is interesting to note that broilers in this study spent less time sitting (see Fig. 4-6) compared to previous studies (Murphy and Preston, 1988; Weeks et al., 2000; Cornetto and Estevez, 2001a). High frequencies of sitting were most prominent in the highest density treatment during week 5. A possible explanation for this is that 18 birds/m^2 surpasses an important threshold in space allowance such that birds housed in this condition do not have adequate space to properly navigate the pen and perform other locomotive behaviors (Estevez, 2007). Also, since it is shown elsewhere that birds in the high density are afflicted with more serious footpad lesions, it is possible that these lesions (or other lameness issues) may have contributed to more frequent sitting earlier in life.

A high frequency of use of the barrier perches was observed in the current study (See Fig. 4-12), although frequency of use declined as age and density increased. Use of the barrier perches was similar across simple and complex barrier treatments, suggesting that both the simple and complex barrier designs were equally attractive, equally accessible, or both. Perching peaked at 4 weeks of age for all density treatments and remained high through week 5 in the lowest density treatment. For example, birds were observed perching nearly one-quarter of the time in the 8 birds/m^2 treatment during week 4. While high perching rates (up to 27%) using higher, more traditional perches have been reported for broilers in the past (Hughes and Elson, 1977), those results come from broilers of 30 years ago, which represent a much different bird than modern broilers in terms of weight and growth rate. More recent reports from Bizeray et al. (2002a) and particularly Le Van et al. (2000), Pettit-Riley and Estevez (2001), and Su et al. (2000) have reported appreciably lower perching rates throughout the rearing period. Additionally, Pettit-Riley and Estevez (2001) reported a stimulating effect of increased density on perch use, whereas here we report that perch use was lower at higher densities. Available perching space may have been somewhat limited in this study at the higher densities as chickens grew larger in size. It is possible that increasing the amount of barrier space may have mitigated the negative effects of density observed in the present study. Interestingly, by week 6, perching had declined to around 10% for all densities, suggesting that by this stage of the rearing cycle even low densities can only go so far in mitigating the effects of increased body size on broiler activity levels.

Contrary to prior speculation (Pettit, 2000), broilers seem to have retained the ability and motivation to demonstrate perching behavior, provided that a proper, better-suited substrate is offered. It is likely that the low height of the barrier perches used in this study translated into higher accessibility and thus greater use. These results suggest that enrichment via barrier perch provision encourages perching behavior, which may translate into improved foot and leg health and decreased aggression and disturbances, thus improving bird welfare.

4.5.2 Use of space

Use of space by broiler chickens is not uniform, with aggregations occurring near walls and other objects that provide a sense of cover and protection (Newberry and Hall, 1990; Cornetto and Estevez, 2001b) as well as near important resources like food and water (Arnould and Faure, 2004). Clustering around the pen periphery can lead to a range of problems including increased disturbances along the pen periphery (Cornetto et al., 2002), interruptions in resting time (Murphy and Preston, 1988), increased aggressive interactions (Cornetto et al., 2002), and exacerbated heat stress (Estevez, 1999; Dozier et al., 2005). The results of this study indicate that providing simple barrier perches affected the spatial distribution of broiler chickens in a way that promoted a more even use of pen space. There are two probable reasons for this effect: first, barrier perches unequivocally attracted birds toward the central space by offering additional behavioral opportunities in the form of perching, opportunities which were located in the pen center as opposed to the periphery. Secondly, as discussed in earlier sections, barrier perches created additional wall space, which may also afford a sense of cover and protection similar to what has been observed with vertical cover panels (Cornetto and Estevez, 2001b), thus providing additional incentive for birds to come into the center of the pen. As indicated earlier, higher occupancy of the central area and subsequent reduction in open space availability is a potential mechanism of action that underlies the decrease in aggression and disturbances in enriched pens in this study.

Regarding effects of density on use of space, proportion of use of the central area was highest at 8 birds/m^2 and declined thereafter as density increased. It is unclear where the critical density cutoff lies in promoting more even use of the pen, though in this study it appears to be between 8 and 13 birds/m^2. In any case, this is lower than the densities traditionally used in commercial production and might serve as additional recommendation that housing birds at lower stocking densities promotes a higher state of welfare, which is in line with past research reporting adverse effects of increased stocking density on health and welfare (Proudfoot et al., 1979; Cravener et al., 1992; Heckert et al., 2002; Pettit-Riley et al., 2002; Dozier et al., 2006; Estevez, 2007).

CHAPTER 5: SUMMARY AND CONCLUSIONS

Natural environments are characterized by a high degree of complexity which governs all aspects of animal life, from resource acquisition and predator avoidance to mate acquisition and spatial navigation. However, environmental complexity is limited in large-scale poultry production facilities, where required travel and foraging effort are generally low and bird movement and activity is hampered in part by high stocking densities (Newberry and Hall, 1990; Estevez et al., 1997; Estevez and Christman, 2006), resulting in inactivity and restriction of natural behavior (SCAHAW, 2000). The goal of this project was to discern whether increasing environmental complexity by providing barrier perches at different stocking densities could improve broiler welfare, in part by encouraging activity, promoting better use of the pen space, improving leg and foot condition, and alleviating fear levels.

Throughout this project we offered evidence that housing broilers at higher densities had a detrimental effect on broiler health and welfare. In the first part of the experiment, measures of leg condition and foot health confirmed that increasing stocking density from 8 to 13 and 18 birds/m^2 resulted in higher fluctuating asymmetry of tibia length and declining footpad and hock health as density increased. These findings are in line with others who have reported similar adverse effects of increased density on both fluctuating asymmetry of tibia length (Van Poucke et al., 2007) as well as on footpad health (Martland, 1985; Berg, 1998; Arnould and Faure, 2004; Dozier et al., 2006; Haslam et al., 2007). The resultant increase in severity of footpad and hock lesions as density increased is particularly pertinent to broiler welfare because lesions contribute to lameness and likely cause pain.

Though density did not have a substantial impact on fear levels as measured by required TI attempts and TI duration, the behavioral measures taken during the second phase of the experiment further confirmed that increasing density had undesirable repercussions for broiler behavior and welfare. Results showed that housing birds at higher densities promoted inactivity, as shown by declining foraging activity when density increased from 13 to 18 birds/m^2. Notably, walking and perching declined and sitting increased during the end of the rearing period (especially weeks 5 and 6) for densities above 8 birds/m^2. As in previous studies (Lewis and Hurnik, 1990; Hall, 2001; Cornetto et al., 2002) the frequency of disturbances was higher as density increased. This is an undesirable behavior that can have consequences for the birds' welfare status, as disturbances have been shown to interrupt resting periods (Murphy and Preston, 1988) and are responsible for a higher frequency of scratches which increase the risk of infection (Frankenhuis et al., 1991). This last effect could have resulted from how density affected broiler use of the available space. Increased density suppressed bird distribution throughout the pen, as seen by the fact that the percentage of birds using the central space was lower in the higher experimental densities compared to the lowest density. Enhancing use of the central space decreases the opportunity for birds to engage in aggressive interactions because underused open spaces provide prime sites for aggressive displays (Cornetto et al., 2002; Pettit-Riley et al., 2002). Furthermore, a more uniform bird distribution at low densities reduces aggregations near the pen periphery where birds are most prone to disturbances (Cornetto et al., 2002), which is desirable from a welfare standpoint.

According to the results of the present study, barrier perches, and particularly simple barriers, appeared to provide some health and welfare benefits to broilers. Foot condition appeared to improve when birds had access to simple barrier perches, as seen by the low frequency of ‘poor’ footpad lesions that characterized this treatment. We also demonstrated that birds reared with complex barrier perches exhibited lower levels of fluctuating asymmetry of tibia length than birds raised without barrier perches, suggesting that birds in the complex barrier treatment may have been less affected by developmental stress. However, complex barriers appeared to have a damaging effect on footpad health compared to the control pens, potentially because the extra arms of the complex barrier occupied increasingly precious space reserves as birds grew, which in turn compromised foot health. This may override any positive effects that the complex barriers had on fluctuating asymmetry, as footpad lesions can be considered to have a more direct impact on broiler welfare. However, the present results demonstrated that this was not an issue with the simple barrier perches, potentially because they occupied less space and thus may have not unnecessarily impeded bird movement throughout the pen. The beneficial impact of providing simple barriers on footpad health suggests that this form of barriers may have effectively allowed broilers to decrease their time on wet litter, particularly since poor litter quality has been noted as a primary causative factor of dermatitis lesions (Martland, 1985; Berg, 1998; Dawkins et al., 2004).

Nevertheless, it must be noted that simple barrier perches did not benefit all traits considered in this study. For example, though enrichment was expected to attenuate fear levels (Jones and Waddington, 1992; Brake et al., 1994; Jones, 1996), broilers raised with simple barriers were most easily induced into TI, suggesting that they were more fearful.

However, TI duration has been considered to be a more meaningful measure of fear levels in broilers (Forkman et al., 2007), and duration was unaffected by barrier treatment in the current study. Therefore, these results suggest that the presence of barrier perches does not appear to demonstrably affect fear levels in broilers, which is in agreement with previous findings (Bizeray et al., 2002b). However, before a more concrete conclusion can be drawn, we recommend a more thorough evaluation of the effects of barrier perches on fear levels in broilers, which may include other measures of fear such as the novel object or open field[3] tests (e.g., as in Jones and Waddington, 1992).

Results from the behavior and use of space study further substantiate the idea that barrier perches yield positive effects. The presence of both simple and complex barrier perches had a substantial effect on the behavioral repertoire of broilers, most notably by providing additional behavioral options in the form of perching, which contributed to an increase in activity levels. Standing was less frequently observed in both barrier perch treatments compared to the control treatment, but this is likely explainable by the fact that broilers reared with barrier perches allocated a substantial portion of that time toward perching. Indeed, barrier perches encouraged much higher rates of perching than what has been recorded in the past for modern broilers (Le Van et al., 2000; Su et al., 2000; Pettit-Riley and Estevez, 2001). For example, in the present study, birds were observed perching nearly one-quarter of the time in the lowest density treatment during the 4th week of age. This demonstrates that, contrary to prior speculation (Pettit, 2000), broilers have retained the ability and motivation to perch, so long as they are provided with adequate enrichment that is biologically relevant (Newberry, 1995) and suited to their

[3] It must be noted that behavior in the open field test does not stem from fear alone but is also motivated by desire (or lack thereof) for social reinstatement (Forkman et al., 2007), so conclusions from this test must be drawn carefully.

nature. It is probable that the low height of the barrier perches made them more accessible to broilers, which enabled greater use.

Though foraging, sitting and preening were not affected by barrier treatment, the effect of barrier perches on behavioral repertoire was not limited to activity levels. For example, barrier perches affected appetitive behaviors, with lower feeding frequencies in the complex barrier treatment and lower drinking frequencies in the simple barrier treatment compared to controls. These results are likely explainable by the fact that resource access may have been better facilitated in the comparatively empty control pens. More importantly, however, barrier perches had a significant impact on aggression and disturbances such that both behaviors were considerably less frequent if broilers were raised with either type of barrier perch. Because higher aggression and more frequent disturbances are undesirable (Cornetto et al., 2002), this is continued evidence that barrier perches are an effective tool for modifying broiler behavior in a positive manner.

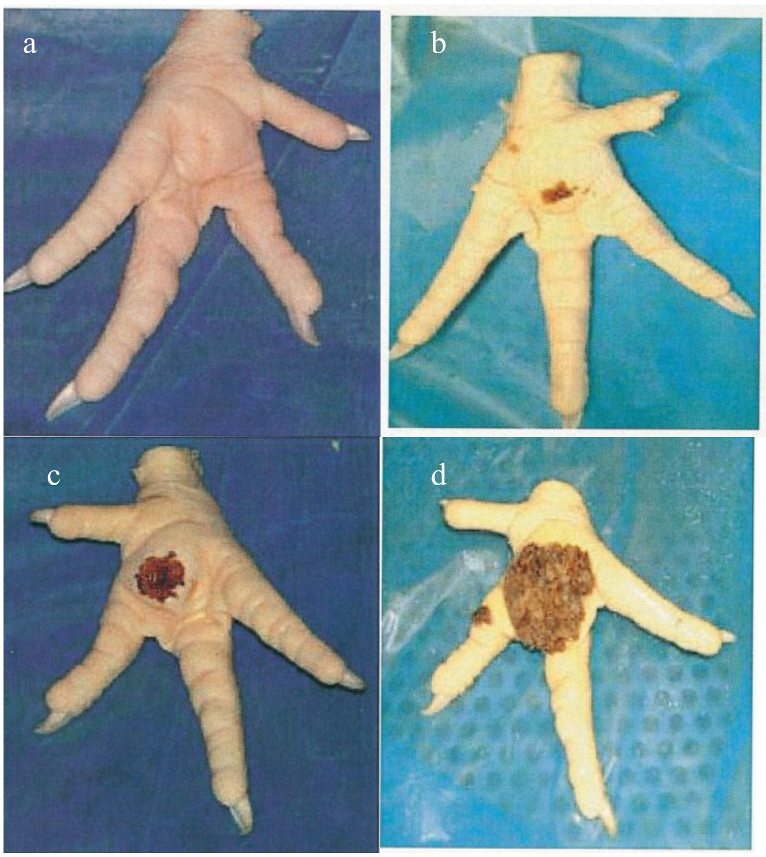
Again, as with density effects on disturbances, the decrease in disturbances and aggression in the enriched pens can be explained by how barriers affected bird distribution throughout the pen. In comparison with control pens, use of the central space was increased when simple barrier perches were provided, suggesting that simple barriers attract birds into the center of the pen in a similar fashion to what has been observed with the use of cover panels (Cornetto and Estevez, 2001b). In doing so, the simple barriers used in the present study decreased the concentration of individuals around the periphery, thus decreasing disturbances and aggression.

The fact that feed conversion, total percent mortality, and final body weight were unaffected by any of the experimental treatments, including barrier perch, indicates that barrier perches did not adversely affect production levels. This is a promising result for barrier perches as it supports the applicability of this form of enrichment into commercial systems. It is extremely difficult to make strides for animal welfare when a proposed change has differential effects on welfare and production costs; however, it appears that, at least in this case, production goals do not need to be sacrificed in order to provide for improved welfare of broilers.

In conclusion, in this study we provided further evidence of the detrimental effects of increasing stocking density on broiler health and welfare, as attested by increased tibia asymmetry, poorer footpad and hock health, suppression of activity levels, increased disturbances and decreased use of space. We also demonstrated that barrier perches, and more specifically, simple barrier perches, appeared to improve broiler welfare, notably by improving footpad health, encouraging a wider behavioral repertoire that led to increased activity levels and decreased aggression and disturbances, and promoting improved use of space. Simple barrier perches may thus be considered as a viable and potentially economical option to improve the welfare of broilers currently in production.

APPENDICES

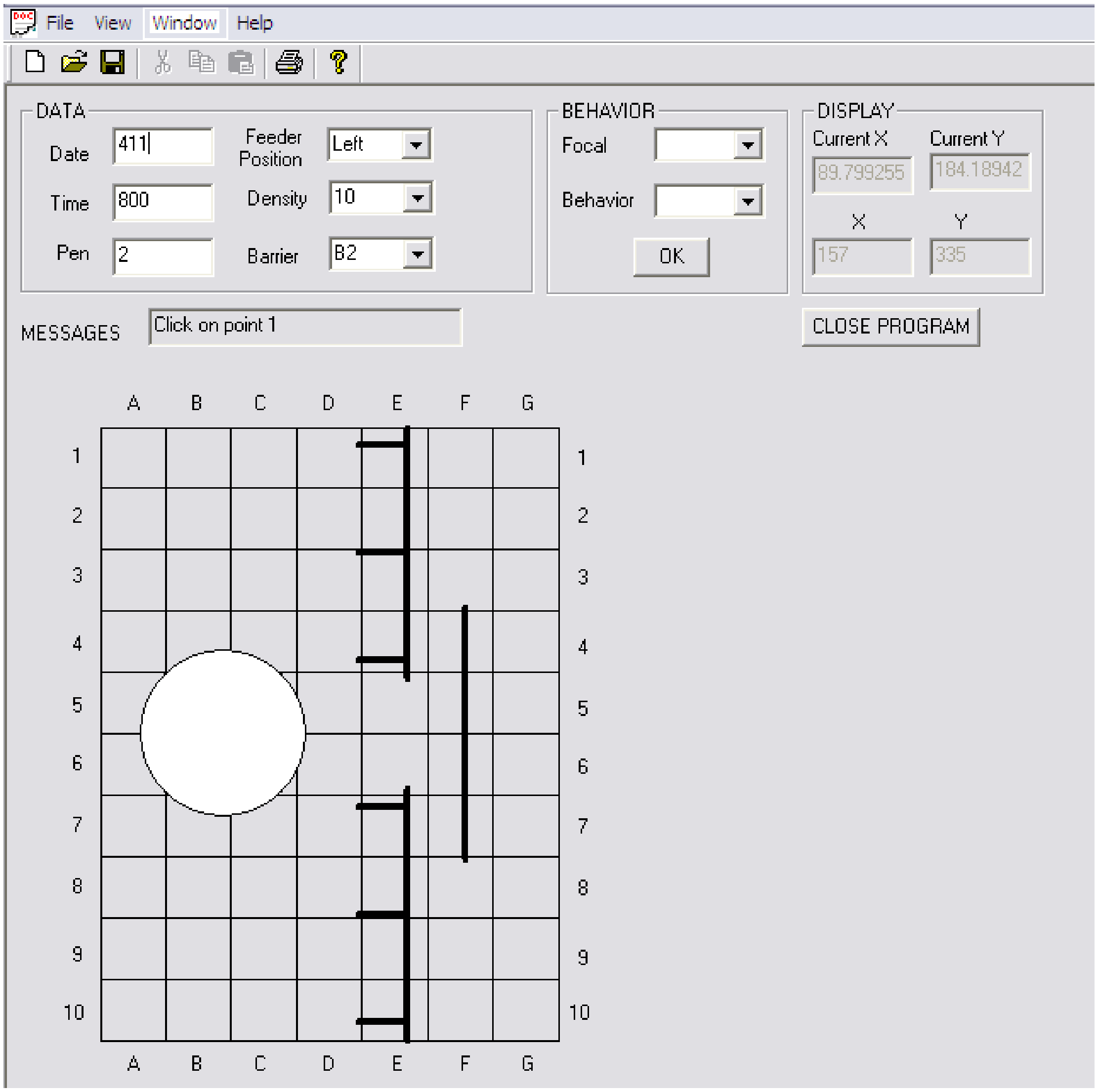
6.1 Photographic scale of footpad dermatitis lesions



Photographic scale used to assess the extent of footpad dermatitis lesions
a) Score 0 b) Score 1 c) Score 2 d) Score 3

Adapted from Pagazaurtundua and Warriss, 2006.

6.2 *Screenshot of the Chickitizer program*



Pen layout was adjusted according to barrier treatment and feeder layout before each pen scan.

BIBLIOGRAPHY

Altmann, J., 1974. Observational study of behavior: sampling methods. Behav. 49, 227-267.

Appleby, M. C., Smith, S. F. and Hughes, B. O., 1992. Individual perching behavior of laying hens and its effects in cages. Br. Poult. Sci. 33, 227-238.

Arnould, C. and Faure, J. M., 2004. Use of pen space and activity of broiler chickens reared at two different densities. Appl. Anim. Behav. Sci. 87, 153-170.

Berg, C.C., 1998. Foot-pad dermatitis in broilers and turkeys – Prevalence, risk factors and prevention. Acta Universitatis Agriculturae Sueciae. Veterinaria, 36. Doctoral Dissertation, Uppsala, Swedish Univ. Agric. Sci.

Bizeray, D., Leterrier, C., Constantin, P., Picard, M., and Faure, J.M., 2000. Early locomotor behaviour in genetic stocks of chickens with different growth rates. Appl. Anim. Behav. Sci. 68, 231-242.

Bizeray, D., Estevez, I., Leterrier, C. and Faure, J.M., 2002a. Effects of increasing environmental complexity on the physical activity of broiler chickens. Appl. Anim. Behav. Sci. 79, 27-41.

Bizeray, D., Estevez, I., Leterrier, C. and Faure, J.M., 2002b. Influence of increased environmental complexity on leg condition, performance, and level of fearfulness in broilers. Poult. Sci. 81, 767-773.

Brake, J., Keeley, T. P. and Jones, R. B., 1994. Effect of age and presence of perches during rearing on tonic immobility fear reactions of broiler breeder pullets. Poult. Sci. 73, 1470-1474.

Brambell, F.W.R., 1965. Report of The Technical Committee To Enquire Into The Welfare Of Animals Kept Under Intensive Livestock Husbandry Systems. HMSO, London.

Campo, J. L., Gil, M. G., Davila, S. G. and Munoz, I., 2005. Influence of perches and footpad dermatitis on tonic immobility and heterophil to lymphocyte ratio of chickens. Poult. Sci. 84, 1004-1009.

Chamove, A. S., 1989. Environmental enrichment: A review. Anim. Technol. 40, 155-` 178.

Clarke, G. M., 1998. Developmental stability and fitness: The evidence is not quite so clear. Am. Nat. 152, 762-766.

Cornetto, T. and Estevez, I., 2001a. Behavior of the domestic fowl in the presence of vertical panels. Poult. Sci. 80, 1455-1462.

Cornetto, T. and Estevez, I., 2001b. Influence of vertical panels on use of space by domestic fowl. Appl. Anim. Behav. Sci. 71, 141-153.

Cornetto, T., Estevez, I. and Douglass, L. W., 2002. Using artificial cover to reduce aggression and disturbances in domestic fowl. Appl. Anim. Behav. Sci. 75, 325-336.

Cravener, T. L., Roush, W. B. and Mashaly, M. M., 1992. Broiler production under varying population-densities. Poult. Sci. 71, 427-433.

Dawkins, M. S., Donnelly, C. A. and Jones, T. A., 2004. Chicken welfare is influenced more by housing conditions than by stocking density. Nature. 427, 342-344.

Dozier, W. A., Lott, B. D. and Branton, S. L., 2005. Live performance of male broilers subjected to constant or increasing air velocities at moderate temperatures with a high dew point. Poult. Sci. 84, 1328-1331.

Dozier, W. A., Thaxton, J. P., Purswell, J. L., Olanrewaju, H. A., Branton, S. L. and Roush, W.B., 2006. Stocking density effects on male broilers grown to 1.8 kilograms of body weight. Poult. Sci. 85, 344-351.

Ekstrand, C., Algers, B., Svedberg, J., 1997. Rearing conditions and foot-pad dermatitis in Swedish broiler chickens. Prev. Vet. Med. 31, 167-174.

Estevez, I., Newberry, R.C., Arias de Reyna, L., 1997. Broiler chickens: a tolerant social system? Etología. 5, 19-29.

Estevez, I., 1999. Cover Panels for Chickens: A Cheap Tool That Can Help You. Poult. Perspect. 1, 4-6.

Estevez, I. and Christman, M.C., 2006. Analysis of the movement and use of space of animals in confinement: The effect of sampling effort. Appl. Anim. Behav. Sci. 97, 221-240.

Estevez, I., 2007. Density allowances for broilers: Where to set the limits? Poult. Sci. 86, 1265-1272.

Faure, J.M. and Jones, R.B., 1982. Effects of sex, strain and type of perch on perching behavior in the domestic fowl. Appl. Anim. Ethol. 8, 281-293.

Forkman, B., Boissy, A., Meunier-Salaun, M.-C., Canali, E., and Jones, R.B., 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Phys. Beh. 92, 340-374.

Frankenhuis, M.T., Vertommen, M.H., and Hemminga, H., 1991. Influence of claw clipping, stocking density and feeding space on the incidence of scabby hips in broilers. Br. Poult. Sci. 32, 227-230.

Greene, J. A., Mccracken, R. M. and Evans, R. T., 1985. A contact-dermatitis of broilers - clinical and pathological findings. Avian Pathol. 14, 23-38.

Hall, A. L., 2001. The effect of stocking density on the welfare and behaviour of broiler chickens reared commercially. Anim. Welf. 10, 23-40.

Harms, R.H., Damron, B.L., and Simpson, C.F., 1977. Effect of wet litter and supplemental biotin and/or whey on the production of foot pad dermatitis in broilers. Poult. Sci. 56, 291-296.

Haslam, S. M., Brown, S. N., Wilkins, L. J., Kestin, S. C., Warriss, P. D. and Nicol, C. J., 2006. Preliminary study to examine the utility of using foot burn or hock burn to assess aspects of housing conditions for broiler chicken. Br. Poult. Sci. 47, 13-18.

Haslam, S. M., Knowles, T. G., Brown, S. N., Wilkins, L. J., Kestin, S. C., Warriss, P. D. and Nicol, C. J., 2007. Factors affecting the prevalence of foot pad dermatitis, hock burn and breast burn in broiler chicken. Br. Poult. Sci. 48, 264-275.

Haye, U. and Simons, P. C. M., 1978. Twisted legs in broilers. Br. Poult. Sci. 19, 549-557.

Heckert, R. A., Estevez, I., Russek-Cohen, E. and Pettit-Riley, R., 2002. Effects of density and perch availability on the immune status of broilers. Poult. Sci. 81, 451-457.

Hoerl, E.N., 2004. Foraging strategies, use of space and aggressive behavior of domestic fowl (Gallus gallus domesticus). M.S. Thesis, Univ. Maryland, College Park.

Hughes, B.O., Wood-Gush, D.G.M., and Morley Jones, R. 1974. Spatial organization in flocks of domestic fowls. Anim. Behav. 22, 438-445.

Hughes, B. O. and Elson, H. A., 1977. The use of perches by broilers in floor pens. Br. Poult. Sci. 18, 715-722.

Jones, R.B., 1986. The tonic immobility reaction of the domestic fowl: a review. World Poultry Sci J. 42, 82-96.

Jones, R.B., 1992. The nature of handling immediately prior to test affects tonic immobility fear reactions in laying hens and broilers. Appl. Anim. Behav. Sci. 34, 247-254. Abstract.

Jones, R.B. and Waddington, D., 1992. Modification of fear in domestic chicks, gallus gallus domesticus, via regular handling and early environmental enrichment. Anim. Behav. 43, 1021-1033.

Jones, R. B., 1996. Fear and adaptability in poultry: Insights, implications and imperatives. World's Poult. Sci. J. 52, 131-174. Abstract.

Jones, R.B., and Mills, A.D., 1983. Estimation of fear in 2 lines of the domestic chick-correlations between various methods. Behav. Process. 8, 243-253.

Kjaer, J. B., Su, G., Nielsen, B. L. and Sorensen, P., 2006. Foot pad dermatitis and hock burn in broiler chickens and degree of inheritance. Poult. Sci. 85, 1342-1348.

Knierim, U., Van Dongen, S., Forkman, B., Tuyttens, F.A.M., Spinka, M., Campo, J.L., and Weissengruber, G.E., 2007. Fluctuating asymmetry as an animal welfare indicator-a review of methodology and validity. Physiol. & Behav. 92, 398-421.

Kestin, S. C., Knowles, T. G., Tinch, A. E. and Gregory, N. G., 1992. Prevalence of leg weakness in broiler-chickens and its relationship with genotype. Vet. Rec. 131, 190-194.

Koene, P., Ruiten, S.V., Bokkers, E., 1999. The effect of increasing broiler behaviour possibilities by giving extra furniture and a slimmer body: the effects of perches and feed restriction. In: Boe, K.E., Bakken, M., Braastad, B.O. (Eds.), Proceedings of the 33rd International Congress of the International Society for Applied Ethology, Lillehammer, Norway, As, 17-21 August 1999. Agricultural University of Norway, Norway, p. 136.

Krause, E.T., Naguid, M. Trillmich, F. and Schrader, L. 2006. The effects of short term enrichment on learning in chickens from a laying strain (*Gallus gallus domesticus*). Appl. Anim. Behav. Sci. 101, 318-327.

Leone, E. H., Estevez, I. and Christman, M. C., 2007. Environmental complexity and group size: Immediate effects on use of space by domestic fowl. Appl.Anim. Behav. Sci. 102, 39-52.

Leone, E.H. and Estevez, I., 2008a. Economic and welfare benefits of environmental enrichment for broiler breeders. Poult. Sci. 87, 14-21.

Leone, E. H. and Estevez, I., 2008b. Space use according to the distribution of resources and level of competition. Poult. Sci. 87, 3-13.

Leone, E. H. and Estevez, I., 2008c. Use of space in the domestic fowl: Separating the effects of enclosure size, group size and density. Anim. Behav. 76, 1673-1682.

LeVan, N. F., Estevez, I. and Stricklin, W. R., 2000. Use of horizontal and angled perches by broiler chickens. Appl. Anim. Behav. Sci. 65, 349-365.

Lewis, N.J. and Hurnik, J.F., 1990. Locomotion of broiler-chickens in floor pens. Poult. Sci. 69, 1087-1093.

Lilburn, M. S., 1994. Skeletal growth of commercial poultry species. Poult. Sci. 73, 897-903.

Lynch, M., Thorp, B. H. and Whitehead, C. C., 1992. Avian tibial dyschondroplasia as a cause of bone deformity. Avian Pathol. 21, 275-285.

Mankovich, N.J. and Banks, E.M., 1982. An analysis of social orientation and the use of space in a flock of domestic fowl. Appl. Anim. Ethol. 9, 177-193.

Marin, R.H., Freytes, P., Guzman, D. and Jones, R.B., 2001. Effects of an acute stressor on fear and on the social reinstatement responses of domestic chicks to cagemates and strangers. Appl. Anim. Behav. Sci. 71, 57-66.

Martland, M. F., 1985. Ulcerative dermatitis in broiler-chickens - the effects of wet litter. Avian Pathol. 14, 353-364.

McGinnis, J. and Carver, J.S., 1947. The effect of riboflavin and biotin in prevention of dermatitis and perosis in turkey poults. Poult. Sci. 26, 364-371.

McLean, K. A., Baxter, M. R. and Michie, W., 1986. A comparison of the welfare of laying hens in battery cages and in a perchery. Res. Dev. Agric. 3, 93-98.

Meehan, C. L. and Mench, J. A., 2007. The challenge of challenge: Can problem solving opportunities enhance animal welfare? Appl. Anim. Behav. Sci. 102, 246-261.

Meyer, W. A. and Sunde, M. L., 1974. Bone breakage as affected by type housing or an exercise machine for layers. Poult. Sci. 53, 878-885.

Mills, A. D. and Faure, J. M., 1991. Divergent Selection for Duration of Tonic Immobility and Social Reinstatement Behavior in Japanese-Quail (Coturnix-Coturnix-Japonica) Chicks. J. of Comp. Psychol. 105, 25-38.

Moller, A. P., 1999a. Asymmetry as a predictor of growth, fecundity and survival. Ecol. Lett. 2, 149-156.

Moller, A. P., Sanotra, G. S. and Vestergaard, K. S., 1999b. Developmental instability and light regime in chickens (Gallus gallus). Appl. Anim. Behav. Sci. 62, 57-71.

Murphy, L. B. and Preston, A. P., 1988. Time-budgeting in meat chickens grown commercially. Br. Poult. Sci. 29, 571-580.

National Agricultural Statistics Service (NASS), Agricultural Statistics Board, U.S. Department of Agriculture. 'U.S. Broiler Industry Structure'; November 27, 2002; http://www.usda.gov/nass.

National Agricultural Statistics Service (NASS), Agricultural Statistics Board, U.S. Department of Agriculture. 'Poultry-Production and Value 2006 Summary'; April 2007; http://www.usda.gov/nass.

Newberry, R. C. and Hall, J. W., 1990. Use of pen space by broiler-chickens - effects of age and pen size. Appl. Anim. Behav. Sci. 25, 125-136.

Newberry, R. C., 1995. Environmental enrichment - increasing the biological relevance of captive environments. Appl. Anim. Behav. Sci. 44, 229-243.

Newberry, R.C., 1999. Exploratory behaviour of young domestic fowl. Appl. Anim. Behav. Sci. 63, 311-321.

Newberry, R. C., Estevez, I. and Keeling, L. J., 2001. Group size and perching behaviour in young domestic fowl. Appl. Anim. Behav. Sci. 73, 117-129.

Pagazaurtundua, A. and Warriss, P. D., 2006. Measurements of footpad dermatitis in broiler chickens at processing plants. Vet. Rec. 158, 679-682.

Palmer, A. R. and Strobeck, C., 1986. Fluctuating asymmetry - measurement, analysis, patterns. Annu. Rev. Ecol. Sys. 17, 391-421.

Pettit, R.L., 2000. Effects of perch availability and stocking density on perching behavior and aggression in broiler chickens. M.S. Thesis, Univ. Maryland, College Park.

Pettit-Riley, R. and Estevez, I., 2001. Effects of density on perching behavior of broiler chickens. Appl. Anim. Behav. Sci. 71, 127-140.

Pettit-Riley, R., Estevez, I. and Russek-Cohen, E., 2002. Effects of crowding and access to perches on aggressive behaviour in broilers. Appl. Anim. Behav. Sci. 79, 11-25.

Proudfoot, F. G., Hulan, H. W. and Ramey, D. R., 1979. Effect of 4 stocking densities on broiler carcass grade, the incidence of breast blisters, and other performance traits. Poult. Sci. 58, 791-793.

Reiter, K. and Bessei, W., 1998a. Effect of locomotor activity on bone development and leg disorders in broilers. Arch. Geflugelk. 62, 247-253. Abstract.

Reiter, K. and Bessei, W., 1998b. Possibilities to reduce leg disorders in broilers and turkeys (Review). Arch. Geflugelk. 62, 145-149. Abstract.

Reiter, K. and Bessei, W., 2000. The behaviour of broilers in response to group size and stocking density. Arch. Geflugelk. 64, 93-98.

Reiter, K. and Kutritz, B., 2001. Behaviour and leg weakness in different broiler breeds. Arch.Geflugelk. 65, 137-141. Abstract.

Rose, N., Constantin, P., Leterrier, C., 1996. Sex differences in bone growth of broiler chickens. Growth Dev. Aging 60, 49-59.

Sanchez, C. and Estevez, I., 1998. The chickitizer, v.4, University of Maryland, College Park, MD, USA.

Sandusky, C. L. and Heath, J. L., 1988. Effect of age, sex, and barriers in experimental pens on muscle growth. Poult. Sci. 67, 1708-1716.

Sanotra, G.S., Lund, J.D., Ersboll, A.K., Petersen, J.S., and Vestergaard, K.S., 2001. Monitoring leg problems in broilers: a survey of commercial broiler production in Denmark. World Poult. Sci. J. 57, 55-69. Abstract.

Scientific Committee on Animal Health and Animal Welfare (SCAHAW)., 2000. The welfare of chickens kept for meat production (broilers) SCAHAW Report No. SANCO.B.3/AH/R15/2000. Brussels: European Commission. Available on the World Wide Web: http://europa.eu.int/comm/food/fs/sc/scah/out39_en.pdf.

Sirri, F., Minelli, G., Folegatti, E., Lolli, S. and Meluzzi, A., 2007. Foot dermatitis and productive traits in broiler chickens kept with different stocking densities, litter types and light regimen. Ital. J. Anim. Sci. 6, 734-736.

Steel, R.G.D. and Torrie, J.H., 1960. Principles and procedures of statistics. McGraw, New York.

Stricklin, W.R., 1995. Space as environmental enrichment. Lab Anim. 24-29.

Su, G., Sorensen, P. and Kestin, S. C., 2000. A note on the effects of perches and litter substrate on leg weakness in broiler chickens. Poult. Sci. 79, 1259-1263.

Tablante, N.L., Estevez, I., Russek-Cohen, E., 2003. Effect of perches and stocking density on tibial dyschondroplasia and bone mineralization as measured by bone ask in broiler chickens. J. Appl. Poult. Res., 12, 53-59.

Thaxton, J. P., Dozier, W. A., Branton, S. L., Morgan, G. W., Miles, D. W., Roush, W. B., Lott, B. D. and Vizzier-Thaxton, Y., 2006. Stocking density and physiological adaptive responses of broilers. Poult. Sci.. 85, 819-824.

Van Poucke, E., Van Nuffel, A., Van Dongen, S., Sonck, B., Lens, L. and Tuyttens, F. A. M., 2007. Experimental stress does not increase fluctuating asymmetry of broiler chickens at slaughter age. Poult. Sci. 86, 2110-2116.

Weeks, C. A., Danbury, T. D., Davies, H. C., Hunt, P. and Kestin, S. C., 2000. The behaviour of broiler chickens and its modification by lameness. Appl. Anim. Behav. Sci. 67, 111-125.