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EFFECTS OF AN ENRICHED AND BARREN ENVIRONMENT ON BEHAVIORAL AND PHYSIOLOGICAL RESPONSES RELATED TO LAYING HEN WELFARE

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ABSTRACT

This study was conducted to determine the effects of housing environment on behavioral and physiological responses related to laying hen welfare. General observations and two behavioral fear assessments (emergence test [EM] and tonic immobility test [TI]) were used as behavioral assessments. A heterophil/lymphocyte ratio was used as a physiological stress indicator. Nine hundred day-old Leghorn chicks were randomly assigned to either a floor pen environment or a commercial cage housing environment. The cage chicks were housed in 20 commercial battery brooder cages (25 per cage) up until four weeks of age and then moved to 39 battery grower cages (10 per cage). At 16 weeks of age, the pullets were moved into 39 commercial layer cages (8 per cage) for the remainder of the experiment. The floor pen birds were continuously housed in 14 individual floor pens (28 per pen) enriched with perches, dust baths, and nest boxes. General behavioral observations were recorded on birds from nine randomly selected floor pens and cages. Both TI and EM were conducted on ten randomly selected hens from the cage environment and ten randomly selected hens from the floor pen environment. Blood collection from twenty randomly selected hens from each environment was used for a heterophil/lymphocyte ratio.

When the two environmental treatments were compared, there were significant differences ($P<0.05$) between the floor pen birds and cage birds for percentage of birds standing and the log odds of sitting behaviors. Although there was a significant ($P<0.05$) interaction present between treatment and time, there were significant differences in

percentage of birds feeding and log odds of other behavior during certain weeks. There was also an interaction present between treatment and time for EM and TI, which again had significant differences between floor pen and cage birdsat certain weeks ($P<0.05$). There was no significant difference across all weeks between the cage and floor pen treatments for average heterophil/lymphocyte ratio. Although interactions were present in some of the assessments, looking at trends within the data reveal that floor pen birds may be housed in an environment that is better suited to meet their welfare needs than birds housed in the commercial cage system.

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CHAPTER I

INTRODUCTION

Concerns about the welfare of domestic poultry have been around since the early 1960s. The origin of these concerns is thought to have been sparked by the transition from litter and free range housing systems to commercial cage housing systems for laying hens (Appleby et al., 2004). Two of the Five Freedoms for farm animals, recommended by the UK's Farm Animal Welfare Council in 1997, are the freedom to express normal behavior and the freedom from fear and distress. An EU directive, published in 1999, placed a ban on all commercial battery cages by 2012. Furnished cages and non-cage systems will only be allowed, and these systems should provide hens with a nest site, perches and a litter area for scratching and pecking. Welfare can be assessed by observation of behaviors and measurement of physiological stress indicators. This study assesses normal hen behaviors in an enriched floor pen environment compared to a barren cage environment. The freedom from fear and distress are also assessed in this study through behavioral fear tests and physiological stress indicators.

In order to assess the hen's normal behavior in floor pens and cage environments, video recordings of the hens in their environments were taken. Because the cage birds' behavior repertoire is very limited and the number of hens housed in each environment, only certain behaviors could be compared between the floor pen birds and cages birds. These behaviors were standing, sitting and feeding. It is also important to assess if animals use the enrichment that is provided in an enriched environment for normal

behaviors. Perch, dust bathing box, and nesting box use were all monitored in the floor pens.

In animals, the internal level of fear can only be assessed through the occurrence of behavioral and/or physiological changes. In order to measure an animal's fear response, methods must be in place to experimentally induce fear. It is best to use a variety of behavioral tests to measure fear response because it is thought that there is no single, straightforward method of measuring general fearfulness (Jones, 1987). In this study, tonic immobility and emergence tests were used to determine fear in chicks and pullets raised in two different housing environments. Tonic immobility is an easily distinguishable phenomenon observed in many different species of animals (Gallup, 1974a). The most common animal tested with tonic immobility is the domestic fowl because of its degree of response, which is easily discriminated from other behaviors (Jones, 1986). The duration of the immobile state is most frequently used when measuring tonic immobility. Typically, the measurement is taken from the time the test subject is released until it regains mobility (Ratner, 1967). An emergence test was also used to determine fear in the chicks and pullets. Emergence tests involve measuring the latency of an animal's movement from a sheltered or preferred environment into an exposed or unfamiliar environment. This test provides a measure of the timidity aspect of fear. The assumption of this test is that more fearful or timid animals will show longer emergence latencies (Jones, 1987).

Stress can be described as an animal's defense mechanism to any situation that causes a defensive response (Selye, 1936). The environment in which an animal lives is

known to be a potential stress stimulus. An individual bird's ability to adapt to an environment depends on the severity of the stress stimulus and the bird's physiological ability to respond properly. Changing a bird's environment can stimulate regulatory processes to attempt to return the bird to a state of homeostasis (Siegel, 1980). The heterophil/lymphocyte ratio is a good measurement of the chicken's perception of stress in its environment. Measuring the heterophil/lymphocyte ratio provides an indication of long-term stress in the environment (Gross and Siegel, 1983). In this study¸ the heterophil/lymphocyte ratio was used to assess stress levels of birds housed in two different environments.

Currently there is limited information available about the effect of different housing environments on general behaviors, fear response, and stress in relation to welfare for laying hens chickens. The aim of this study is to investigate the effects of a floor pen environment and commercial cage environment on behavioral and physiological responses in efforts to assess laying hen welfare.

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CHAPTER II

LITERATURE REVIEW

Welfare

Welfare concerns with poultry production have been around since the early 1960's. These concerns are thought to have started with the transition of free range housing systems to commercial cage housing systems (Appleby et al., 2004). Public awareness of intensive production methods was increased by publications such as the Brambell Report in the United Kingdom (Her Majesty's Stationery Office, 1965). The publication of the report by the Brambell Committee led to the formation of the Farm Animal Welfare Council (FAWC) in 1979. The purpose of this council was to review the welfare of farm animals on agricultural land, at market, in transit and at the place of slaughter. The council also advised the British Government on any legislative changes that were necessary (FAWC, 2007). In 1997, the Farm Animal Welfare Council established the "five freedoms" to evaluate welfare conditions of animals. These freedoms are considered to be ideal states for animals, not standards for acceptable welfare, and can be applied in many different situations.

The Five Freedoms are:

1. Freedom from hunger and thirst - by ready access to fresh water and a diet to maintain full health and vigour.

2. Freedom from Discomfort - by providing an appropriate environment including shelter and a comfortable resting area.

3. Freedom from Pain, Injury or Disease - by prevention or rapid diagnosis and treatment.

4. Freedom to Express Normal Behaviour - by providing sufficient space, proper facilities and company of the animal's own kind.

5. Freedom from Fear and Distress - by ensuring conditions and treatment which avoid mental suffering.

The first three of the five freedoms do not cause as much public concern as the last two. Production practices typically cover the first three concerns with no problems. It is the freedom to express normal behavior and freedom from fear and distress that the public is most concerned with today. Welfare can be assessed by observation of behaviors, laboratory testing and measurement of physiological stress indicators. This study assesses normal behaviors in an enriched floor pen environment compared to a barren cage environment.

There are three scientific approaches to understanding animal welfare. The first approach focuses on the importance of how an animal feels, placing emphasis on emotions such as pain, suffering and pleasure. The second approach places emphasis on biological function in which the animal's fitness and health is assessed by productivity indicators such as growth, milk yield, reproduction, disease and injury. The third approach focuses on the concern for naturalness. For example, a concern if an animal

should be kept in an environment within which its species has evolved with respect for its nature (Duncan and Fraser, 1997). Freedom from fear and distress are also assessed in this study through behavioral fear tests and physiological stress indicators. Although all three are valuable methods, the behavioral assessment is the only one practical for use under field or commercial conditions.

Normal Behaviors

Behaviors that are used to sustain an animal's physiological equilibrium are known as maintenance behaviors. These maintenance behaviors include generalized feeding, drinking, resting, and comfort behaviors, such as behaviors involved with care of the bird's plumage (Appleby et al., 2004).

Foraging

Wild and feral poultry are very active and most of their active time is devoted to foraging and consuming food. Jungle fowl, raised under semi-natural conditions, spend a large proportion of their time performing foraging behaviors (Dawkins, 1989). Common components of foraging behaviors of chickens are pecking and scratching. Scratching is commonly performed using both feet while moving backwards in a quick motion. The birds then peck at anything edible that is exposed by scratching. This is considered to be the appetitive component of feeding behavior, while the actual picking up and swallowing of food is the consummatory behavior. Pecking and scratching are performed in loose litter, if it is available. Birds housed in conventional commercial cages do not have access to loose material, but instead these birds spend a substantial proportion of time either feeding or manipulating the food in the trough with their beaks

(Appleby et al., 2004). Manipulation of food is usually performed in two ways: food is either drawn back toward the bird and piled up at the back of the trough or the bird flicks its beak back and forth with vigorous movements causing some of the food to end up outside of the trough where it is wasted. For cage birds, this is the appetitive component of feeding behavior because it is the only substrate the birds can access (Appleby et al., 2004).

In the absence of an appropriate substrate for foraging, food wastage can be a major issue of economical importance. A number of solutions to this problem have been adopted commercially to help resolve the issue. Beak trimming is one solution that has been used, because this removes the tip of the beak and food can no longer be caught under the beak hook and flicked out of the feeder (Appleby et al., 2004). However, beak trimming is not allowed in all countries. For example, this practice has been banned since 1992 in Switzerland (FAWC, 2007). Wastage can also be minimized by feeder design. This can be accomplished by placing a wire grid at the level of the food so that the birds have to peck through the spaces in order to feed or by having a spiral along the bottom of the trough that prevents flicking. Using a deep, narrow feed trough that is replenished by an automatic conveyer system may also reduce food wastage. In response to these concerns, the European Union now requires furnished cages with a suitable substrate for performing foraging behaviors (Appleby et al., 2004).

Dust Bathing

Another natural behavior of chickens is dust bathing. Materials appropriate for dust bathing are not included in conventional commercial cages and this deprivation is

significant for the performance of normal behavior patterns (Vestergaard, 1997). The purpose of dust bathing is to clean the plumage and keep the feathers in good condition (Borchelt and Duncan, 1974). Birds typically dust bathe in wood shavings or other floor litter, but if a finer material such as sand or peat moss is available, they prefer to use this finer substrate. This is because finer materials are better for penetrating the feathers to reach the downy portion of the plumage (Shields et al., 2004). It has also been seen that dust bathing may appear as a vacuum or sham activity when appropriate substrate is not provided or on a wire floor (Vestergaard, 1982). On average, mature hens perform a bout of dust bathing for approximately 0.5 hours every other day (Shields et al., 2004).

In 1997, Vestergaard et al., investigated the potential stress involved in the nonperformance of dust bathing by depriving female white leghorn laying hens access to sand. The hens were reared and housed in either cages with sand floors or cages with wire floors for 2.5 years. These hens were then switched between environments. The birds raised on the sand were moved into wire floor cages and the birds raised on the wire floor were switched to a sand floor. The birds that were switched from a sand floor to wire floor cages had significantly fewer bouts of dust bathing after switching environments. The opposite was true with the birds raised on the wire floor. They had significantly higher incidence of dust bathing after switching to a sand floor. There was also a significant increase in plasma corticosterone levels for birds raised on the sand floor and then switched to a wire floor cage. These results revealed that laying hens are stressed when an appropriate stimulus for the release of a specific behavior pattern, such as dust bathing, is missing (Vertergaard et al., 1997).

Perching

Perching is another natural behavior of birds, and commercial cage housing systems do not typically provide birds with access to perches. Perching or roosting has evolved in the hen's natural habitat as a behavior to avoid predation during the night. Even though domestic hens in commercial conditions are not threatened by predators, the motivation for perching behavior remains high (Wichman et al., 2007). It is thought that birds that have access to perches are less fearful, and that perches provide a sense of security for birds (Brake et al., 1994). During the daytime, perches are used for resting, preening and as a retreat for subordinate birds to avoid aggressive encounters (Cordiner and Savory, 2001). In 2002, Olsson and Keeling, demonstrated that Lohmann Selected Leghorn laying hens are highly motivated to gain access to perches at night. This study found that hens would push through a weighted door to gain access to perches. Therefore, they concluded that hens are motivated to use a perch for night-time roosting, and hens should be housed with access to perches. Because of this strong motivation to perch, hens will struggle and crowd very closely on perches when space is limited. For adult hens, a perch space allowance of 140 mm per bird for most breeds is necessary (Appleby, 1995). As of January 1, 2010, the United Egg Producers are requiring a minimum of 152.4mm (6 inches) of elevated perch space per bird for cage-free egg production methods certified under United Egg Producer's guidelines (United Egg Producers, 2003).

Nesting

Although nesting is a normal behavior of laying hens, appropriate areas for nesting are not provided in conventional cage housing systems. All other housing systems for laying poultry include nest boxes. Systems with nest boxes allow the birds to express behaviors associated with laying site selection and oviposition. Darkness and seclusion are thought to be important in a bird's selection of a nest box (Appleby et al., 2004). The substrate provided in the nest box is also important in the attractiveness to hens. Struelens et al., (2005) found that hens preferred peat moss and artificial turf substrates to a coated wire mesh floor for laying eggs. Hens prefer loose nesting material if it is available, such as wood savings in nest boxes (Appleby et al., 1988).

Production concerns arose with birds performing the appropriate behaviors in the inappropriate locations when both a nest box and dust box were present in a housing system. If hens dust bathed in the nest box this could result in broken eggs, and eggs laid in the dust box can be difficult to collect. Smith et al., (1993) investigated this problem, and found that hens in laying cages provided with both a nest box containing wood shavings and a dust bath containing sand very rarely laid eggs in the dust bath and were never recorded dust bathing in the nest box. This study concluded that if the appropriate substrates for the behavior are provided in the right location, the birds will perform the behavior in the appropriate location. It is now generally accepted in many parts of Europe that providing hens with a nest box is essential to satisfying welfare requirements (Struelens et al., 2005).

Fear Assessment

There is no generally accepted definition of fear when dealing with animals. Fear is usually listed in the emotions category of behaviors. In 1959, Miller proposed that "fear is a drive, like hunger or thirst, which may motivate either adaptive or maladaptive behavior, and that a sudden reduction in the strength of fear serves as a reward to reinforce immediate preceding responses". Salzen, in 1979, suggested that there are two distinct concepts associated with fear; a "fear behavior" and a "fear state". He concluded that all fear behaviors function to protect the animal from immediate or potential physicochemical damage. Behavior associated with fear would best be classified as a large repertoire of responses that can be changed and integrated with each other to produce an optimal strategy for dealing with a particular threat (Jones, 1987).

There have been many multi-step fear response models proposed, but the majority are similar. The first step in a fear response behavior is an orientation period which is usually associated with physiological changes such as heart rate, respiration, blood flow, etc. These changes help the body to prepare for a response to the novel or frightening stimulus. If the stimulus causes a high degree of fear, the orientation reaction can change into a fight, flight or fright reaction (Sokolov, 1960; Hinde, 1966; Archer 1976). Methods used to estimate fearfulness and the interpretation or responses to fear-evoking stimuli are controversial. In animals, the internal level of fear can only be assessed by behavioral and/or physiological changes. In order to measure fearfulness, methods to induce fear behaviors experimentally must first be established. One approach is to frighten animals experimentally and then observe the subsequent reactions. This works under the assumption that certain stimuli or manipulations will cause more or less fear

than others. There is, however, no single, straightforward method of measuring general fearfulness. Therefore, it is important to use a number of tests and to measure a variety of behavioral patterns and physiological parameters (Jones, 1987).

Tonic Immobility

Tonic immobility is an easily distinguishable phenomenon observed in many different species of animals (Gallup, 1974a). The most common animal tested for tonic immobility is the domestic fowl because of its degree of response, which is easily discriminated from other behaviors (Jones, 1986). It is also known as animal hypnosis and is induced by a short period of physical restraint, which is usually administered by holding an animal down on a flat surface. The state of an animal being in tonic immobility is characterized by motor inhibition, intermittent periods of eye closure, changes in heart and respiration rate, altered electroencephalographic patterns, tremors in the extremities, and reduced responsiveness to external stimulus (Gallup, 1974a). The animal typically struggles and tries to escape when tonic immobility is first induced, but after a few seconds, the animal adopts an immobile posture that may last for a few seconds up to a few hours after being restrained (Jones, 1986).

It has been proposed that there are three stages or levels of tonic immobility which have been characterized in the domestic chick. The first stage is associated with shrill vocalizations and continuously open eyes, this stage occurs at the beginning of tonic immobility and again just before the immobility state is terminated. The second stage is associated with reduced vocalization and eye flutters. The third stage is characterized by silence, complete eye closure, and occasional body twitching and head

bobbing. The third stage is also predictive of long-term immobility (Rovee and Luciano, 1973).

There are two different levels to characterize tonic immobility in the adult hen. The first level is known to be one of behavioral inhibition, which lasts from induction of tonic immobility until the first alert head movement. This is also thought to be linked to Rovee and Luciano's third stage of tonic immobility in the chick. During the second level of tonic immobility the hen is alert and may vocalize. The hen may also make several scanning motions with its head before righting itself (Jones and Faure, 1981).

The duration of the immobile state is the most frequently used measure for tonic immobility. Typically the measurement is taken from the time the test subject is released until it regains mobility (Ratner, 1967). Other methods include the restraint time in the measurement. For the measurement to be used objectively, the subject must be immobilized in a position other than upright so that so that termination of immobility can be easily determined by a righting response. Minor movements of limbs, eyes, and neck that occur during the immobile state offer no relation to the termination of the overall reaction (Gallup 1974a). It was thought that one explanation for the tonic immobility response was inversion. However, it is now known that vestibular involvement is unlikely because tonic immobility can be induced in chicks in a lateral, upright, or ventral position (Rovee and Luciano, 1973). Also, in frogs, removal of ears had no effect on tonic immobility responses (McBride and Klemm, 1969). For chickens, a trough can be used to induce tonic immobility. The preferable posture, however, is to restrain the bird laterally on one side or the other on a flat surface. Placing the bird dorsally will leave the

subject in an unstable position that can lead to rolling to one side. This may cause a premature termination of the experiment. Many researchers have adopted an upper time limit for conducting the tonic immobility test. This is due to the possibility of the test lasting for several hours. This will conserve time, but it may also sacrifice important data (Gallup, 1974a).

Tonic immobility has been widely used as a parameter for estimating fearfulness, and it is considered to be well correlated with fear. From an ecological standpoint it is thought that tonic immobility may be, or may have been, involved in predator-prey relationships as a defense mechanism. Gallup (1974b) showed that tonic immobility has an unusually large heritability component in chickens. Gallup has also shown that different genetic strains of birds exhibit different responses to tonic immobility. Albentosa et al., (2003), showed that strains of White Leghorn chickens had significantly longer tonic immobility durations than Columbian Blacktails, ISA Browns, and Ixworths. They also found that the number of inductions was influenced by genetic strain. Ixworths required more inductions than White Leghorns, but the ISA Browns and Columbian Blacktail strains had similar results. The tonic immobility response is thought to be prolonged by procedures that are intended to increase fear. These procedures may include shock, loud noise, simulated predator encounters and suspension over a visual cliff. The tonic immobility response, however, is also thought to be shortened by fear reducing procedures such as taming, habituation, companion presence and tranquilizers (Gallup, 1974a). Jones and Faure (1981) tested the differences in tonic immobility responses for one year old laying hens housed in cages versus pens. They found that

susceptibility to tonic immobility was similar in both groups, but pen-housed birds showed significantly shorter latencies to the first leg and head movements. The pen-housed birds also had a significantly shorter duration of immobility than the caged birds. These results suggest that caged birds are more fearful than pen-housed birds (Jones and Faure, 1981).

Emergence Test

Emergence tests are used to determine the latency of an animal to move from a preferred, familiar, or sheltered environment into an unfamiliar or exposed environment. This test is thought to provide a measurement of the timidity aspect of fearfulness. Emergence tests work under the assumption that more fearful animals will exhibit a longer latency of emergence than bolder or less fearful animals (Jones, 1987a). The emergence test has been used widely in rodents to obtain a measure of fear (Einon and Tye, 1975; Gallate et al., 2003; Lalonde and Strazielle, 2009). Erhard and Mendl, in 1999, conducted an experiment on 3 week old piglets to investigate the tonic immobility fear response in a challenging situation. The challenging situation was an emergence test. Experimental subjects were 29 pigs from three litters. Each subject was tested with an emergence test that was immediately followed by a tonic immobility test over four consecutive days. The results demonstrated that there was a link between tonic immobility and emergence time only on the first day. This indicated that tonic immobility reveals something about the behavior of pigs that are faced with a challenging situation for the first time. The response to tonic immobility can be regarded as reflecting an element of activity in a challenging situation such as emergence from the box. Pigs

with low susceptibility to tonic immobility responded more quickly in the emergence test, while those with high susceptibility respond more slowly in the emergence test.

The emergence test has also been used in avian species. In 2008, Davis et al. investigated the fear response of offspring from divergent quail stress response line hens treated with corticosterone during egg formation. In this study, both the emergence test and tonic immobility test were used to assess fear response. Forty-eight high stress and forty-eight low stress quail hens were implanted with either a corticosterone implant or a control implant that contained no corticosterone. These hens were then paired with a non-sibling, same line male and housed in battery cages. The pairs were then allowed to mate and egg collection took place for three weeks. The eggs were then incubated at the same time and allowed to hatch. The juvenile offspring were tested with an emergence test on days 21 and 23. The results demonstrated that the offspring from the control hens had a significantly shorter average emergence time than the offspring from the corticosterone implanted hens. There was no significant difference, however, for average emergence time between the two stress lines. This demonstrates that there are many different factors that can affect fear response including hormonal influences.

In 2008, Gharreb et al. conducted a study to investigate the effect of strain and age on tonic immobility, emergence time and social reinstatement characteristics. They also investigated the consistency of individual behavioral characteristics over time, such as fear and sociality in laying hens. One hundred ISA Brown and one hundred Lohmann Tradition chicks were obtained six hours after hatching. Behavioral assessments were

conducted on the same 20 randomly selected birds from each strain at each time point. For the emergence test, birds were placed in a closed start box and allowed a two minute acclimation period. The door was then removed and the latency to complete emergence was recorded. The emergence box was placed on a 160 cm runway that contained a goal box at the end with stimulus birds from the same pen as the test subject. The tested bird had to travel the length of the runway and enter the goal zone after emergence from the start box. The time from complete emergence from the start box until the bird entered the goal zone was considered to be the social reinstatement test. The results from this study demonstrated that ISA Brown birds had a longer tonic immobility duration and latency of emergence from the start box as well as a slower reinstate time with companions than the Lohmann Tradition birds. This strain difference indicates that these behavioral responses are genetically and strain influenced. Also, during the rearing period, tonic immobility duration increased with age from week 3 to week 10 in both lines. The ISA Brown birds showed an increase in tonic immobility durations from week 13 to week 20, which could reflect an effect of maturation. The longer tonic immobility duration during the rearing period and before maturity compared with older ages in both lines could be explained as a helpful strategy for young chicks to avoid the higher risk of predation. The emergence time decreased as the birds increased in age until week 20 in individuals that were tested repeatedly. This could be due to maturation or the effect of habituation. The researchers also thought that the decreased latency to leave the start box in older birds could be explained by their greater body weight or more likely, experience dependent reductions in separation distress and the expression of social reinstatement behavior that

accompanied repeated testing. There was an increase in the emergence time after week 20 in repeatedly tested individuals in both strains which could be explained by age changes after maturity. The conclusion of the study was that the intra-situational consistency of individual tonic immobility, emergence time and latency of social reinstatement response in commercial laying hens reveals that these behavioral characteristics are behavioral strategies used by individuals in challenging situations such as predator attack, isolation or social stress. A behavioral strategy applies to individual differences in behavior which are consistent when repeated in a specific situation. In other words, behavioral strategies are situation-dependent.

Physiological Stress Indicators

Stress can be described as an animal's defense mechanism to any situation that causes a defensive response (Selye, 1936). An environment in which a bird lives includes a combination of many factors. These factors could be external such as humidity, light, or temperature. These factors could also be internal such as parasites, compromised immune system, or disease. The environment in which an animal lives is known to be a potential stress stimulus. An individual bird's ability to adapt to an environment depends on the severity of the stress stimulus and the bird's physiological ability to respond properly. Changing a bird's environment can stimulate regulatory processes to attempt to return the bird to a state of homeostasis. Regulatory processes can be classified into two different types, specific and nonspecific. These two processes happen simultaneously, and one process may have an effect on the other process. A specific regulatory process will be noticed in response to a particular condition. An

example of a specific regulatory process would be when the environmental temperature increases causing the bird's internal body temperature to increase. The rise in internal body temperature causes the surface blood vessels to dilate to permit greater blood flow to the skin for more rapid heat dissipation. This would be a specific response to the environmental stressor of heat. A non-specific regulatory process is when the animal responds in a generalized manner regardless of the environmental stressor. This causes the animal to go into a state of general stress (Siegel, 1980).

Corticosterone

Parts of the nervous and endocrine systems are responsible for producing responses to environmental stress. The postganglionic neurons and adrenal medullary tissues form the part of the neurogenic system that is involved in stress response. The neurogenic system involves a more immediate response to a stressor with the release of epinephrine and norepinephrine from the adrenal medulla. This response causes an immediate increase in blood pressure, muscle tone, nerve sensibility, respiration rate, and blood sugar (Siegel, 1980). The reaction has been termed the "fight or flight" response (Cannon, 1929). Essentially all of the circulating epinephrine and 70-80% of norepinephrine comes from the adrenal gland (Lacombe and Jones, 1990).

The hypothalamic-pituitary-adrenal axis (HPA) forms the part of the endocrine system involved in stress responses. The HPA is composed of direct influences and feedback interactions among the hypothalamus, pituitary gland, and the adrenal glands. The endocrine system causes more of a delayed response to stressors because it involves the release of hormones. The incoming stimulus that is neural or blood-borne causes an

increase in the hypothalamic production of corticotropin-releaseing hormone (CRH). CRH stimulates the anterior pituitary gland to increase the production of adrenocortioctropin (ACTH). The ACTH is then released into the blood and moves to stimulate adrenal cortical tissue cells. This increases the production and release of steroid hormones. The steroid hormone associated with stress in poultry is corticosterone (Siegel, 1980).

Plasma corticosterone concentrations can be measured in birds to indicate the degree of stress they experience (Fraisse and Cockrem, 2006). Exposing chickens to various stressful or frightening situations, such as extreme temperature changes, water and food deprivation, handling and immobilization has been shown to cause an increase in plasma corticosterone concentrations (Etches, 1976). Plasma corticosterone levels in caged hens have been reported to be lower (Koelkebeck and Cain, 1984), higher (Gibson et al., 1986), or similar to (Craig et al., 1986) coricosterone concentrations in hens in other housing systems. Corticosterone levels have also been used as a common or physiological indicator of fear. This was demonstrated by the measurement of plasma corticosterone and fear behavior together in hens (Jones et al., 1988) and Japanese quail (Jones et al., 1994, 2000). Jones et al. (1988) showed that fearful behavior in hens can be induced by increasing plasma corticosterone levels using a coricosterone treatment. The most common behavior tests performed to measure fearfulness are the tonic immobility test and response to a novel object or environment test (Jones, 1996). One study found that the duration of tonic immobility was greater in white hens versus brown hens (Gallup

et al., 1976). The white hen strains were said to be more fearful and flighty than the brown hen strains (Murphy, 1977).

There are several challenges with using corticosterone as a stress indicator in chickens. It has been shown that plasma corticosteroids begin to increase within 45 seconds of being restrained by hand, and increases some six-fold within eight minutes in domestic chickens. It has also been shown that there is a clear diurnal rhythm associated with plasma corticosterone levels in laying hens. The corticosterone levels peaked at 2-3ng/ml at the beginning of the light period and fell to a level of 0.5ng/ml at the end of the light period. In laying hens, plasma corticosterone was shown to be elevated 100 minutes prior to oviposition, reaching a peak of 4ng/ml at 44 minutes before egg laying. The hens returned to normal levels of coricosterone one hour after laying (Beuving and Vonder, 1978). If factors such as time of day, oviposition and age-related changes are controlled, then the measurement of plasma corticosterone concentrations and their comparison with resting levels may allow meaningful analysis of the corticosterone response to fear or stress (Jones, 1987). However, in this study all of these varying factors could not be controlled. Therefore, corticosterone was not used as a stress indicator in this study.

Elevated levels of corticosteroids in the body have been known to cause outward symptoms associated with long-term stress. Some symptoms include reduced inflammatory response, gastrointestinal lesions, and lower antibody response. If an animal is exposed to a prolonged amount of stress, bodily activities associated with reproduction, growth and infection resistance may shutdown (Jones, 1987). Cortocosterone has been suggested to increase the activity of adrenal

phenylethanolamine-N-methyl transferase which is known to speed up the conversion of norepinephrine to epinepherine (Zachariasen and Newcomer, 1975). This indicates that high levels of corticosterone may provide a pathway for replenishing the adrenal with catecholamines under stressful situations. Cortcosteroids can also cause an increase in plasma glucose concentrations and enhance liver glycogenolysis in a variety of avian species (Siegel, 1980).

Heterophil/Lymphocyte ratio

The heterophil/lymphocyte ratio is a good measurement of the chicken's perception of stress in its environment. Measuring the heterophil/lymphocyte ratio provides an indication of long-term stress in the environment, where as the plasma corticosterone concentrations are thought to be a better indicator of short term stress (Gross and Siegel, 1983). The average number of heterophils in the adult, female White Leghorn is 13.3 per 100 cells, and the average number of lymphocytes is 76.1 (Sturkie, 1965). There are many different ways a heterophil/lymphocyte ratio for avian blood can be determined. In 1983, Gross and Siegel used two different methods to determine the heterophil/lymphocyte ratio for birds that were fed corticosterone. The first method used a hemocytometer in which cells were counted within three hours of collection. In the other method, cells were counted by examining a blood smear prepared by centrifugation with a Larc Spinner. The blood smears were stained seven days prior to counting with May- Grönwald-Giemsa stain. The heterophil/lymphocyte ratio was determined by dividing the number of heterophils by the number of lymphocytes counted out of a total count of 50 cells. Using these two methods, Gross and Siegel determined that the

number of lymphocytes decreased and the number of heterophils increased in blood samples taken from white leghorns fed coricosterone supplemented feed (Gross and Siegel, 1983). It was also determined that the variability in the heterophil/lymphocyte ratio decreased by increasing the total number of cells counted.

In 2000, Elston et al. conducted an experiment to determine cage type preference and heterophil/lymphocyte ratio of laying hens in two different cage environments. One cage type was an open-sided cage and the other cage type was a solid-sided cage. Blood was collected from 24 Hy-Line W36 White Leghorn laying hens that were 45 weeks old. Blood samples were obtained from 12 hens that were housed long term in open-sided cages and 12 hens that were housed long term in solid-sided cages. Smears were prepared on duplicate glass microscope slides, and stained using a Leukostat stain. A total count of 100 white blood cells were counted to determine the heterophil/lymphocyte count. Elston et al. determined that birds housed in solid-sided cages generally had a higher heterophil/lymphocyte ratio than birds housed in open-sided cages. These results indicate that open-sided cages may be less stressful than solid-sided cages for laying hens.

There are many factors that may have an effect on heterophil/lymphocyte ratios in laying hens. Campo et al. conducted an experiment in 2008 to assess the effects of housing systems and cold stress on heterophil/lymphocyte ratios in different breeds of chickens. The two different housing systems were litter pens with or without access to an outdoor area. This was to simulate the difference between birds housed in an indoor area that would be in a controlled temperature versus free-range birds that could be exposed to

extreme temperatures. Blood samples were collected from 5 different Spanish breeds and a population of White Leghorns housed in two different systems. The blood smears were prepared with a May-Grünwald-Giemsa stain approximately 2-4 hours after methyl alcohol fixation. A total count of 100 leukocytes were counted and the heterophil/lymphocyte count was calculated. These researchers concluded that the housing system did affect the heterophil/lymphocyte ratio of birds. In general, birds that were allowed access to the outdoors had a lower heterophil/lymphocyte ratio. There were some differences between the different breeds indicating that breed could influence the heterophil/lymphocyte ratio. In the second part of the experiment, cold-stressed birds had a higher heterophil/lymphocyte ratio. Although there were again some differences in the breeds, the results still indicated exposure to cold temperatures caused a higher heterophil/lymphocyte ratio. Heterophil/lymphocyte ratio has been used in many experiments as a measure of stress which can be a cause of malicious behavior in poultry. A heterophil/lymphocyte ratio was used to assess stress in White Lohman Selected Leghorn hybrids housed in pens with or without long-cut straw for foraging and hens given different types of feed. These researchers were trying to determine if stress from a housing environment or diet could be a cause of feather pecking. Providing foraging material or a feed that is conducive to foraging is thought to reduce feather pecking tendencies. Feather pecking is an abnormal behavior which is known to decrease production and increase mortality in laying hens. Heterophil/lymphocyte ratios were found to be higher in groups housed without straw than groups housed with straw (El-Lethey et al., 2000). This again indicates that housing conditions can have an effect on

heterophil/lymphocyte ratios, therefore, having an effect on stress and welfare of laying hens.

The conventional cage housing system that is used most commonly in production is a very controversial welfare issue. The major concern with this housing system is the animals' ability to express normal behaviors in this environment. Nesting, perching, dust bathing and foraging are all important normal behaviors performed by laying hens. The conventional cage housing systems deprive laying hens the ability to perform these normal behaviors. Therefore, the enriched environment in this study contains nest boxes, perches, dust bathing boxes and wood shavings on the floor. Also, it is of concern whether the animals are stressed and in a state of constant fearfulness when housed in cages. Therefore, in efforts to assess laying hen welfare in a commercial cage housing system compared to a floor pen environment, behavioral assessments, fear response assessments and a physiological assessment were all used in this study. Each of these assessments can be linked back to the animals' welfare through the 5 freedoms established by the UK in 1997. With the combination of each of these assessments, a fair recommendation on the welfare of animals housed in these systems can be made.

CHAPTER III

COMPARISON OF AN ENRICHED AND BARREN ENVIRONMENT ON GENERAL BEHAVIORS IN THE HOME ENVIRONMENT AND EGG PRODUCTION OF COMMERCIAL LAYING HENS

Objective

The objective of this study was to investigate the effect of housing environment (enriched compared to barren) on general behaviors in the home environment and egg production in efforts to assess welfare in Hy-line® (W-36) laying hens from 6 to 35 weeks of age.

Materials and Methods

I. Experimental Treatments

On the first day of the project, 900 Hy-Line® W-36 day-old female chicks were wing banded for identification and divided into two environmental treatment groups. The chicks were randomly assigned to either an enriched or barren environment. Chicks assigned to the barren environment treatment were housed in 20 Petersime® battery brooder cages (25 chicks per cage) for the first 4 weeks. The battery brooder cages were 99 X 69 X 25 cm (39 X 27 X 10 in) with a level floor (273 cm^2 per bird). All birds were beak trimmed at 10 days of age. The chicks were then moved to 39 battery grower cages (10 pullets per cage) to simulate a commercial pullet cage system until 16 weeks of age. The battery grower cages were 61 X 58 X 38 cm (24 X 23 X 15 in) with level a floor (354 cm^2 per bird). At 16 weeks of age, the pullets were moved into 39 commercial VAL-CO™ layer cages (8 pullets per cage) for the remainder of the experiment. The

layer cages were 61 X 58 X 38 cm (24 X 23 X 15 in) with a 7.5° sloping wire floor (442 cm^2 per bird). Chicks assigned to the enriched environment were placed in 14 individual 3 X 2 m (9 X 6 ft) floor pens (28 chicks per pen 2143 cm^2 per bird) containing: 10 nest boxes that were 31 X 33 X 18 cm (12 X 13 X 7 in.) each, 254 cm (100 in) of 1.5 cm (0.6 in) diameter perches and a 61 X 64 X 15cm (24 X 25 X 6 in.) dust bathing box filled with peat moss. Birds housed in the enriched environment remained in the floor pens throughout the entire course of the study. United Egg Producer's guidelines were followed for bird density, perch specifications and nest space. Lighting, feeding and environmental temperature specifications were provided by Hy-line® commercial management guide. All birds were given ad libitum access to feed and water.

II. General Behavioral Observations

General observations were recorded on 9 randomly selected floor pens and 9 randomly selected cages during weeks 6, 10, 14, 19, 23, 27, 31 and 35. Video collection was performed over a 3 day period between 0800 and 1200 hours. Each selected floor pen and cage was videotaped for a total of 60 minutes. The observers set up the video cameras and then exited the facility to avoid observer effect on the bird's behavior. The first 15 minutes of video was ignored to allow time for the birds to recover from the observers entering and exiting the facility. The remaining forty-five minutes of video was analyzed for selected behaviors.

Scan Sampling

Scan samples were performed on all individuals in the group at two minute sample points from 15 minutes to 55 minutes of each video. The number of individuals in the group performing the selected behaviors was counted and recorded at each sample point. The selected behaviors for both floor pens and cages were: sitting, standing, feeding and other. The floor pen birds had an additional three categories in which they could be classified and counted. These behaviors were perching, nesting and dust bathing. A bird was counted in the other category if it was visible and performing a behavior that was other than sitting, standing or feeding in the cage. A bird was counted in the other category in the floor pens if it was performing a behavior other than sitting, standing, feeding, perching, nesting or dust bathing. For the purpose of comparison and analysis between the two environments; perching, nesting and dust bathing were added to the other category for the floor pen birds. A bird was classified as sitting if it was in a sitting or resting position on the floor of the cage or pen. Standing was defined as when a bird was in a standing position on the floor of the cage or pen. A bird was classified as feeding when it was standing at the feeder with its head in a downward position. If a bird was standing at the feeder and its head was in an upright position it was counted as standing. For the floor pen birds, a bird was counted as perching if it was standing or sitting on the designated perching areas. Nesting was defined to be when a bird was occupying a nest box. A bird was counted as dust bathing when it occupied the designated dust bathing box. Also at each sample point the total number of visible birds was counted and recorded. The total number of birds observed performing each behavior

for all 21 sample points was recorded and divided by 21 possible sample points. This number was then adjusted for comparison between the floor pen and cage environments by dividing it by the average number of birds visible. Because of the unequal number of birds in the floor pens and cages, an adjustment had to be made for comparison of floor pen birds and cage birds. These numbers could then be compared through statistical analysis to compare a percentage of birds performing the selected behavior in their home environments.

Interval / One-Zero Sampling

A one-zero sampling method was used to record certain behavioral events. This method was used at two minute sample intervals from 15 minutes to 59 minutes. The birds were observed during the intervals. If a certain behavior occurred during the interval a "1" was recorded. If the behavior did not occur a "0" was recorded. The designated behaviors that were observed were; wing flapping, wing preening, foraging, displacement at the feeder, aggressive pecking and non-aggressive pecking. Wing flapping was defined as when the bird flapped either one or both wings, or was flying. Wing preening was defined as when the bird's head rotated around and preened either of its wings. Foraging was defined as when the bird scratched at the floor with its feet and then pecked the floor of its home environment. Displacement at the feeder was defined as when one bird approached the feeder and caused an adjacent bird to move away from the feeder. Aggressive pecking was defined as when a bird pecked at any area on another bird in an aggressive manner causing the other bird to react by moving away or retaliating. Non-aggressive pecking was defined as when one bird pecked at any area on

another bird that caused no reaction from the other bird. After the entire video was observed, the total number of intervals that the behavior occurred was calculated and recorded. This number was then divided by 21, which is the number of possible intervals in which the behavior could have occurred. This number was then considered to be the one-zero score for each behavior. The scores from the floor pens could not be statistically compared to the scores of the cages because of the unequal numbers of birds in the two environments. Therefore, no further analyses were conducted on this data.

III. Production Assessment

Egg Collection and Production

Egg collection was performed on a daily basis, and the daily numbers were compiled to yield weekly egg production numbers. Egg production was analyzed on a weekly basis to avoid discrepancies in daily egg collection time. The total number of eggs collected per week was divided by the average number of hens in each environment per week. This yielded a hen week number which is the number of eggs produced per hen per week.

IV. Statistical Analysis

In order to satisfy the normality assumption for analysis, the scan sampling data for "sitting" and "other" behavior was transformed into log odds using a logit function [ln(P/(1-P))]. A two factor-factorial analysis was conducted to determine if there were significant treatment effects or interactions. Follow-up tests were conducted for treatment combinations within each week using Fisher's least significant difference

(LSD). All analyses were conducted using JMP, Version 8.0.1. SAS Institute Inc., Cary, NC, 1989-2009.

V. Animal Welfare Compliance

All procedures associated with the birds in this study were approved (App 2009-030) by the Clemson University Institutional Animal Care and Use Committee.

Results

General Observations- Scan Sampling

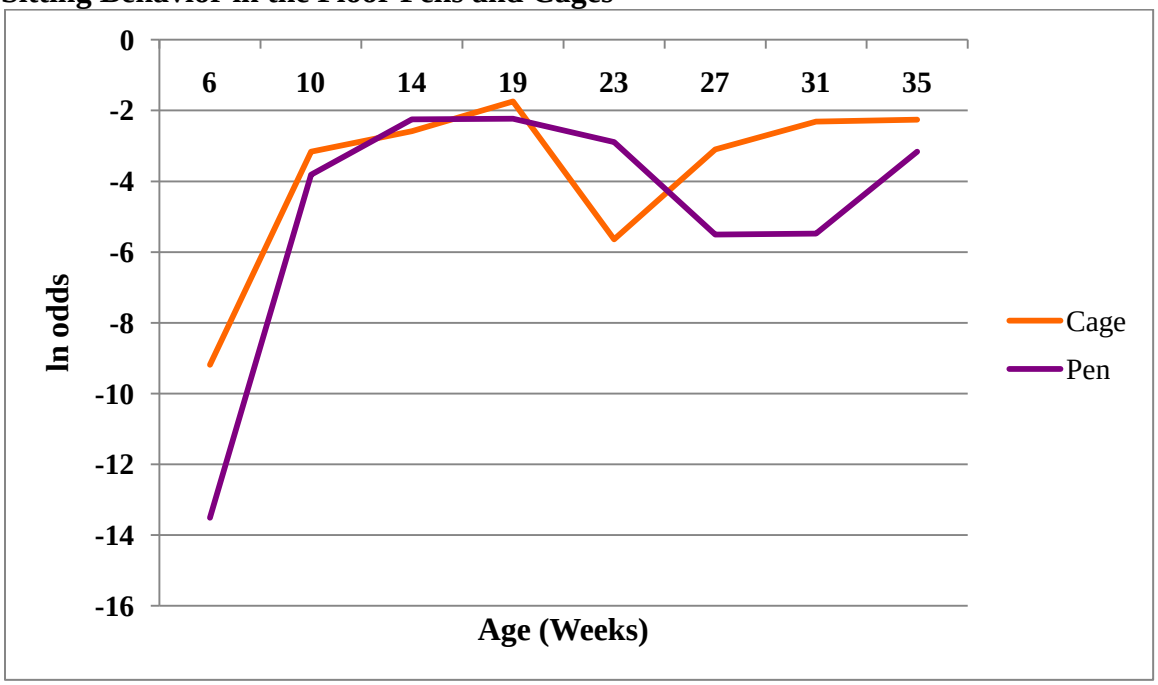
Results from the two-factor factorial analysis for the log odds of sitting behavior appear in Table 3.1. No significant interaction between the treatment and time was present in the log odds of sitting behavior as illustrated in Figure 3.1. There was a significant difference in log odds between the floor pen and cage environments on sitting behavior ($P<0.05$). There was also a significant difference in sitting behavior across the weeks ($P<0.05$).

Table 3.1. ANOVA Table for Log Odds Sitting Behavior

Source	DF	Sum of Squares	F Ratio	Prob > F
Trt.	1	84.0042	5.1986	0.0243*
Week	7	1115.6572	9.8632	<.0001*
Week*Trt	7	152.0094	1.3439	0.2350
Error	128	2068.3586		

*($P<0.05$)

Figure 3.1. Log Odds Interaction Plot of Time and Environmental Treatment on Sitting Behavior in the Floor Pens and Cages



The estimated average log odds and standard error of sitting behavior for each week of sampling appear in Table 3.2. The Fisher's LSD p-values from the transformed log odds sitting behavior data are also presented in Table 3.2. The log odds of birds sitting was significantly smaller (P<0.05) for floor pen birds than for cage birds during week 6.

Table 3.2. Mean* Proportion of Birds Sitting, Standard Error and P-values for Floor Pen Birds versus Cage Birds

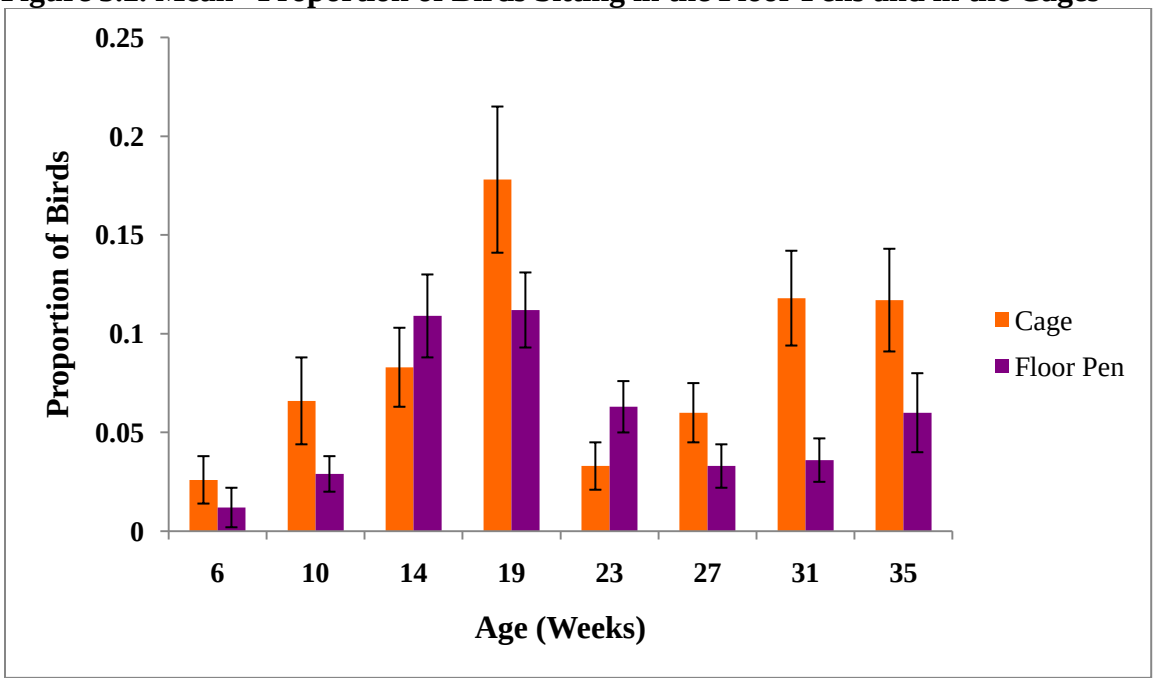
Week	Floor Pen (Mean ± SE)	Cage (Mean ± SE)	P-value (Significance P<0.05[+])
6	-13.512 ± 2.869	-9.191 ± 2.89	0.02426[+]
10	-3.819 ± 0.279	-3.16 ± 0.408	0.72856
14	-2.249 ± 0.216	-2.582 ± 0.225	0.86092
19	-2.229 ± 0.238	-1.743 ± 0.301	0.79789
23	-2.894 ± 0.238	-5.64 ± 1.92	0.14971
27	-5.507 ± 1.925	-3.103 ± 0.342	0.20675
31	-5.478 ± 1.94	-2.316 ± 0.364	0.09769
35	-3.16 ± 0.339	-2.257 ± 0.289	0.63434

*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample points. At each sample point, the ratio of the behaviors observed to the birds that were visible was recorded.

[+](P<0.05)

Figure 3.2. shows the proportion of birds performing sitting behavior from each week for floor pen birds and cage birds.

Figure 3.2. Mean* Proportion of Birds Sitting in the Floor Pens and in the Cages



*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample points. At each sample point, the ratio of the behaviors observed to the birds that were visible was recorded.

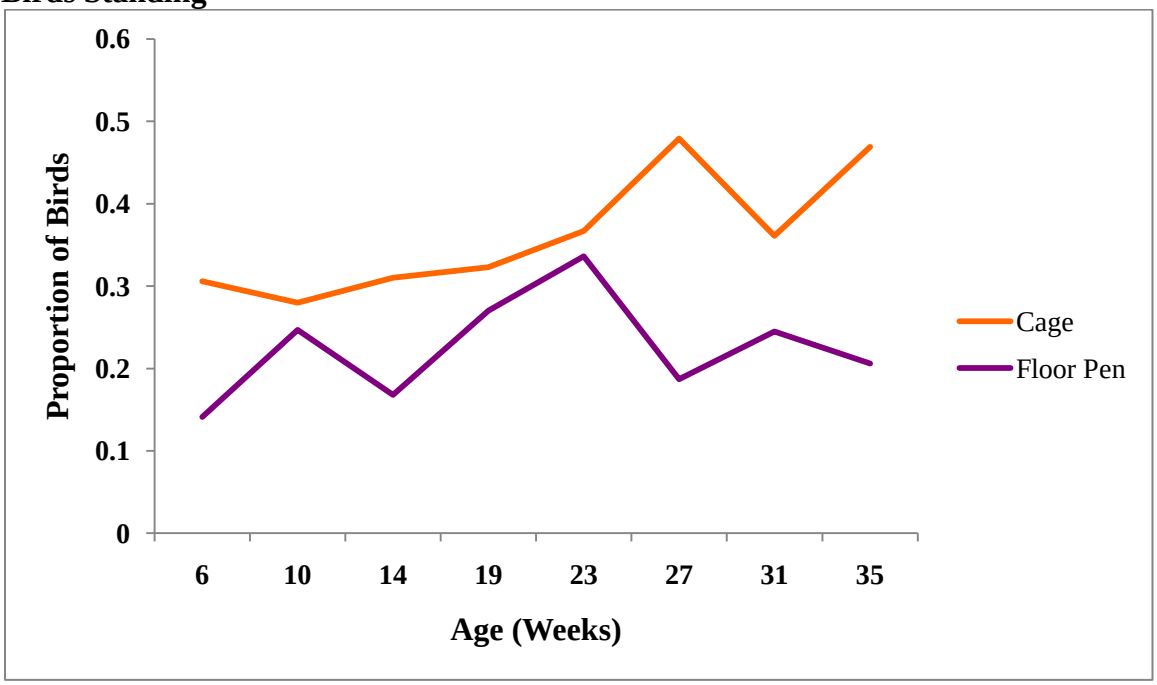
Results from the two-factor factorial analysis for standing behavior appear in Table 3.3. No significant interaction between the treatment and time was present in the average percentage of birds standing as illustrated in Figure 3.3 ($P<0.05$). There was a significant difference between the floor pen and cage treatments on standing behavior ($P<0.05$).

Table 3.3. ANOVA Table for Standing Behavior

Source	DF	Sum of Squares	F Ratio	Prob > F
Trt.	1	0.12159771	5.4964	0.0206*
Week	7	0.28322104	1.8289	0.0871
Week*Trt	7	0.31715016	2.0479	0.0539
Error	128	2.8317694		

*($P<0.05$)

Figure 3.3. Interaction Plot of Time on Environmental Treatment for Proportion of Birds Standing



The mean proportion of birds standing and standard error for each week of sampling appear in Table 3.4. The Fisher's LSD p-values are also presented in Table 3.4. The average proportion of birds standing was significantly smaller ($P<0.05$) for floor pen birds than for cage birds during weeks 6, 14, 27 and 35 of sampling (Figure 3.4).

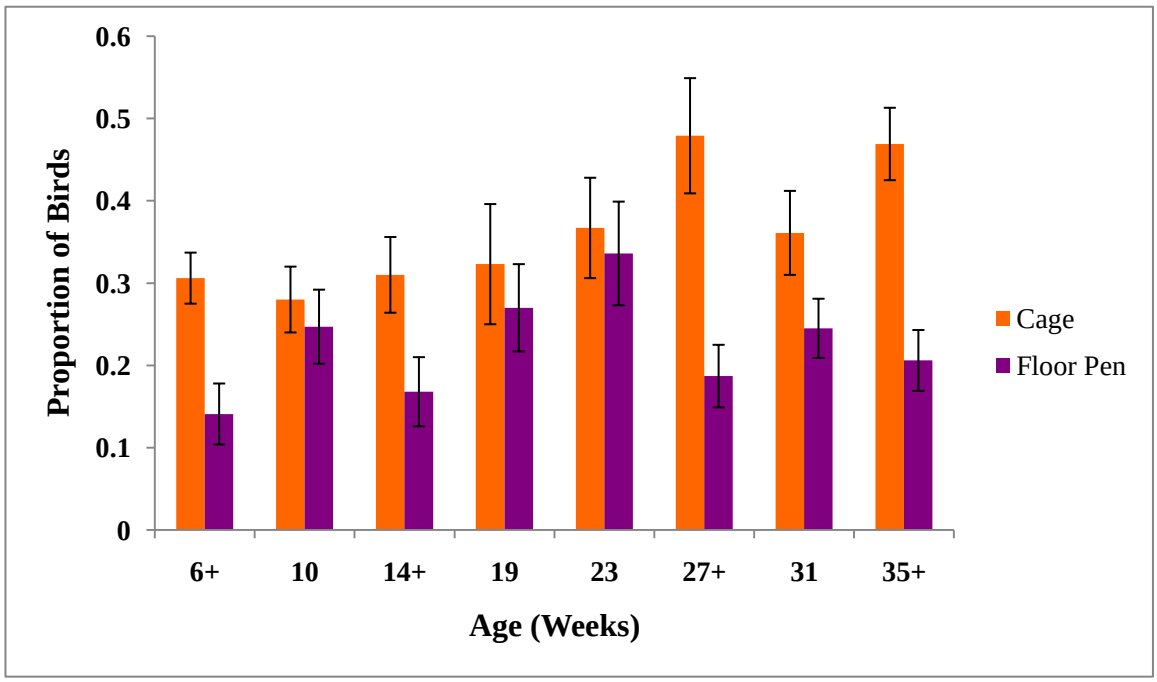
Table 3.4. Mean* Proportion of Birds Standing, Standard Error and P-values for Floor Pen Birds versus Cage Birds

Week	Floor Pen (Mean ± SE)	Cage (Mean ± SE)	P-value (Significance $P<0.05^{+}$)
6	0.141 ± 0.037	0.306 ± 0.031	0.02059^{+}
10	0.247 ± 0.045	0.28 ± 0.04	0.64018
14	0.168 ± 0.042	0.31 ± 0.046	0.04483^{+}
19	0.27 ± 0.053	0.323 ± 0.073	0.45466
23	0.336 ± 0.063	0.367 ± 0.061	0.66247
27	0.187 ± 0.038	0.479 ± 0.07	0.00006^{+}
31	0.245 ± 0.036	0.361 ± 0.051	0.09991
35	0.206 ± 0.037	0.469 ± 0.044	0.00026^{+}

*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample points. At each sample point, the ratio of the behaviors observed to the birds that were visible was recorded.
$^{+}(P<0.05)$

Figure 3.4 shows the proportion of birds performing standing behavior from each week for floor pen birds and cage birds.

Figure 3.4. Mean* Proportion of Birds Standing in the Floor Pens and in the Cages



*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample points. At each sample point, the ratio of the behaviors observed to the birds that were visible was recorded.
$^{+}$($P<0.05$)

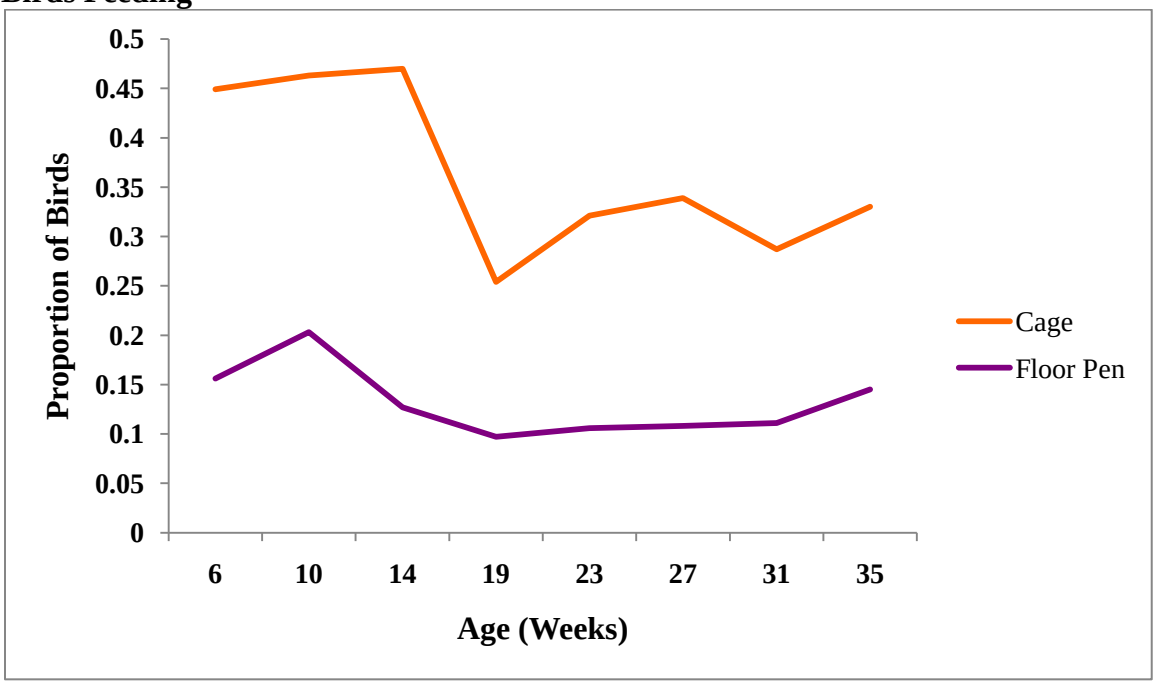
Results from the two-factor factorial analysis for feeding behavior appear in Table 3.5. There is a significant interaction ($P<0.05$) between week and environmental treatment on feeding behavior as illustrated in Figure 3.5. This indicates that the enriched floor pen and barren commercial cage environmental treatments did not have a consistent effect on the feeding behavior throughout all the weeks.

Table 3.5. ANOVA Table for Feeding

Source	DF	Sum of Squares	F Ratio	Prob > F
Trt.	1	0.38754236	88.3268	<.0001*
Week	7	0.40102664	13.0572	<.0001*
Week*Trt	7	0.12778470	4.1606	0.0004*
Error	128	0.5616123		

*(P<0.05)

Figure 3.5. Interaction Plot of Time on Environmental Treatment for Proportion of Birds Feeding



The mean proportion of birds feeding and standard error for each week of sampling appear in Table 3.6. The Fisher's LSD p-values are also presented in Table 3.6. The average proportion of birds feeding was significantly smaller ($P<0.05$) for floor pen birds than for cage birds during all weeks of sampling (Figure 3.6).

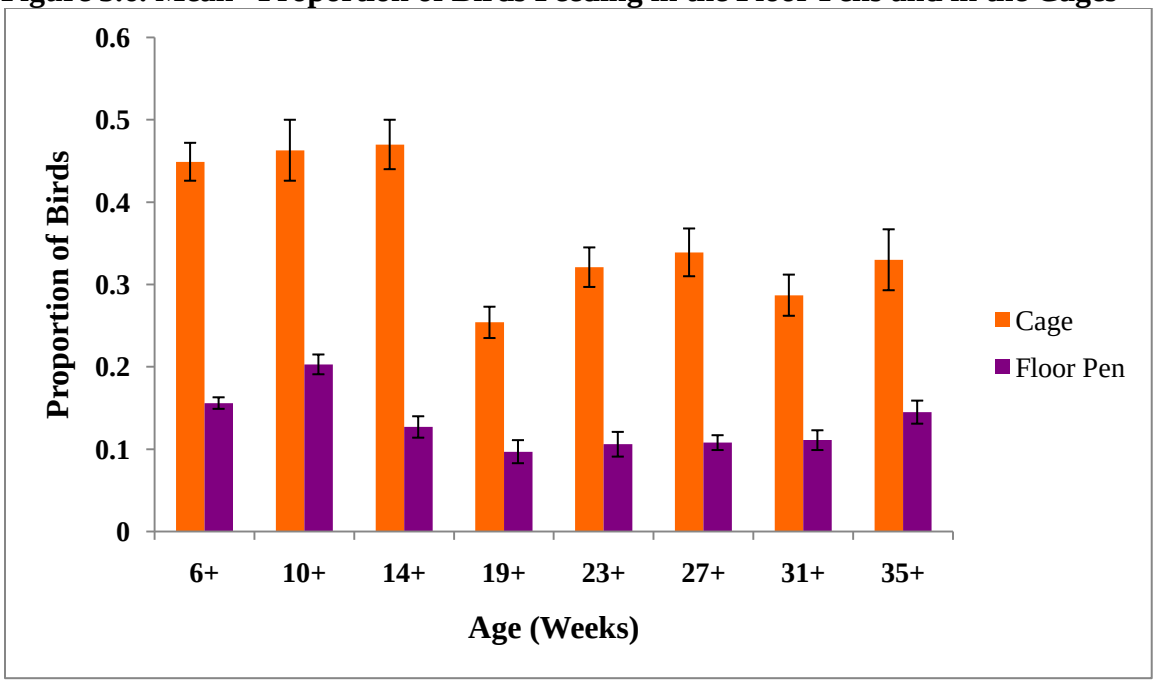
Table 3.6. Mean* Proportion of birds Feeding, Standard Error and P-values for Floor Pen Birds versus Cage Birds

Week	Floor Pen (Mean ± SE)	Cage (Mean ± SE)	P-value (Significance $P<0.05^{+}$)
6	0.156 ± 0.007	0.449 ± 0.023	$<.0001^{+}$
10	0.203 ± 0.012	0.463 ± 0.037	$<.0001^{+}$
14	0.127 ± 0.013	0.47 ± 0.03	$<.0001^{+}$
19	0.097 ± 0.014	0.254 ± 0.019	$<.0001^{+}$
23	0.106 ± 0.015	0.321 ± 0.024	$<.0001^{+}$
27	0.108 ± 0.009	0.339 ± 0.029	$<.0001^{+}$
31	0.111 ± 0.012	0.287 ± 0.025	$<.0001^{+}$
35	0.145 ± 0.014	0.33 ± 0.037	$<.0001^{+}$

*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample points. At each sample point, the ratio of the behaviors observed to the birds that were visible was recorded.
$^{+}(P<0.05)$

Figure 3.6. shows the proportion of birds performing feeding behavior from each week for floor pen birds and cage birds.

Figure 3.6. Mean* Proportion of Birds Feeding in the Floor Pens and in the Cages



*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample points. At each sample point, the ratio of the behaviors observed to the birds that were visible was recorded.

$^{+}(P<0.05)$

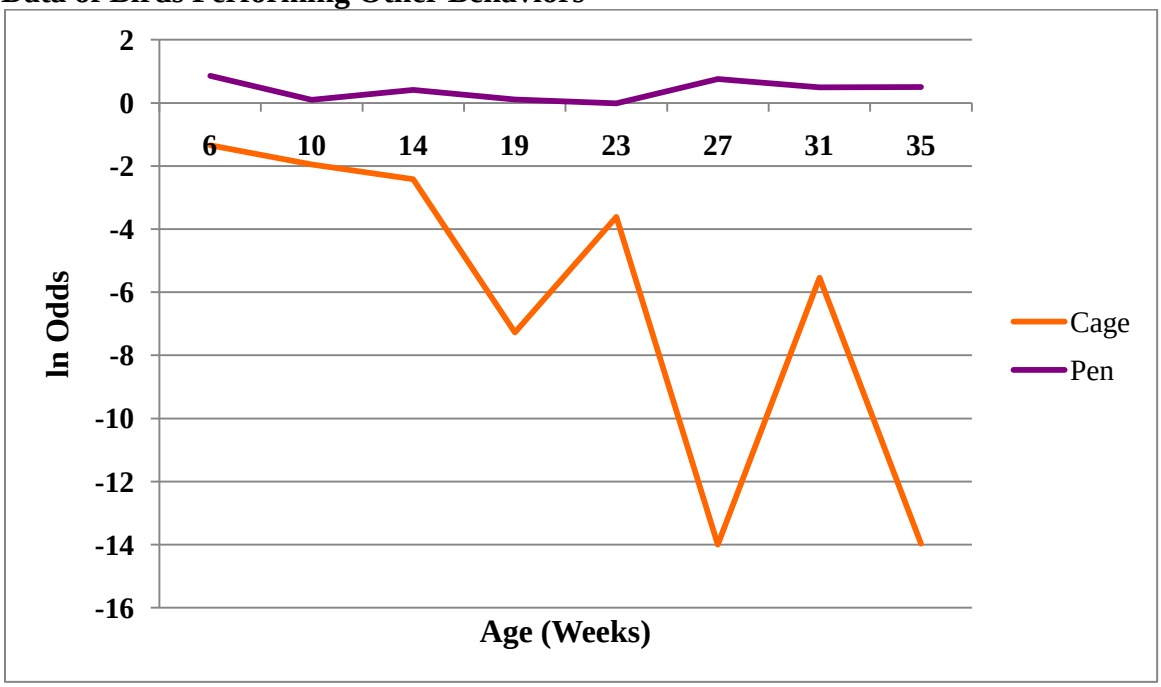
Results of the two-factor factorial analysis for the log odds of other behaviors appear in Table 3.7. There was a significant interaction between week and environmental treatment on log odds of other behaviors ($P<0.05$). This indicates that the enriched floor pen and barren commercial cage environmental treatments did not have a consistent effect on the log odds of other behaviors throughout all the weeks as illustrated in Figure 3.7.

Table 3.7. ANOVA Table for Log Odds Other Behavior

Source	DF	Sum of Squares	F Ratio	Prob > F
Trt.	1	21.76143	0.7995	0.3729
Week	7	807.78623	4.2397	0.0003*
Week*Trt	7	866.93699	4.5501	0.0001*
Error	128	3483.9793		

*($P<0.05$)

Figure 3.7. Interaction Plot of Time on Environmental Treatment for Transformed Data of Birds Performing Other Behaviors



The estimated average log odds of birds performing other behaviors and standard error for each week of sampling appear in Table 3.8. The Fisher's LSD p-values from the transformed odds data are also presented in Table 3.8. The log odds of birds performing other behaviors was significantly smaller (P<0.05) for cage birds than for floor pen birds during weeks 19, 27, 31 and 35 of sampling.

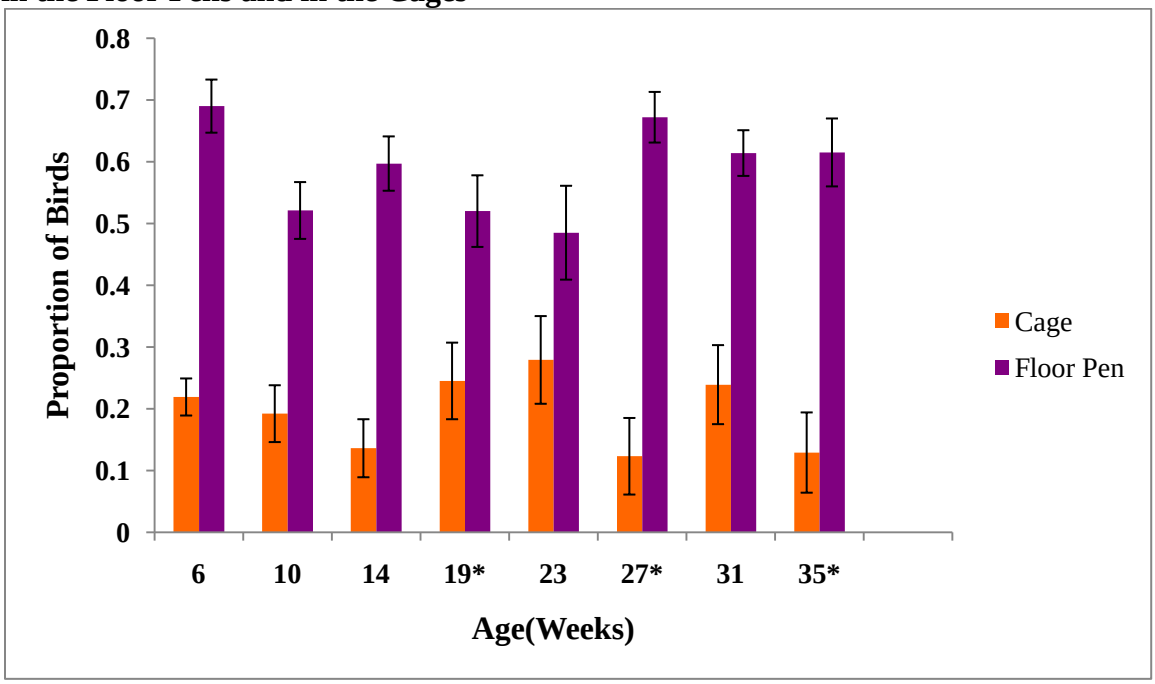
Table 3.8. Mean* Log Odds of Other Behaviors, Standard Error and P-values for Floor Pen Birds versus Cage Birds

Week	Floor Pen (Mean ± SE)	Cage (Mean ± SE)	P-value (Significance P<0.05[+])
6	0.858 ± 0.2	-1.341 ± 0.174	0.37292
10	0.096 ± 0.196	-1.952 ± 0.514	0.40657
14	0.41 ± 0.185	-2.422 ± 0.455	0.25165
19	0.108 ± 0.251	-7.274 ± 3.363	0.00323[+]
23	-0.011 ± 0.348	-3.612 ± 2.237	0.14557
27	0.76 ± 0.186	-13.997 ± 3.363	<.0001[+]
31	0.49 ± 0.163	-5.538 ± 2.907	0.01559[+]
35	0.498 ± 0.233	-13.971 ± 3.376	<.0001[+]

*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample points. At each sample point, the ratio of the behaviors observed to the birds that were visible was recorded.
[+](P<0.05)

Figure 3.8. shows the proportion of birds performing other behaviors from each week for floor pen birds and cage birds.

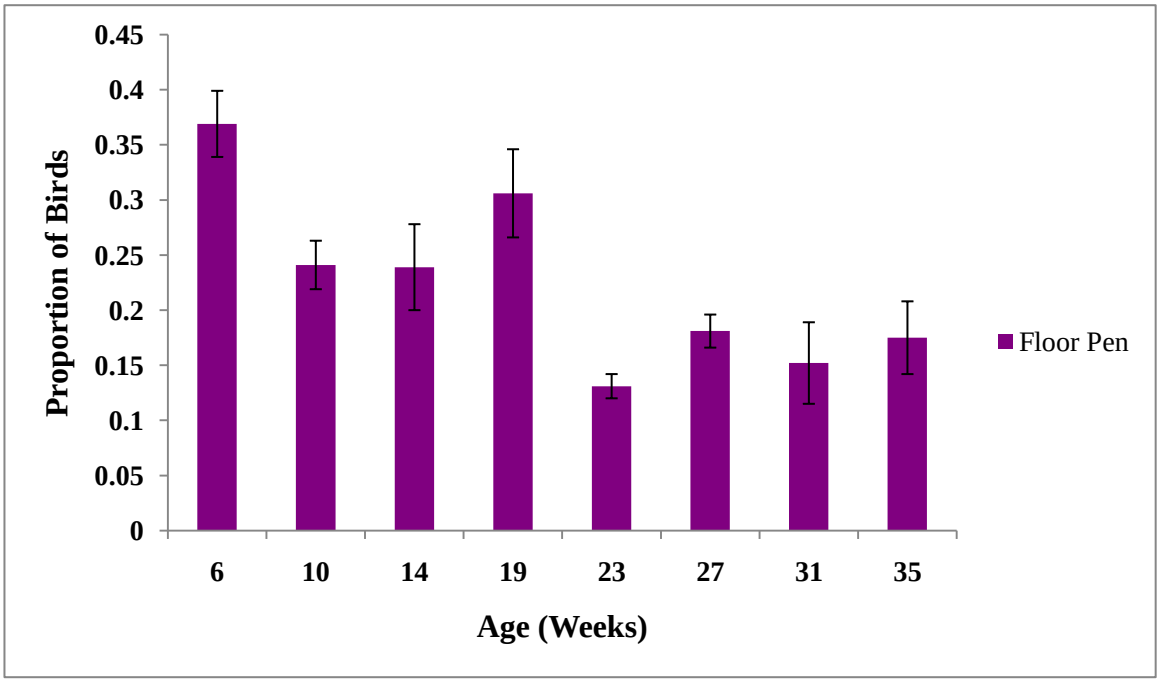
Figure 3.8. Mean Proportion of Birds Performing a Behavior Other than Specified in the Floor Pens and in the Cages



*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample points. At each sample point, the ratio of the behaviors observed to the birds that were visible was recorded.

Figure 3.9. shows the proportion of birds performing perching behaviors from each week for birds housed in the floor pens or enriched environment.

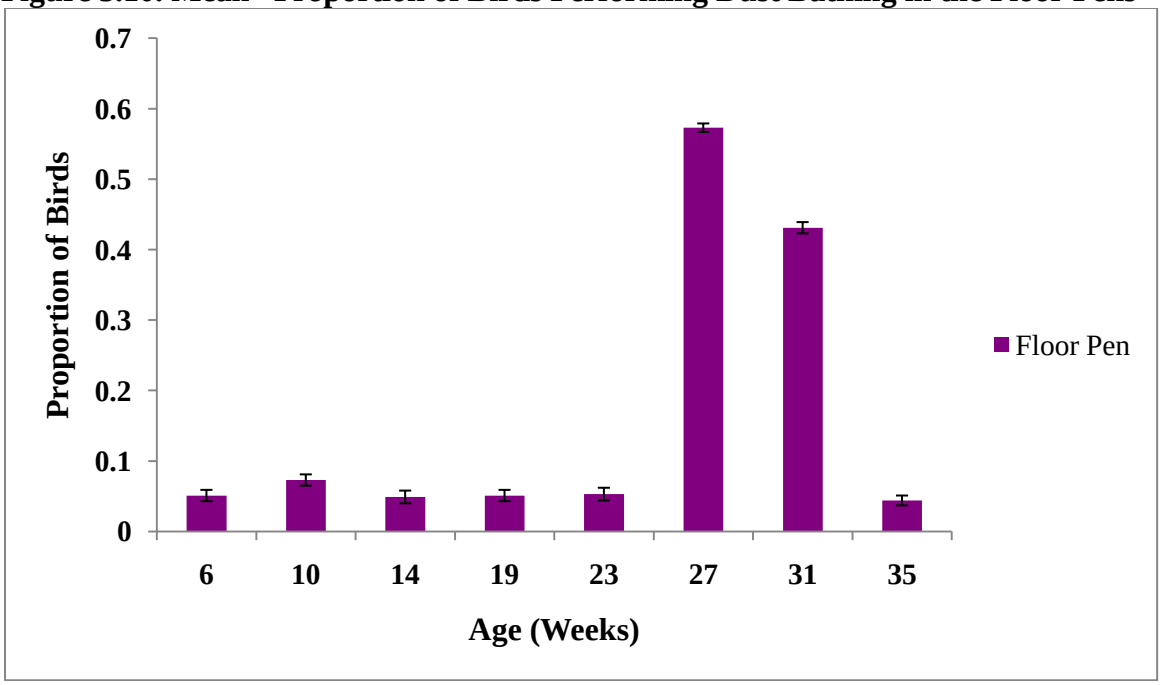
Figure 3.9. Mean* Proportion of Birds Performing Perching in the Floor Pens



*Each mean represents an average of birds in nine pens. For each pen, there were 21 sample points. At each sample point, the ratio of the behaviors observed to the birds that were visible was recorded.

Figure 3.10. shows the proportion of birds performing dust bathing behavior from each week for birds housed in the floor pens or enriched environment.

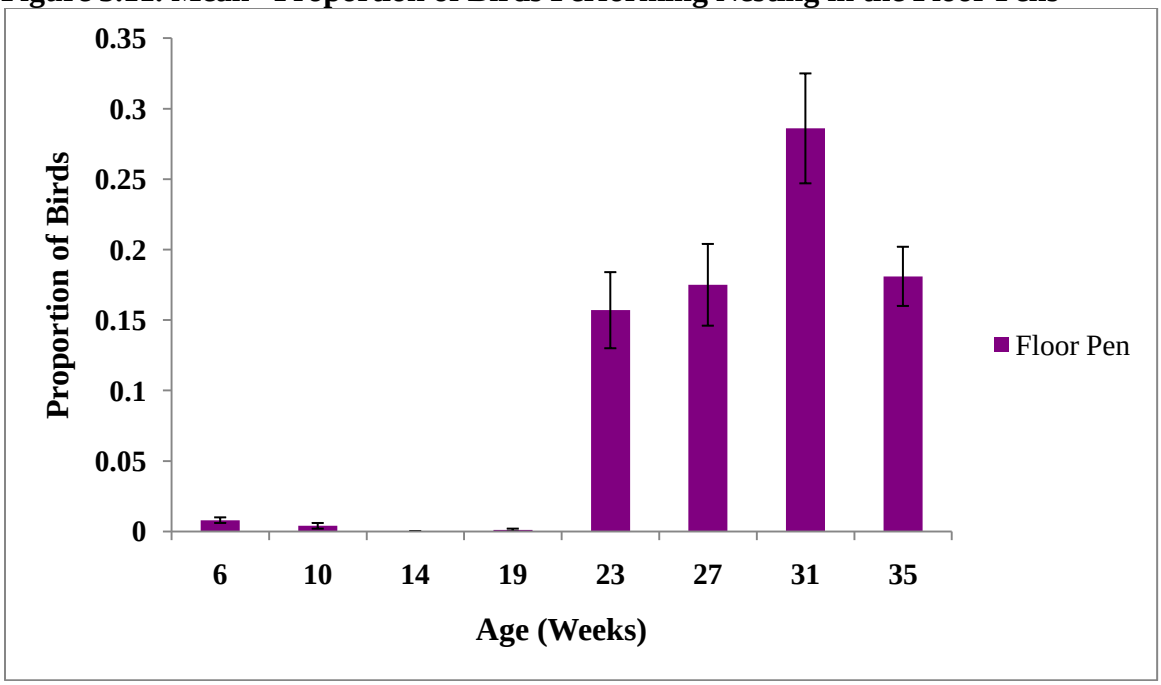
Figure 3.10. Mean* Proportion of Birds Performing Dust Bathing in the Floor Pens



*Each mean represents an average of birds in nine pens. For each pen, there were 21 sample points. At each sample point, the ratio of the behaviors observed to the birds that were visible was recorded.

Figure 3.11. shows the proportion of birds performing nesting behaviors from each week for birds housed in the floor pens or enriched environment.

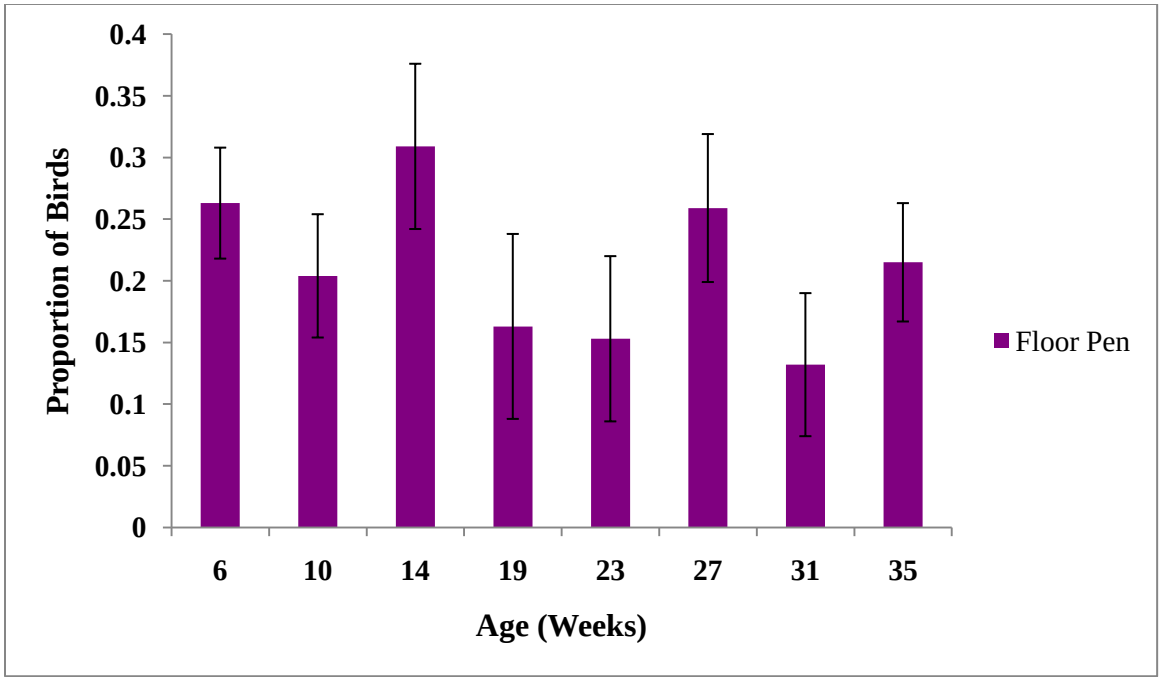
Figure 3.11. Mean* Proportion of Birds Performing Nesting in the Floor Pens



*Each mean represents an average of birds in nine pens. For each pen, there were 21 sample points. At each sample point, the ratio of the behaviors observed to the birds that were visible was recorded.

Figure 3.12. shows the proportion of birds performing other than specified behaviors from each week for birds housed in the floor pens or enriched environment. The specified behaviors were sitting, standing, feeding, perching, dust bathing and nesting.

Figure 3.12. Mean* Proportion of Birds Performing Other than Specified Behaviors in the Floor Pens

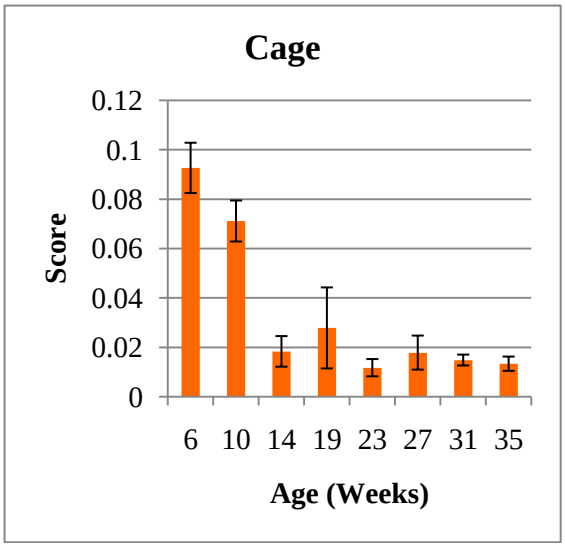


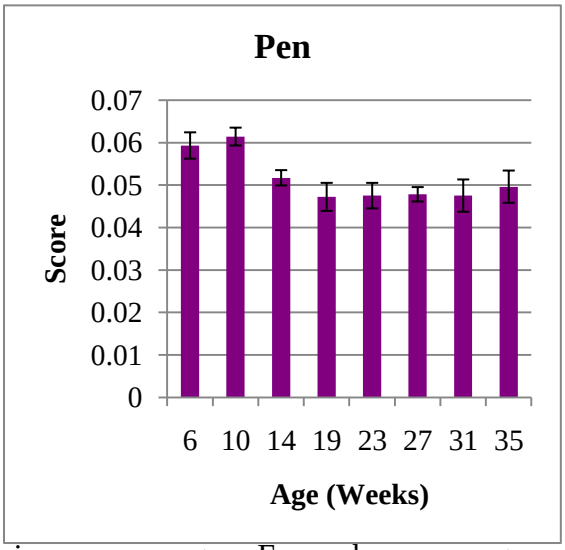
*Each mean represents an average of birds in nine pens. For each pen, there were 21 sample points. At each sample point, the ratio of the behaviors observed to the birds that were visible was recorded.

General Observations- Interval Sampling

Figures 3.13 and 3.14 show the mean one-zero scores for wing flapping behaviors in cage and floor pen birds.

Figure 3.13 and Figure 3.14 Mean* Wing Flapping Scores for Cage and Floor Pen Birds

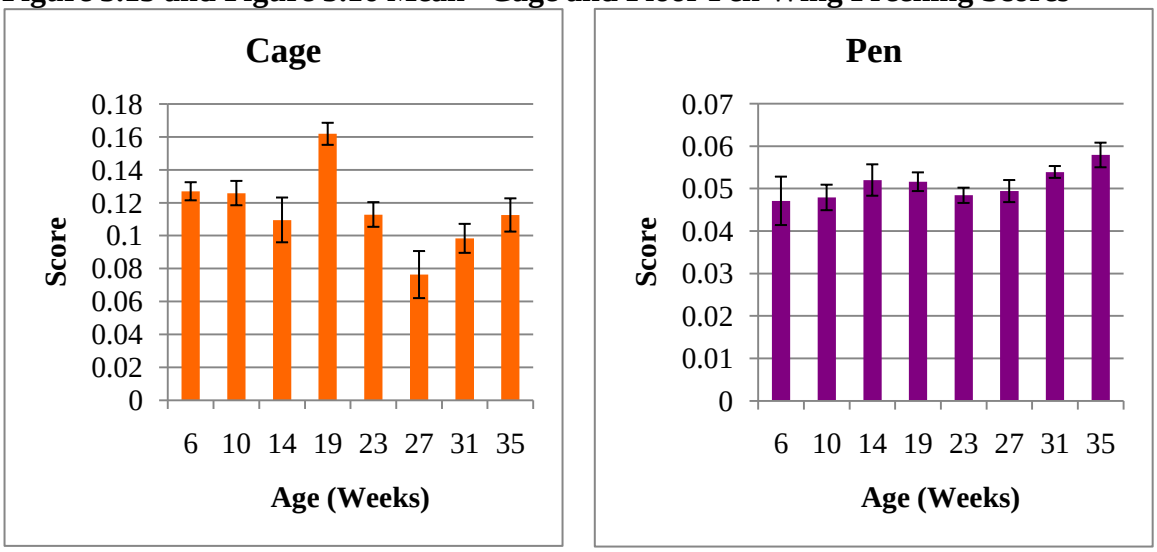




*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample intervals. The ratio of the intervals that the behavior occurred to 21 possible sample intervals was recorded.

Figures 3.15 and 3.16 show the mean one-zero scores for wing preening behaviors in cage and floor pen birds.

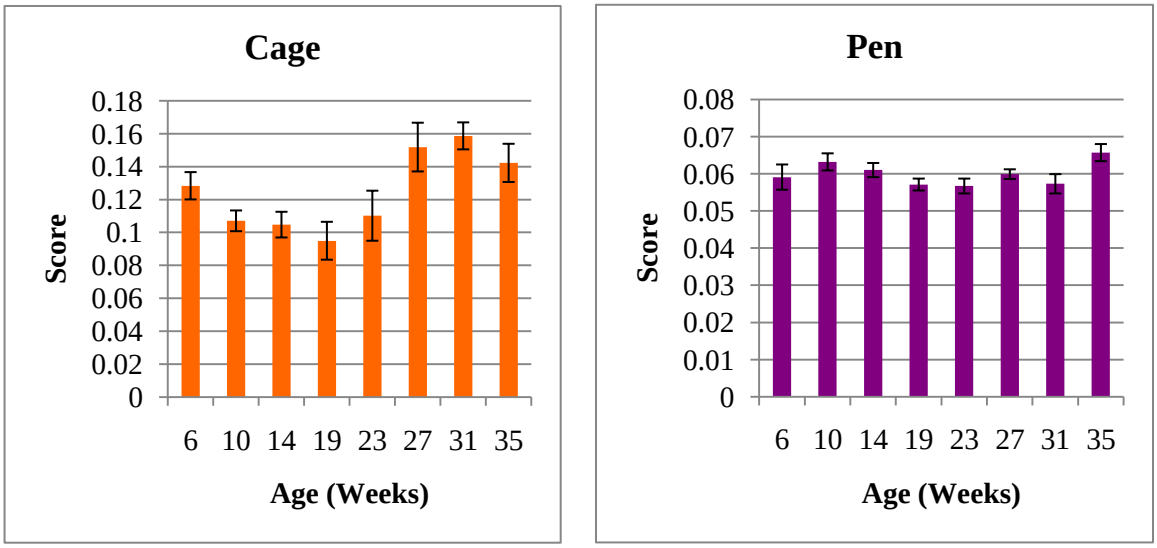
Figure 3.15 and Figure 3.16 Mean* Cage and Floor Pen Wing Preening Scores



*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample intervals. The ratio of the intervals that the behavior occurred to 21 possible sample intervals was recorded.

Figures 3.17 and 3.18 show the mean one-zero scores for foraging behaviors in cage and floor pen birds.

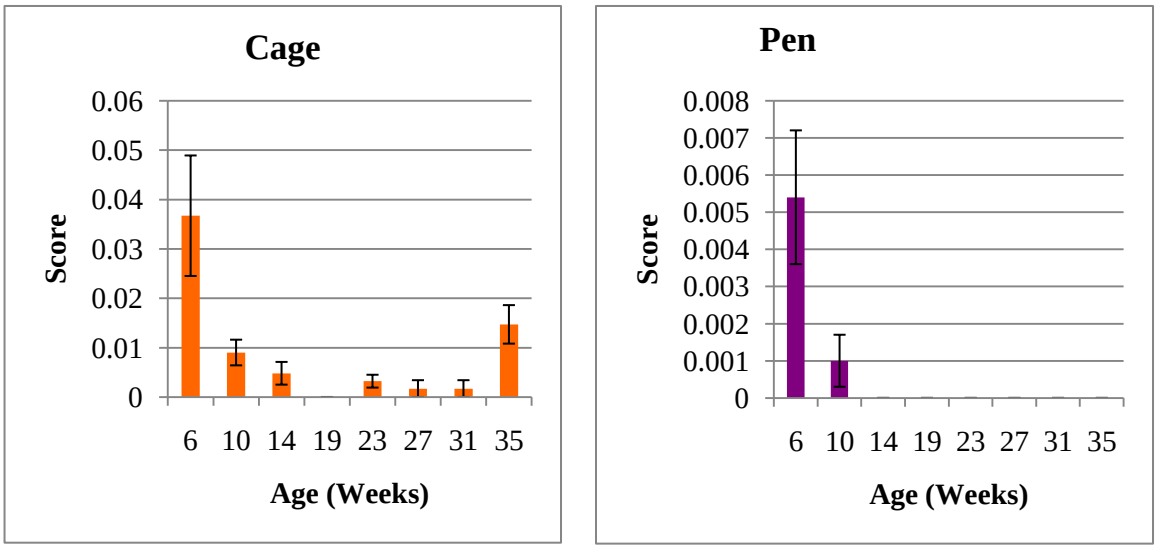
Figure 3.17 and Figure 3.18 Mean* Cage and Pen Foraging Scores



*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample intervals. The ratio of the intervals that the behavior occurred to 21 possible sample intervals was recorded.

Figures 3.19 and 3.20 show the mean one-zero scores for displacement behaviors at the feeder for cage and floor pen birds.

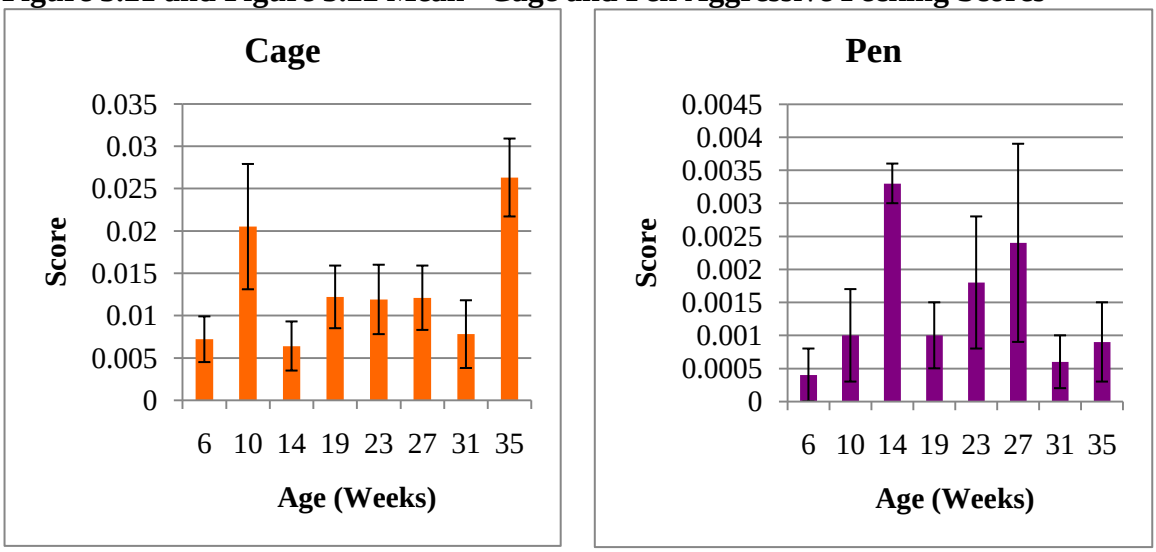
Figure 3.19 and Figure 3.20 Mean* Cage and Pen Displacement at the Feeder Scores



*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample intervals. The ratio of the intervals that the behavior occurred to 21 possible sample intervals was recorded.

Figures 3.21 and 3.22 show the mean one-zero scores for aggressive pecking behaviors in cage and floor pen birds.

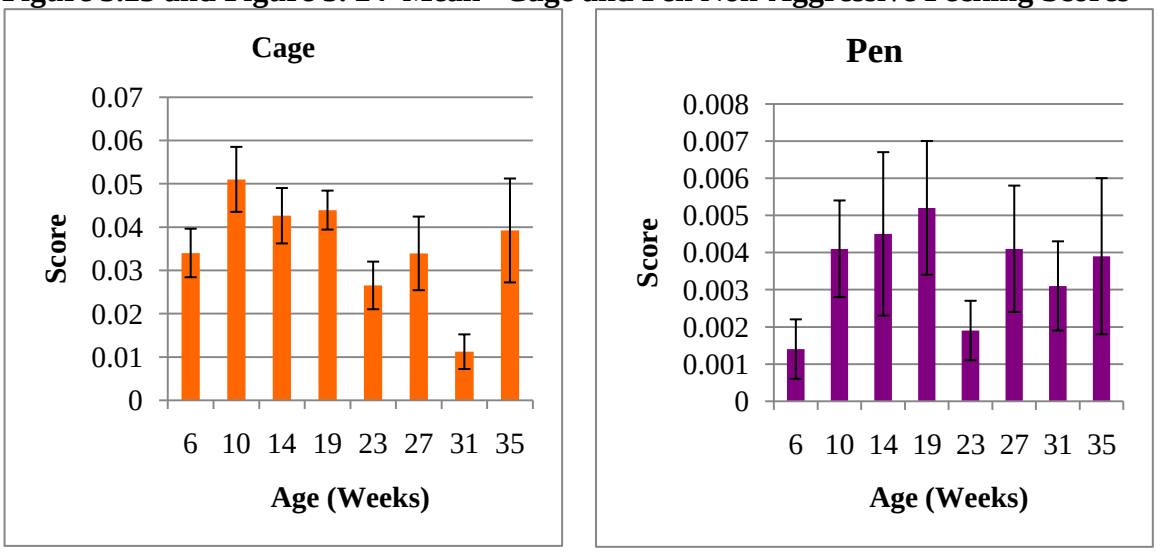
Figure 3.21 and Figure 3.22 Mean* Cage and Pen Aggressive Pecking Scores



*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample intervals. The ratio of the intervals that the behavior occurred to 21 possible sample intervals was recorded.

Figures 3.23 and 3.24 show the mean one-zero scores for non-aggressive pecking behaviors in cage and floor pen birds.

Figure 3.23 and Figure 3. 24 Mean* Cage and Pen Non-Aggressive Pecking Scores

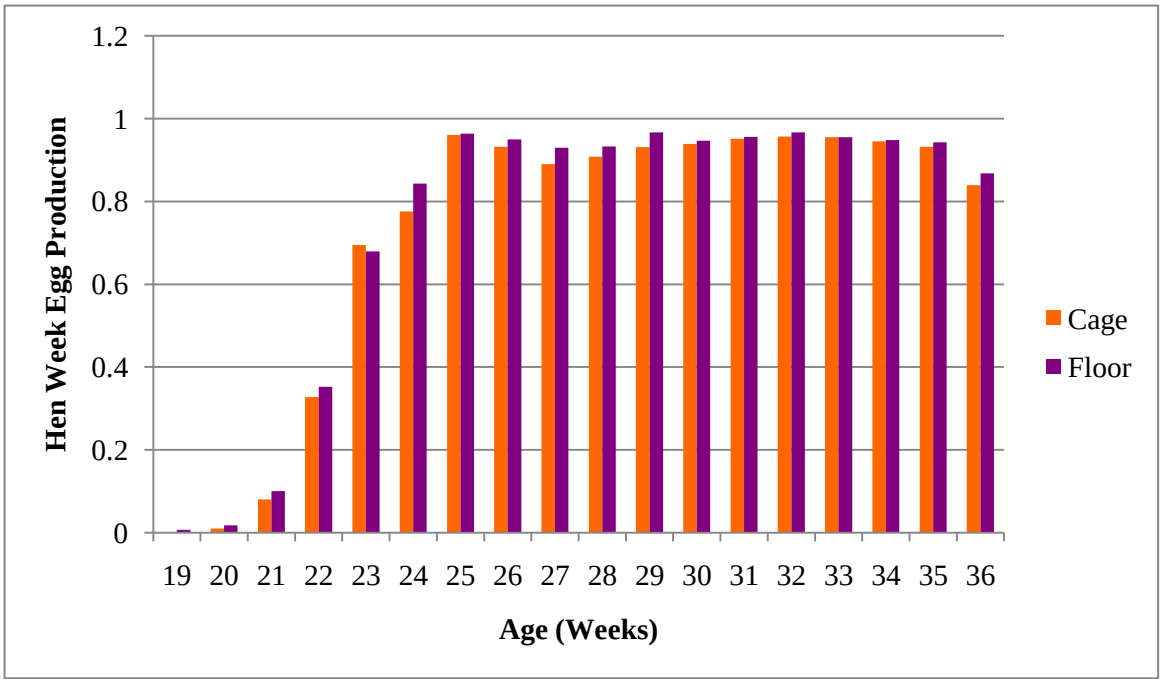


*Each mean represents an average of birds in nine pens or cages. For each pen or cage, there were 21 sample intervals. The ratio of the intervals that the behavior occurred to 21 possible sample intervals was recorded.

Egg Production

Figure 3.25. shows the mean hen week egg production for birds housed in the floor pens and birds housed in cages.

Figure 3.25. Mean* Hen Week Egg Production for Floor Pen and Cage Birds



*Each mean represents the average of eggs collected from 14 pens and 35 cages.

Discussion and Conclusions

There was a significant difference ($P<0.05$) between floor pen birds and cage birds for the log odds of sitting behavior. There was also a significant difference ($P<0.05$) in the percentage of birds standing between the floor pen and the cage environments. Although, a significant interaction ($P<0.05$) was present in the feeding behavior, the cage birds had a significantly higher ($P<0.05$) percentage of birds feeding than the floor pen birds did in all weeks of sampling. A possible explanation for the cage

birds having a higher percentage of birds sitting, standing and feeding is stocking density. When the cage birds were moved at week 16 into the layer cages, they had 442 cm^2 per bird. The floor pen birds had 2143 cm^2 per bird in the floor pens. The cage birds are, therefore, stocked at a much higher density and have less room per bird than the floor pen birds. Carmichael et al., in 1999, investigated the effect on behavior and welfare of systematically varying the amount of space per bird at higher stocking densities than typical of commercial conditions in a multilevel perch system. They found that birds spent significantly more time standing when the stocking density was increased from 9.9 birds per m^2 to 19 birds per m^2. Density was not found to have an effect on feeding or resting behavior. It is, however, possible that this difference was not detected because they only used one environmental treatment which was a multilevel perchery system. In our study, we compared two completely different housing environments. In the floor pens the birds had access to perches, nest boxes and dust bathing boxes. If a bird in the floor pen was in one of these areas it was not counted as standing or sitting, even if it was doing so, in the nest boxes, dust bathing box or on the perches. It is possible that, if given a choice, birds will prefer to use these areas rather than just standing or sitting on the floor. This also accounts for the "other" behaviors. Birds in the floor pens not only have more space per bird to perform behaviors but they also have a variety of different areas they can occupy in the floor pens.

Albentosa et al. (2002), found that comfort behaviors increased as stocking density decreased. Comfort behaviors, such as wing flapping, feather ruffling, dust bathing and preening are important for keeping the plumage well groomed in both natural

and artificial conditions. The performance of these comfort behaviors varies among different housing systems. This is mainly due to stocking density, because these behaviors require a large area of space for performance (Appleby et al., 2004). An assessment of dust bathing in the dust bathing box was used in the pens to determine if this enrichment was readily utilized by the birds. This assessment, however, did not count the percentage of birds performing dust bathing on the floor in the wood shavings. Only birds using the specified dust bathing box were counted as dust bathing. Shields et al. (2004) found that birds performed dust bathing in wood shavings or other floor litter, but if a finer substrate was provided, the birds preferred to dust bathe in the finer substrate. It is thought that the finer substrate penetrates through the feathers to reach the downy portion of the plumage better than a thicker substrate. It is possible that percentage of birds performing dust bathing was not recorded accurately because of possible dust bathing in the wood shavings litter on the floor.

Although, no statistical analysis was performed on the percentage of birds occupying the nesting boxes in the floor pens, it was expected that an increase in activity in the nesting boxes would occur around the onset of lay. Egg production began in the floor pens when the birds were 19 weeks of age. Providing nest boxes to hens is important in helping to prevent egg laying on the floor. When eggs are laid on the floor, they are easily broken and often dirty (Appleby, et al., 2004). Baxter, in 1994, found that hens are motivated to find nesting sites and if access was denied, hens became frustrated. It appears that perching behavior slightly decreases as the birds grow older, and it is possible that not all perching behavior was recorded. All video recordings were

performed between 0800 and 1200 hours. Birds spend more time perching at night than during the day (Savory, et al. 2002). Providing birds with early access to perches is important for normal spatial skills development. In 2000, Gunnarsson, et al., found that rearing without early access to perches impairs the spatial cognitive skills of the domestic hen. Early access to perches may influence the behavior of the adult hen in two different ways. The first is that the use of perches at an early age increases the muscle mass and bone strength of birds, so that they can more easily use the perches. The second is that the use of perches helps to develop the cognitive skills necessary for moving around in a three-dimensional space (Gunnarsson, et al., 2000). Providing chicks with early access to perches may also influence the amount of eggs that are laid on the floor instead of nest boxes. Chicks raised in environments without perches have been found to lay more eggs on the floor when compared to chicks raised with access to perches (Appleby et al., 1988).

It appears that the score of wing flapping in the cages decreases over time, and in the floor pens, wing flapping remains constant over the weeks. This could be due to the increase in the stocking density of the birds in the cages. As the birds grow and mature, they have less space available per bird. It is possible that the decrease in wing flapping in the cages is associated with the birds not having enough space to perform this behavior. Battery cages restrict movement more than any other production system. Restricting movement results in the prevention of specific behavior patterns, because most behavior patterns require more space than just standing. Frustration and physiological consequences can arise from restriction of movement (Appleby et al., 2004). Knowles

and Broom, in 1990, found that wing movement and flying were completely absent in battery cages when compared to other housing systems. They also found that birds housed in cages had weaker bones than birds housed in other systems such as percheries.

Wing preening, foraging, displacement of others at the feeder, aggressive and non-aggressive pecking behaviors all have similar trends between floor pen birds and cage birds. Displacement of others at the feeder appears to be higher during week six than any other week for both floor pen birds and cage birds. It is possible that this higher level of displacement at the feeder during week six is due to the birds establishing a social hierarchy. Guhl, in 1958, observed that chicks began play fighting as early as two weeks of age. True fighting was seen at six weeks of age, and the average age of pecking-order formation was nine to ten weeks for females. It is possible this pecking-order formation was occurring during week six with displacement of others at the feeder, and this behavior may have increased over the next few weeks before decreasing at week ten. At week ten recordings, it is possible that the pecking-order had already been established. Once the social hierarchy is established, it remains relatively stable (Guhl, 1958). When the cage birds were moved during week 4, it is possible that the pecking order had not yet been established. When the cage birds were again moved during week 16, they had to establish a new pecking order in the laying cages. It is possible that this was not detected by displacement at the feeder behavior observations during week 19, because a new pecking order within a small group can be established within hours (Craig, 1981).

No statistical analysis was performed on egg production data. It appears that egg production in the cages and floor pens is similar. Egg production, however, did begin at an earlier age (week 19) in the floor pens than the cages (week 20). Environmental conditions have been shown to have an effect on egg production. Pohle and Cheng (2009), found that environmentally enriched cages caused a left shift in the onset of peak production in hens. Egg production was not the primary focus of this study, therefore further investigation is needed to assess if there are any statistical differences between the two environments.

It appears from scan sampling that cage birds had a higher percentage of birds sitting on the floor, standing and feeding than the floor pen birds. This could be due to the floor pen birds having more options and space to perform other behaviors such as perching, dust bathing or occupying the nest boxes. From the literature, it appears that providing laying hens with perches, a place to dust bathe and nest boxes is important to meet normal behavioral needs and improve welfare. A comparison of these behaviors was unable to be made between the floor pens and cages because battery cages do not provide appropriate areas for these normal behaviors. The one-zero sampling data was unable to be statistically analyzed due to different bird numbers in the floor pens and cages. The trend of wing flapping, which is a comfort behavior, appears to decrease across the weeks in the cages. Perhaps this trend occurs because of the increased stocking density in the cages which may not allow adequate space for performing this behavior. From these general behavioral observations, it appears that the floor pen birds have the ability to perform more natural behaviors because of the increased amount of

space per bird and with the enrichment of perches, dust bathing box and nest boxes. Because of the ability to perform more natural behaviors, the floor pen birds are housed in an environment that better suits the welfare needs of laying hens. It is, however, important to include a physiological assessment as well as fear assessments before making a final conclusion about laying hen welfare in cages and floor pens.

CHAPTER IV

COMPARISON OF AN ENRICHED AND BARREN ENVIRONMENT ON TWO WELFARE RELATED FEAR RESPONSE BEHAVIORS OF COMMERCIAL LAYING HENS

Objective

The objective of this study was to investigate the effect of housing environment (enriched compared to barren) on behavioral fear response in efforts to assess welfare in Hy-line® (W-36) laying hens from day 1 to 32 weeks of age.

Materials and Methods

I. Experimental Treatments

On the first day of the project, 900 Hy-Line® W-36 day-old female chicks were wing banded for identification and divided into two environmental treatment groups. The chicks were randomly assigned to either an enriched or barren environment. Chicks assigned to the barren environment treatment were housed in 20 Petersime® battery brooder cages (25 chicks per cage) for the first 4 weeks. The battery brooder cages were 99 X 69 X 25 cm (39 X 27 X 10 in) with a level floor (273 cm^2 per bird). All birds were beak trimmed at 10 days of age. The chicks were then moved to 39 battery grower cages (10 pullets per cage) to simulate a commercial pullet cage system until 16 weeks of age. The battery grower cages were 61 X 58 X 38 cm (24 X 23 X 15 in) with level a floor (354 cm^2 per bird). At 16 weeks of age, the pullets were moved into 39 commercial VAL-CO™ layer cages (8 pullets per cage) for the remainder of the experiment. The layer cages were 61 X 58 X 38 cm (24 X 23 X 15 in) with a 7.5° sloping wire floor (442

cm^2 per bird). Chicks assigned to the enriched environment were placed in 14 individual 3 X 2 m (9 X 6 ft) floor pens (28 chicks per pen 2143 cm^2 per bird) containing: 10 nest boxes that were 31 X 33 X 18 cm (12 X 13 X 7 in.) each, 254 cm (100 in) of 1.5 cm (0.6 in) diameter perches and a 61 X 64 X 15cm (24 X 25 X 6 in.) dust bathing box filled with peat moss. Birds housed in the enriched environment remained in the floor pens throughout the entire course of the study. United Egg Producer's guidelines were followed for bird density, perch specifications and nest space. Lighting, feeding and environmental temperature specifications were provided by Hy-line® commercial management guide. All birds were given ad libitum access to feed and water.

II. Behavioral assessments

Emergence test

Ten birds from each environment were randomly selected at weeks 2, 4, 8, 12, 17, 21, 25, 29 and 33 for testing. Each bird was taken individually to a testing area that resembled the bird's home environment. The bird was then placed in an emergence box and the exit door was closed for 60 seconds. Three different size boxes were used throughout the experiment to adjust to the body size of the birds. For weeks 2 and 4 of testing, the emergence box was 18 X 18 X 18 cm (7 X 7 X 7 in) with an opening that was 10 X 15 cm (4 X 6 in). For weeks 8 and 12, the emergence box was 31 X 31 X 31 cm (12 X 12 X 12 in) with an opening that was 15 X 15 cm (6 X 6 in). For the remainder of the weeks, the emergence box was 31 X 31 X 31 cm (12 X 12 X 12 in) with an opening that was 18 X 20 cm (7 X 8 in). After the 60 seconds, the door was opened and latency until complete emergence was recorded in seconds. After the exit door was opened, the

observer stepped out of sight and made observations on a video monitor that was connected to a camera outside the emergence box. Complete emergence was determined when the bird's entire body had exited the emergence box. The maximum time allowed for emergence was 600 seconds. If the bird did not emerge within the 600 seconds, the test was terminated. After the emergence test, a red leg band was placed on the leg of each bird for identification purposes. Each bird was only used once for the emergence test and no other behavioral assessments were performed on that bird.

Tonic immobility

Ten birds from each environmental treatment were randomly selected and taken individually to another room for testing at weeks 2, 4, 8, 12, 17, 21, 25, 29 and 33. Tonic immobility was performed on a covered table to create a soft surface for the birds. Each bird was manually restrained on its left side for 15 seconds then released. If the bird remained immobile, the observer stepped out-of-sight and watched from a video camera and monitor that was mounted above the table. Tonic immobility was considered to be induced if the bird remained immobile for a minimum of 10 seconds. A maximum of 5 attempts were made to induce tonic immobility in each bird. Recovery from tonic immobility was considered to be when the bird self-righted. Records were made on tonic immobility duration in seconds, as well as, number of inductions. The maximum time allowed for recovery was 900 seconds. If the bird did not recover within 900 seconds, the test was terminated. After the tonic immobility test, a dark blue leg band was placed on the leg of each bird for identification purposes. Each bird was used only once for the tonic immobility test and no other behavioral assessments were performed on that bird.

III. Statistical Analysis

A two factor-factorial analysis was conducted to determine if there were significant treatment effects or interactions. Follow-up tests were conducted for treatment combinations within each week using Fisher's least significant difference (LSD). All analyses were conducted using JMP, Version 8.0.1. SAS Institute Inc., Cary, NC, 1989-2009.

IV. Animal Welfare Compliance

All procedures associated with the birds in this study were approved (App 2009-030) by the Clemson University Institutional Animal Care and Use Committee.

Results

Emergence Test

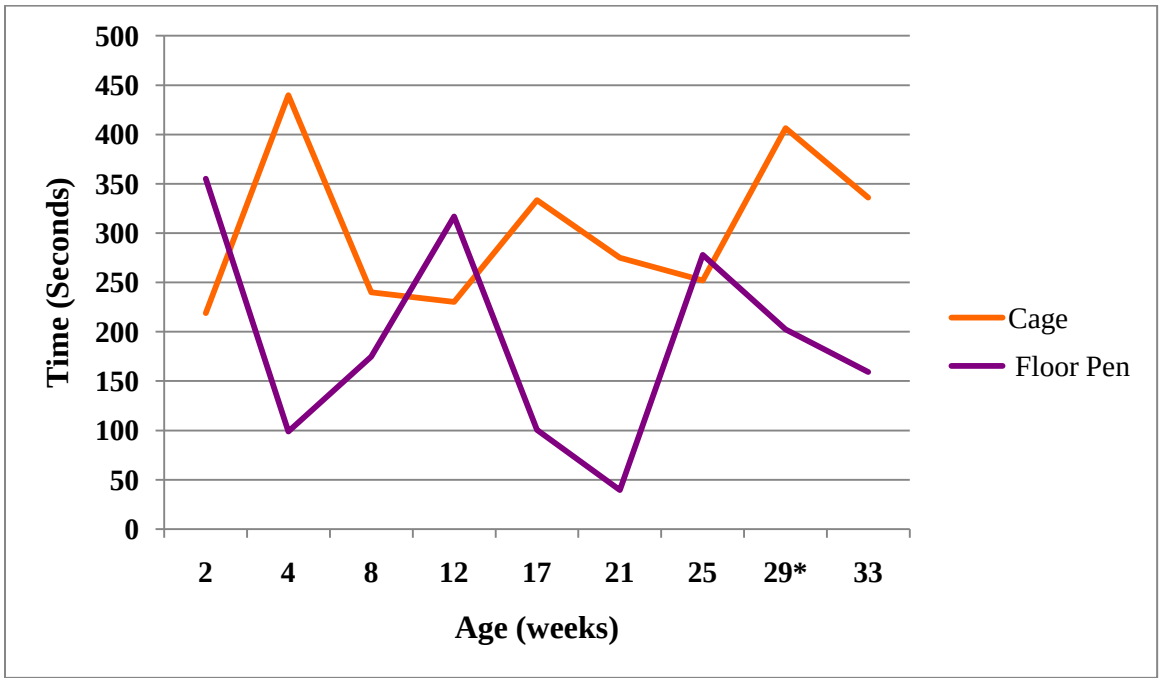
Results from the two-factor factorial analysis for the emergence test appear in Table 4.1. There is a significant interaction ($P<0.05$) between week and environmental treatment on the latency of emergence in the emergence test as illustrated in Figure 4.1. This indicates that the enriched floor pen and barren commercial cage environmental treatments did not have a consistent effect on the emergence fear response throughout all the weeks.

Table 4.1. ANOVA Table for Emergence Test

Source	DF	Sum of Squares	F Ratio	Prob > F
Trt.	1	92752.2	2.0190	0.1573
Week	8	338544.1	0.9212	0.5006
Week*Trt	8	1085578.1	2.9538	0.0041*
Error	162	7442331.9		

*($P<0.05$)

Figure 4.1. Interaction Plot of Time and Environmental Treatment on Emergence Time



The mean emergence time in seconds, standard error and Fisher's LSD p-values for each week of sampling appear in Table 4.2. The average latency of emergence was significantly shorter ($P<0.05$) for floor pen birds than for cage birds during weeks 4, 17, 21 and 29 (Figure 4.2).

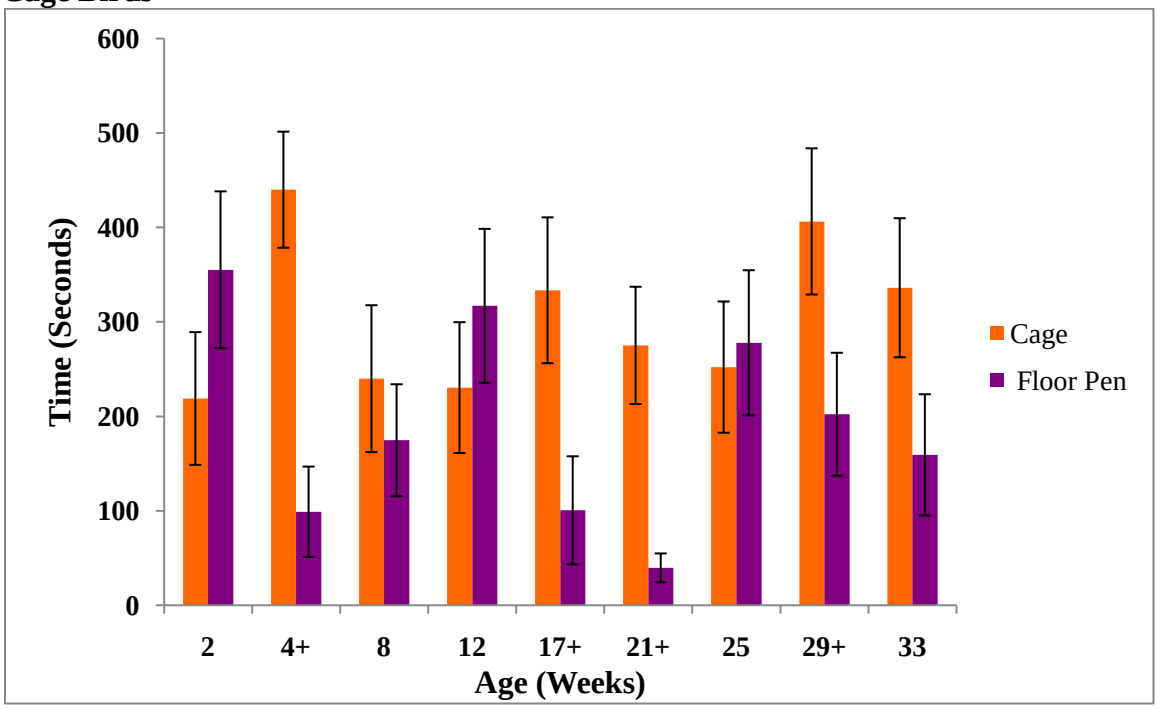
Table 4.2. Mean* Emergence Time in Seconds, Standard Error and Fisher's LSD P-values for Floor Pen and Cage Birds

Age (Weeks)	Floor Pen (Mean ± SE)	Cage (Mean ± SE)	P-value (Significance $P<0.05^{+}$)
2	355.1 ± 70.3	218.9 ± 83	0.1573
4	99 ± 61.4	439.9 ± 47.8	0.0005^{+}
8	174.7 ± 77.7	239.9 ± 59.3	0.4974
12	316.9 ± 69.2	230.4 ± 81.5	0.3682
17	100.5 ± 77.2	333.4 ± 57.2	0.0162^{+}
21	39.6 ± 62.1	275.1 ± 15.3	0.0151^{+}
25	277.8 ± 69.5	252.1 ± 76.8	0.789
29	202.2 ± 77.4	406.3 ± 65	0.0347^{+}
33	159.2 ± 73.6	336.1 ± 65	0.0668

*Each mean represents an average of emergence durations for 10 birds
$^{+}$($P<0.05$)

Figure 4.2. shows the average emergence time from each week for floor pen birds and cage birds.

Figure 4. 2. Mean* Latency of Emergence Duration in Seconds for Floor Pen and Cage Birds



*Each mean represents an average of emergence durations for 10 birds
$^{+}$(P<0.05)

Tonic Immobility

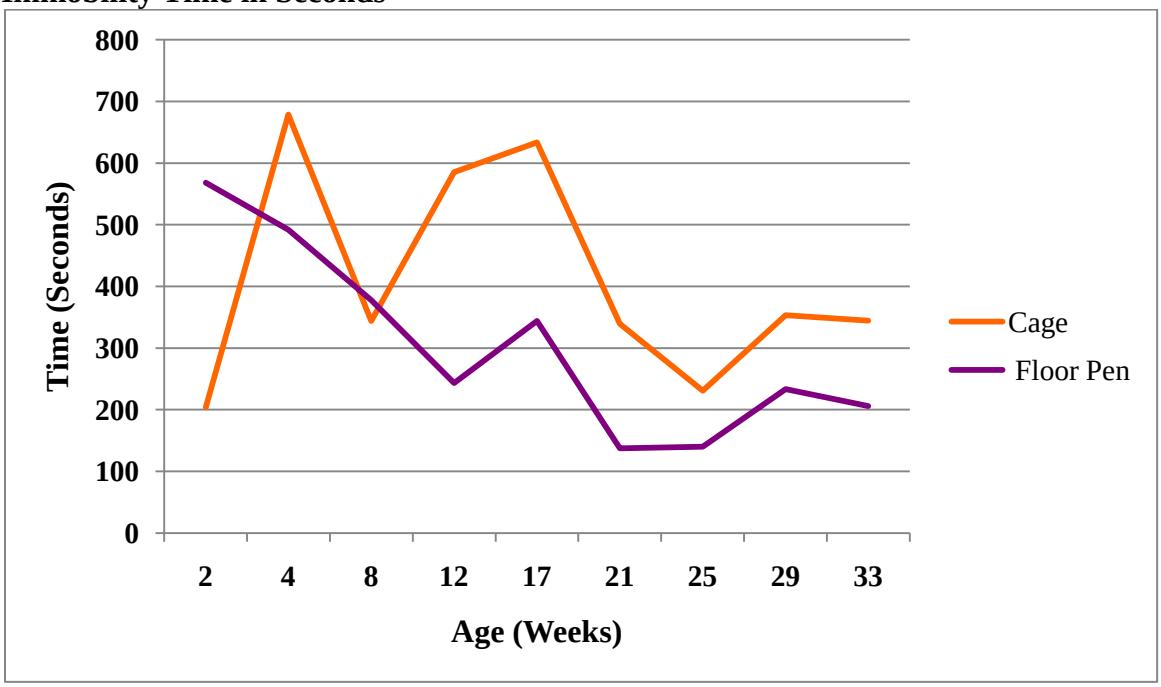
Results of the two-factor factorial analysis for the tonic immobility test appear in Table 4.3. There is a significant interaction between week and environmental treatment on the duration of tonic immobility in the tonic immobility test ($P<0.05$). This indicates that the enriched floor pen and barren commercial cage environmental treatments did not have a consistent effect on the tonic immobility fear response throughout all the weeks as illustrated in Figure 4.3.

Table 4. 3. ANOVA Table for Tonic Immobility

Source	DF	Sum of Squares	F Ratio	Prob > F
Trt.	1	589194.7	8.7483	0.0036*
Week	8	2460689.1	4.5670	<0.0001*
Week*Trt.	8	1608030.6	2.9845	0.0039*
Error	157	10573930		

*(P<0.05)

Figure 4.3. Interaction Plot of Time and Environmental Treatment on Tonic Immobility Time in Seconds



The mean tonic immobility duration in seconds, standard error and Fisher's LSD p-values for each week of sampling appear in Table 4.4. The average tonic immobility duration was significantly shorter ($P<0.05$) for floor pen birds than for cage birds during weeks 12 and 17 (Figure 4.4). The average tonic immobility duration was significantly shorter ($P<0.05$) for cage birds than for floor pen birds during week 2 (Figure 4.4).

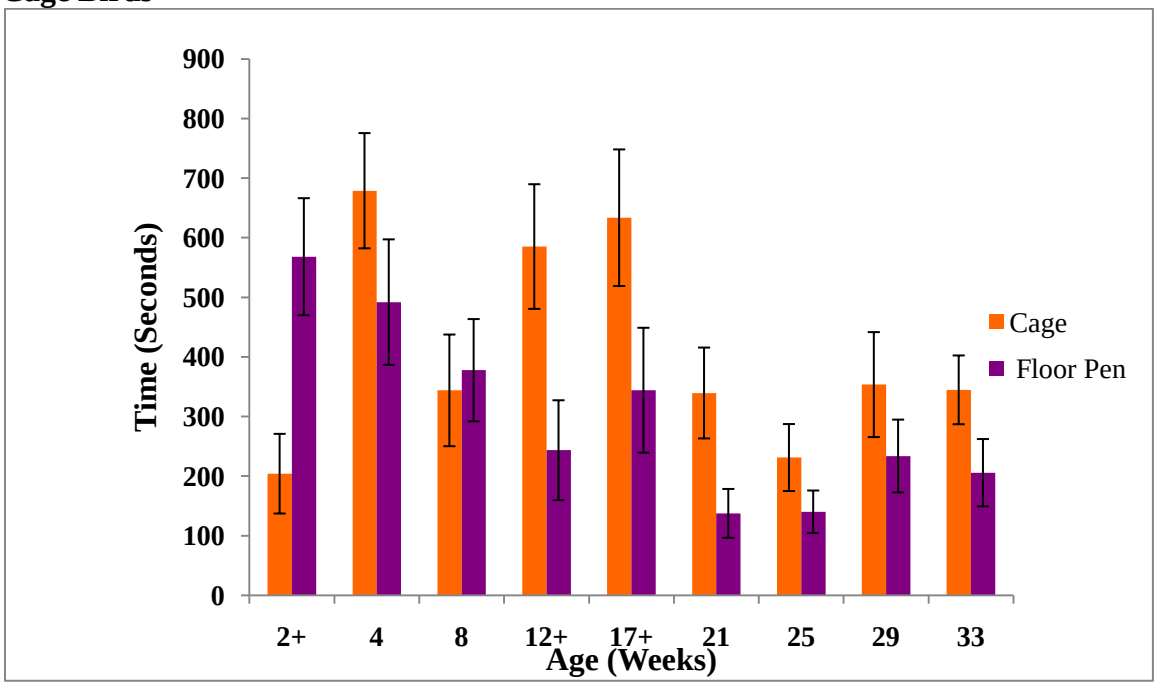
Table 4.4. Mean* Recovery Time from Tonic Immobility in Seconds, Standard Error and Fisher's LSD P-values for Floor Pen and Cage Birds

Age (Weeks)	Floor Pen (Mean ± SE)	Cage (Mean ± SE)	P-value (Significance $P<0.05^{+}$)
2	568.1 ± 66.8	204 ± 98.1	0.0036^{+}
4	491.9 ± 96.7	678 ± 105.3	0.1190
8	377.7 ± 93.7	343.8 ± 85.8	0.7706
12	243.4 ± 104.6	585.1 ± 83.8	0.0037^{+}
17	344 ± 114.6	633.5 ± 104.8	0.0137^{+}
21	137.4 ± 76.2	339.4 ± 41	0.0837
25	140.1 ± 56.2	231.111 ± 35.7	0.4465
29	233.7 ± 88.1	353 ± 61.1	0.3036
33	205.7 ± 57.7	344.667 ± 56.5	0.2456

*Each mean represents an average of tonic immobility durations for 10 birds
$^{+}$($P<0.05$)

Figure 4.4. shows the average tonic immobility duration from each week for floor pen birds and cage birds.

Figure 4.4. Mean Tonic Immobility Recovery Time in Seconds for Floor Pen and Cage Birds



*Each mean represents an average of tonic immobility durations for 10 birds
+(P<0.05)

Discussion and Conclusion

Emergence Test and Tonic immobility

There was a significant ($P<0.05$) difference of average emergence time between floor pen birds and cage birds at weeks 4, 17, 21, and 29 of sampling (Table 4.2). There was a significant ($P<0.05$) difference of average duration of tonic immobility between floor pen birds and cage birds at weeks 2, 12 and 17. The differences in average emergence times and tonic immobility durations in selected weeks could indicate an age related effect of environmental conditions on chicks and pullets. Many changes occur as a bird grows and matures, and environmental enrichment has been shown to cause significant changes in behavior and learning. Krause et al. (2006) showed that short term enrichment of housing conditions in chicks 6 weeks of age could have positive immediate effects on behavior by reducing behaviors that are likely to reflect fearfulness and by positively affecting learning performance.

There were significant treatment-by-time interactions in both the emergence and tonic immobility tests. It is possible that the treatments themselves caused these interactions. The birds in the enriched floor pens experienced a consistent environment throughout the study. In contrast, the birds in the cage system were moved two times. First, the birds were moved from battery brooder cages in one building to grower battery cages in the grower/layer house during week 4. The second move within the grower/layer house was from grower battery cages to layer cages during week 16. It is possible that these moves in environments for cage birds could have had an effect on the results of the fear response assessments.

For the tonic immobility test, the trend in the older pullets' tonic immobility duration (weeks 17-33) could indicate a possible point at which fear response levels off and no dramatic changes are taking place. This could also be associated with possible changes that occur when the birds reach maturity. The floor pen birds follow the same trends as the cage birds from week 17-33. An increase in mean tonic immobility time for both environments at week 17 could indicate a possible crucial point in development. With both environments showing an increase at week 17, an age related effect could be indicated. These results coincide with the findings of Gharreb et al. in 2008, who found changes in both tonic immobility duration as well as emergence time around the age that the birds began to reach maturity. It is thought that during rearing age, a higher fear response could be explained as a helpful strategy for young chicks to avoid the higher risk of predation. It was shown that the age-related increase in tonic immobility duration may be associated with the approach of sexual maturity and the birds' endocrine changes (Campo and Carnicer, 1993).

From weeks 12-33, it appears that the environment does have a consistent effect on the average recovery time for tonic immobility, with the cage birds having an apparent trend towards a longer average recovery time than the floor pen birds. These results are consistent with Jones and Faure's findings in 1981 that birds raised in cages has a longer average recovery time from tonic immobility then birds raised in floor pens.

Although the ability to detect treatment difference was compromised due to the interaction of time and treatment that was present in both the emergence and tonic immobility tests, it appears that the cage birds had a longer latency of emergence as well

as a longer tonic immobility duration than the floor pen birds. It is possible that this difference could have been significant with an increase in the number of birds tested as well as more frequent testing. Because of time limitations associated with the behavioral assessments and not wanting to reuse birds for additional assessments, a small sample size was chosen to be analyzed. It has been shown that repeated measures on individuals can cause a habituation effect (Gallup, 1974b). A more distinct difference between the two environmental treatments might be shown with a larger sample size.

Fearful behavior in animals is now widely regarded as an undesirable state of suffering and has been linked back to welfare through the five freedoms established by the UK in 1997 (Farm Animal Welfare Council). It is important to investigate the effects of housing environment on the fear response in laying hens so that welfare may be improved. A clear conclusion about welfare cannot be made from the two behavioral fear response assessments alone. It is important to include general observations from the home environment as well as a physiological assessment.

CHAPTER V

COMPARISON OF AN ENRICHED AND BARREN ENVIRONMENT ON HETEROPHIL/LYMPHOCYTE RATIOS OF COMMERCIAL LAYING HENS

Objective

The objective of this study was to investigate the effect of housing environment (enriched compared to barren) on heterophil/lymphocyte ratios in efforts to assess welfare in Hy-line® (W-36) laying hens from 9 to 34 weeks of age.

Materials and Methods

I. Experimental Treatments

On the first day of the project, 900 Hy-Line® W-36 day-old female chicks were wing banded for identification and divided into two environmental treatment groups. The chicks were randomly assigned to either an enriched or barren environment. Chicks assigned to the barren environment treatment were housed in 20 Petersime® battery brooder cages (25 chicks per cage) for the first 4 weeks. The battery brooder cages were 99 X 69 X 25 cm (39 X 27 X 10 in) with a level floor (273 cm^2 per bird). All birds were beak trimmed at 10 days of age. The chicks were then moved to 39 battery grower cages (10 pullets per cage) to simulate a commercial pullet cage system until 16 weeks of age. The battery grower cages were 61 X 58 X 38 cm (24 X 23 X 15 in) with level a floor (354 cm^2 per bird). At 16 weeks of age, the pullets were moved into 39 commercial VAL-CO™ layer cages (8 pullets per cage) for the remainder of the experiment. The layer cages were 61 X 58 X 38 cm (24 X 23 X 15 in) with a 7.5° sloping wire floor (442

cm^2 per bird). Chicks assigned to the enriched environment were placed in 14 individual 3 X 2 m (9 X 6 ft) floor pens (28 chicks per pen 2143 cm^2 per bird) containing: 10 nest boxes that were 31 X 33 X 18 cm (12 X 13 X 7 in.) each, 254 cm (100 in) of 1.5 cm (0.6 in) diameter perches and a 61 X 64 X 15cm (24 X 25 X 6 in.) dust bathing box filled with peat moss. Birds housed in the enriched environment remained in the floor pens throughout the entire course of the study. United Egg Producer's guidelines were followed for bird density, perch specifications and nest space. Lighting, feeding and environmental temperature specifications were provided by Hy-line® commercial management guide. All birds were given ad libitum access to feed and water.

II. Physiological assessment

Heterophil/Lymphocyte Ratio

Twenty birds from each environment were randomly selected at weeks 9, 13, 18, 22, 26, 30 and 34 for blood collection. Each bird was taken individually to the blood collection area that was located away from the other birds. The bird was then restrained on its side and blood was collected from the brachial vein with a syringe and needle. Two blood smears per bird were made immediately after blood collection. Blood smears were made by removing the needle from the syringe and placing a small drop of blood close to the frosted end of a clean glass slide that was on a flat surface. A second clean slide was then used as a "spreader" by placing the spreader slide against the surface of the slide with the blood droplet at a 30-45° angle. The spreader slide was then drawn back to contact the drop of blood. The blood was allowed to spread and fill the angle between the two slides. The spreader slide was pushed at a moderate speed forward until all of the

blood has been spread into a moderately thin film. All sides were allowed to air dry before being stained with Self Buffered Differential Wright-Giemsa Stain (Camco Quik Stain® II). The slides were dipped in the stain for 10 seconds, and then dipped in distilled water (pH 6-7) for 20 seconds. The slides were allowed to air dry again, and a cover slip was placed over the smear. Each blood smear was then examined with a microscope. An area containing a single layer of cells was identified under a lower magnification for accurate cell counting. A differential count was performed under 1,000 X magnification (oil immersion lens) by moving back and forth across the smear in a pattern that avoided counting the same area repeatedly. A total of 100 white blood cells were counted and identified as either heterophils or lymphocytes on each slide. The heterophil/lymphocyte ratio was calculated by dividing the number of heterophils by the number of lymphocytes for each slide. The ratios from the two slides for each bird were averaged to give one ratio per bird for analysis.

III. Statistical Analysis

A two factor-factorial analysis was conducted to determine if there were significant treatment effects or interactions. All analyses were conducted using JMP, Version 8.0.1. SAS Institute Inc., Cary, NC, 1989-2009.

IV. Animal Welfare Compliance

All procedures associated with the birds in this study were approved (App 2009-030) by the Clemson University Institutional Animal Care and Use Committee.

Results

Results of the two-factor factorial analysis for the heterophil/lymphocyte ratio appear in Table 5.1. A significant interaction between the treatment and time was not present in the average heterophil/lymphocyte ratios (Figure5.1.). There was not a significant environmental treatment effect on the average heterophill/lymphocyte ratios. There was, however, a significant difference in heterophil/lymphocyte ratios across the weeks ($P<0.05$).

Table 5.1. ANOVA Table for Heterophil/Lymphocte Ratio

Source	DF	Sum of Squares	F Ratio	Prob > F
Trt.	1	0.00240604	0.5821	0.4469
Week	6	0.68431585	27.5930	<0.0001*
Week*Trt	6	0.01062256	0.4283	0.8589
Error	126	0.5208065		

*($P<0.05$)

Figure 5.1. Interaction Plot of Time and Environmental Treatment on Heterophil/Lymphocyte Ratios

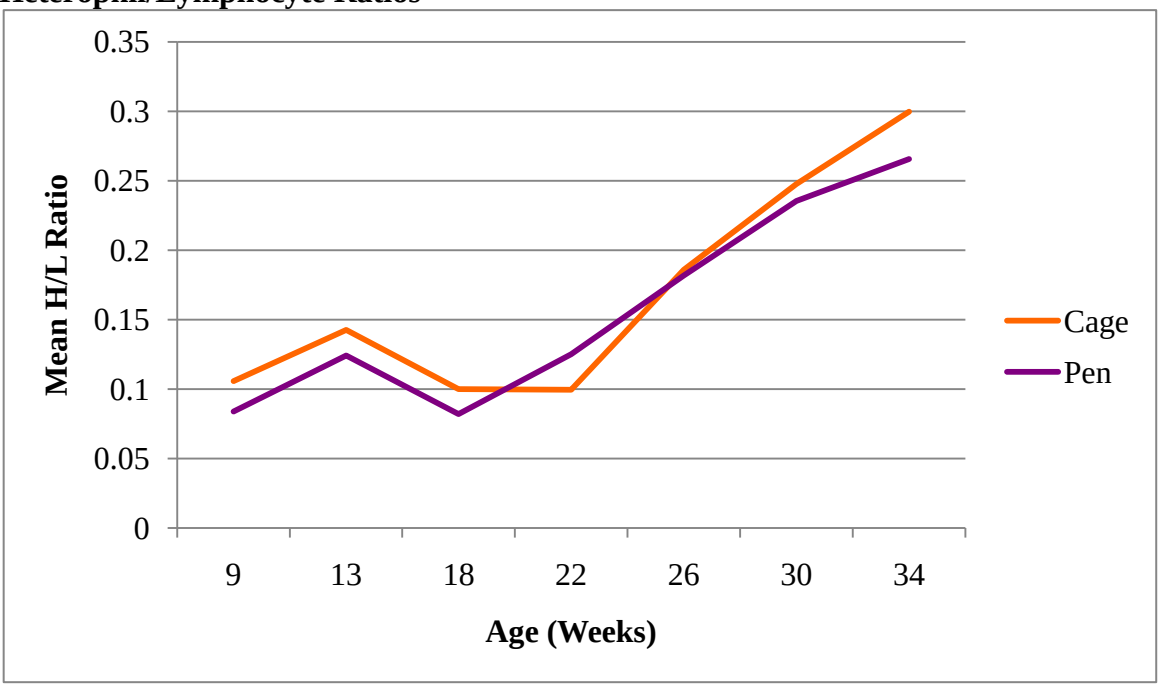
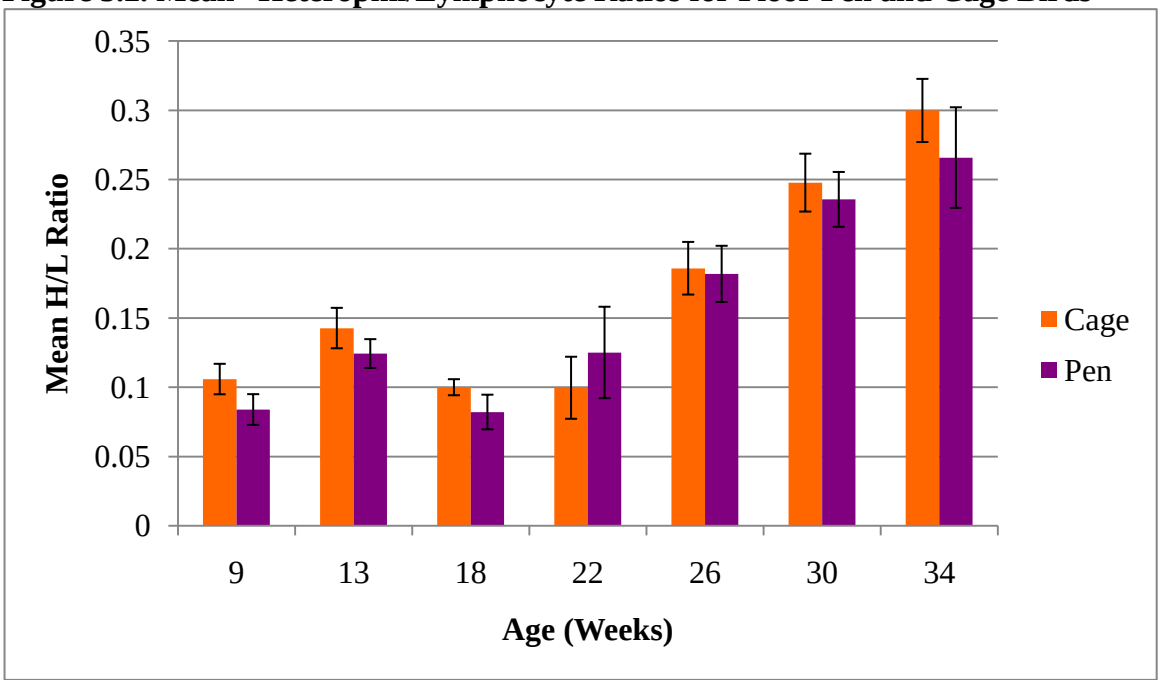


Figure 5.2. shows the average heterophil/lymphocyte ratio from each week for floor pen birds and cage birds.

Figure 5.2. Mean* Heterophil/Lymphocyte Ratios for Floor Pen and Cage Birds



*Each mean represents an average of 20 birds' heterophil/lymphocyte ratios.

Discussion and Conclusion

There was not a significant difference in the average heterophil/lymphocyte ratio between the floor pen birds and commercial cage birds at any week. There was, however, a significant difference across the weeks for the average heterophil/lymphocyte ratio.

Although not statistically analyzed, it appears from general observation of Figure 5.1. that floor pen birds and cage birds follow similar patterns for average heterophil/lymphocyte ratios. The floor pen birds and cage birds appear to have a parallel pattern of heterophil/lymphocyte ratios for weeks 9 through 18. Both floor pen

birds housed in the enriched environment and cage birds housed in the barren environment demonstrate an increase in average heterophil/lymphocyte ratios from week 18 through the rest of the study.

The heterophil/lymphocyte ratio is a good measurement of the chicken's perception of stress in its environment. Measuring the heterophil/lymphocyte ratio is a good indication of long-term stress in the environment (Gross and Siegel, 1983). Since the heterophil/lymphocyte ratio is a good indicator of stress, the results from this study indicate that there is no difference in the stress levels of the birds in the two different environmental treatments. Even though no statistical difference was detected, birds housed in the cage system appear to have slightly higher average heterophil/lymphocyte ratios than the birds housed in the floor pens in six out of the seven sampling weeks. Elston et al., in 2000, found a difference in Hy-line® W-36 hens housed in solid sided cages versus open sided cages that were 45 weeks of age. Their study found that birds housed in the solid sided cages had higher heterophil/lymphocyte ratios. It is possible, in our study, that a difference could have been detected in the floor pen birds and cage birds if they had been housed in the selected environments for a longer period of time.

The increase of heterophil/lymphocyte ratios with age and maturity is consistent with Burton and Harris' findings in 1969. They found that young chicks and quails show slightly lower heterophil/lymphocyte ratios than adults.

Because heterophil/lymphocyte ratios are good indicators of stress (Gross and Siegel, 1983) and can be influenced by environment (Elston et al., 2000; El-Lethey et al., 2000), a heterophil/lymphocyte ratio was used in this study to determine if any

differences were present between birds housed in floor pens and birds housed in cages. No significant differences, however, were found in average heterophil/lymphocyte ratios between the enriched floor pen birds and the barren commercial cage birds. This indicates that the stress levels of the birds housed in the different environments are similar. This assessment alone is not adequate to make a clear conclusion about the birds' welfare in the different environments. Therefore, this heterophil/lymphocyte assessment will be included with behavioral observations and fear assessments in order to make a conclusion about laying hen welfare in floor pens and cages.

CHAPTER VI

DISSCUSSION, CONCLUSIONS AND FUTURE WORK

There were significant interactions ($P<0.05$) between treatment and time for feeding and log odds of other behaviors. There were, however, significant differences ($P<0.05$) between the floor pen birds and cage birds during certain weeks. Standing behavior and log odds of sitting behavior demonstrated significant differences ($P<0.05$) between the floor pen birds and cage birds. Significant interactions were present between time and treatment for emergence test and tonic immobility test, which both demonstrated treatment differences at certain weeks. There were no significant differences between floor pen birds and cage birds for heterophil/lymphocyte ratios.

Even though there were significant interactions present in some of the assessments, it is important to recognize trends that appear among the data. Cage birds appear to have more birds sitting, standing and feeding than floor pen birds. This is to be expected considering the options that the floor pen birds have available. It is possible that birds prefer to occupy the perch, nest boxes or dust bathing box instead of sitting or standing on the floor. Also, the percentage of floor pen birds may be less because they have the option and space to perform other behaviors. It also appears that the cage birds may be more fearful than the floor pen birds from looking at trends from the emergence and tonic immobility test data. A more clearer difference might have been seen with a survival analysis of the data. Although very small and only numerically, it appears that the cage birds have higher heterophil/lymphocyte ratios than the floor pen birds, and

during the last week of testing, there seems to be more of a difference between the two treatments than any other week of sampling. These results may indicate that the floor pen environment is better suited to meet the welfare needs of laying hens than the cage environment.

This study covered a lengthy amount of time, and during this time many changes were occurring as the birds grew older and matured. With the significant differences that were present in some of the weeks, an age effect could be the explanation. Also, the cage birds experienced two moves throughout the study, at week 4 they were moved into grower cages and at week 16 they were moved into the layer cages. More clear differences between the treatments may have been seen if the focus had been on a more specific time frame or if both treatments experienced environmental moves or changes throughout the study. Because of not wanting to reuse birds and time limitations associated with some assessments, a small sample size was used for some assessments. Therefore, more clear differences may have been seen with increased sample sizes. Also the birds' individual personalities could have had an effect on the behavioral responses. Even if the environment is the same, individuals may consistently act differently from one another. Individual personalities could be an explaination for the amount of variation that was observed in the different behavioral assessments.

Currently, there is not much information available regarding the welfare of laying hens in floor pens compared to commercial cage housing systems. Therefore, it is important for further research to be done to improve the welfare of production laying hens.

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