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12-2015

Broiler Breeder Mating Behavior and the Effects of Pre-Incubation Embryo Integrity Modifications on Broiler Performance

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Broiler Breeder Mating Behavior and the Effects of Pre-incubation Embryo Integrity
Modifications on Broiler Performance

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Poultry Science.

by

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University of Arkansas
Bachelor of Science in Agricultural, Food, and Life Sciences, 2013

December 2015
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

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Abstract

Modern broiler breeders have been observed displaying significantly less mating behaviors than their ancestral fowl. Effective mating behavior translates into fertile hatching eggs that ultimately become broilers processed for market. The poultry industry has made significant changes in genetics, environment, housing, and diets of commercial poultry. However, there are some concessions made in genetic selection and streamlined management to produce larger, fast-growing, efficient birds. Aggression in broiler breeder males is believed to be a sign of high fertility. However, highly aggressive males pose a threat to the welfare of their flock mates, and may also impede frequency of mating and actually lower flock fertility. With improvements in efficiency of broiler breeders and their offspring, hatching of fertile eggs has become entirely mechanized to allow for incredible egg volumes to be incubated in a single facility. Some eggs may need to be stored for long periods of time, hatcheries are forced to set eggs from pre- and post-peak production flocks, or eggs laid on the floor area of the breeder house. These common management practices can pose potential threats to the developing embryo, and the performance of the subsequent chicks.

Frequency of mating behavior may be genetic strain-dependent, as observed by some integrators. Therefore, a study was developed to record and analyze the behaviors of two different strain crosses of broiler breeders in a commercial setting. This study differs from those previously conducted because the subjects were reared in commercial broiler breeder houses. In a second study, embryo integrity modifications are known to alter embryo development and have significant effects on hatchability, chick quality, and performance. A study was developed to test the effects of prolonged egg storage, broiler breeder flock age, and location of oviposition on hatchability, 14-day livability, and performance.

It was concluded that there were significant differences in aggression for one strain cross of broiler breeders. More studies with different crosses should be conducted in order to give conclusive evidence to the industry. As expected, egg storage and flock age had some significant effects on hatchability and growth performance. However, no differences were determined for location of oviposition.

Acknowledgements

I am very #blessed to have an amazing support system for which none of this would have been possible without. Thank you to the following:

My graduate committee members: Dr. Bramwell, for affording me the opportunity to go to graduate school. Dr. Clark, for much-appreciated encouragement and jokes laced with historical references and tactical military strategy. Dr. Goodwin, for showing me that being a professional does not mean compromising your true self.

Doug and Laura, for their guidance and assistance on the farm. Leasea, for making sure I had the eggs needed to complete my trials.

Mom and Dad, for giving me the tools to succeed. That's why you're the boss.

My family and friends, for relentless encouragement and love.

Liz, for putting up with me for the last four years, McAlister's dates, and Razorback Baseball.

Gary and Sara, for their life-coaching, friendship, and open doors for the past six years.

Mrs. Donna Delozier, for making sure all of her graduate students were always taken care of.

The "Bird Brains:" Michael, Josh, Zach, and Josh, for providing constant entertainment and being the brothers I never ~~wanted~~ had.

"Just remember to stay relentless. Don't stop running until it's finished. It's up to you the rest is unwritten." – Beyoncé Knowles-Carter

Dedication

Dedicated in memory of my grandfather Gerald G. "Tank" Goodwin (June 13, 1927 – March 26, 2002).

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I.   Introduction

From the wild jungle fowl, to the domesticated chicken, to the dual-purpose breeds that developed the modern broiler, the poultry industry has advanced by leaps and bounds to provide affordable protein to a hungry world. *Gallus gallus* var. *domesticus,* the modern broiler, has come a long way from the jungle fowl and the more common backyard fowl. The modern broiler breeder behaves less like the "normal" version of a domestic chicken and more like a well-oiled machine. Broilers are designed to efficiently convert feedstuffs to meat for the global consumer which means that the broiler's life span is not long enough for it to reach sexual maturity, so mating behaviors are rarely observed in flocks of the broilers themselves. Even their parents, broiler parent stock, the broiler breeders, do not display mating behaviors as frequently as do wild birds and backyard chickens. While some behaviors are similar between ancestral and modern fowl, many have become of secondary importance to more cost-effective factors like growth rate and feed conversion ratio. Hatchability, livability, growth performance, and processing yield supersede most concern and evaluation of behaviors associated with mating and incubation. Although much of the competition for mates has been nearly eliminated in broiler breeders, there are some residual mating behaviors that are concurrent with wild fowl that can still be observed in commercial broiler breeder flocks.

The responsibility of natural incubation has been completely removed from the hen and assumed by the highly efficient incubation equipment that still attempts to replicate the natural incubation of the egg. A developing embryo is very sensitive to its surrounding environment both during and before the "incubation process" is commenced. When temperature, and the presence of pathogenic contamination change, the embryo's rate of development changes accordingly. The embryo does not truly stop its growth, but simply slows down its metabolism until conditions are more suitable for development and growth. Too many changes in the rate of development can result in the embryo starting and slowing growth until it, essentially, "runs out of steam." This translates into higher instances of, primarily, early embryo mortality with some increase in late dead embryos. Storing eggs for extended periods of time may

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result in such slowed growth that the embryo depletes its usable energy resources and experiences cell degradation. Moreover, age of the broiler breeder flock can impact the quality of the sperm that penetrate the egg's germinal disc due to less frequent mating's and possibly less ideal *in vivo* sperm storage in the hens sperm storage tubules. This can result in malformed and/or early dead embryos or infertile eggs. The observable tendency of a chicken is to lay her egg in a place where she feels safe. However some flocks observe a higher tendency for hens to lay their eggs in the floor space or slats rather than the nest box. Eggs that come in contact with litter can be contaminated with pathogens that can cause enteric diseases like colibacillosis, coccidiosis, and salmonellosis that are present in the litter. High instances of contamination and exploding eggs in the hatchery can result in significant hatch loss. Most of these factors discussed have to do with the influence of humans and automation of the poultry industry.

The human influences may be impacting the natural behavior and development of our broilers and broiler breeders as well as the natural development of the embryo. Genetic selection is a game of 'give and take' as selecting for bigger, higher yielding birds, overall mating behaviors and fertility can be impacted and vice versa (Emmerson, 1997; Mangle and Stamps, 2001; Zuidhof *et al*., 2014). The industry has made significant improvements in feed conversion and growth rate since 1957 (Zuidhof *et al.*, 2014). However, selection for these traits can have adverse effects such as ascites syndrome and reduced reproduction rate (Emmerson, 1997), while Mangle and Stamps (2001) also reported these trade-offs exist. Management practices might be affecting flocks, as well, with feed restriction believed by some integrators to increase instances of aggression in broiler breeders. In contrast, some growers believe there is a positive correlation with aggressive males and high fertility. However excessive aggression can pose a threat to the welfare of the breeder flocks and possibly management personnel.

Egg storage at the farm and in the hatchery is thought to perhaps alter the quality and quantity of chicks produced and placed as broilers. In order to fill chick orders and to adjust the number of eggs set to work around employee holidays or simply over production, hatcheries may be forced into a situation where there is a need to store eggs for up to 14 days prior to incubation. This prolonged storage may

cause adverse effects on the developing embryo, and the subsequent quality and quantity of chicks hatched. The systems of rearing replacement broiler breeders and the housing systems in the breeder house may reduce the necessity for competition and courtship in mating. This is evident when comparing the mating behaviors of wild poultry to broiler breeders and even some other species of domesticated poultry (Wood-Gush, 1954). Managers, service technicians, and growers spend day after day caring for their flocks. Their observations of chick quality, bird behavior, and overall health assists researchers in determining the factors that need to be scientifically analyzed. This way, the industry and academia can work together to improve the livelihood of the birds, growers, and integrators.

Studies that observe and evaluate the effects of either neutral or courtship behaviors in poultry have been conducted for almost a century. Current literature, as reviewed in the following, summarizes potential struggles and solutions the poultry industry may have regarding mating behavior and pre-incubation embryo development. Mating behavior can be broken down into courtship, competition and aggression, fertility, and genetic strain. Pre-incubation embryo development can be heavily impacted by length of egg storage, age of broiler breeder flock, and location of oviposition within the broiler breeder house.

II.   Mating Behavior

Mating behavior of birds widely varies from species to species. Birds-of-paradise (family, Paradisaeidae) display elaborate, colorful, and vocal displays. Peacocks (genus, *Pavo*) have evolved ornate male feathering with the purpose to attract a potential mate. The female gender of these birds largely hold the responsibility of choosing a mate based upon their courtship strategy and displays. The modern broiler breeders have almost evolved backwards in terms of mating behavior and display of courtship. This may be due to selection of egg production, growth, and feed conversion traits rather than traits associated with courtship (Emmerson, 1997). Modern rearing practices for broiler breeders eliminate the necessity for extensive time spent attracting a mate. This is due to the typical stocking

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density of 7-9 % males in a breeder house. Males are constantly surrounded by females and have little competition in finding a potential mate. Therefore, the act of choosing a mate has largely shifted from the female to the male, meaning the female often has little impact on the act of being successfully mated by the male. In broiler breeder houses, a male will often decide which female he wishes to breed, run her down, and mate with her. Therefore, the necessity of true courtship has been greatly reduced. Aggression, on the other hand can be said to have increased over time (Millman *et al.*, 2000). Breeder managers often observe existing flocks that are more aggressive than others. Why has mating behavior decreased so much in broiler breeders and what is causing a significant increase in aggressive males in recent years?

Courtship and aggressive behaviors are the main factors that are investigated in chicken mating behavior research, while using visual cues like waltzing, tidbitting, and wing flapping can determine the frequency of courtship behaviors in a flock. Aggressive broiler breeders can pose an animal welfare concern for the entire flock from injuries due to fighting or forced mating and "bullying" of other males and females. These males, particularly those who are highly aggressive at a young age, will often force females to hide from them in nest boxes or the slat area. This action is called "slatting" and can negatively impact fertility by decreasing overall flock mating activity. Aggressive males can also deter or interrupt other males' mating which, similarly impacts overall flock fertility. Highly aggressive individuals pose a risk of injury or death to other flock mates (Kajer and Mench, 2003). Some flock managers hold the belief that males who display more instances of courting and aggression (dominance) are more fertile than subordinate males. One study by Millman and Duncan (2000b) proved that broiler breeders did not display as high levels of aggressiveness to a male model as males from an Old English Game fighting cock strain. Morphological characteristics such as comb and wattle size may indicate higher male fertility (McGary *et al*, 2002). However, Swaffar *et al.* (2007) does not agree with these findings and determined that these external characteristics were not correlated to teste weight and volume. Other factors, such as feed restriction and genetic strain, might also influence the frequency of courtship and aggressive behaviors (Siegel, 1972; Brillard, 2004).

A.   Courtship Behaviors

Common courtship behaviors in broiler breeders include, "waltzing," "wing flapping," "tidbitting," "feather ruffling," "head shaking," preening, "circling," "cornering," and "the rear approach." Each of these behaviors have extensive definitions and can even overlap (Wood-Gush, 1954; Kajer and Mench, 2003). It has been observed that males with more floor space had increased frequencies of courtship behaviors, completed mating, and aggressive acts (Kratzer and Craig, 1980). It can be concluded that the more space a male has, the more active he is therefore allowing for more aggression and courtship behaviors exhibited.

Several researchers have sought to define and find necessities for different aspects of mating behavior. One such scientist, Wood-Gush (1954) investigated the Brown Leghorn male's courtship and its impact on frequency of hen crouches. While waltzing, specifically, was found to be statistically insignificant for eliciting hen crouches, it was discovered that more active and forceful males elicited the highest amount of hen crouches. This may be due to the established "pecking order" within the flock. High ranking males outperform lower ranking males for female attention. Females may also sense the dominance of a male and behave more submissively toward him. Wood-Gush also defined certain behaviors as either "threat display movements" or "displacement reactions." Threat displays were determined to be akin with the behaviors observed in cock fighting. Non-courtship waltzing, neck feather flaring, wing flapping, and other behaviors help the male appear bigger and stronger than other males therefore, making him a more formidable opponent. In these cases, males were attempting to drive off other competing males in order to gain the attention of the females. Displacement reactions, such as post-copulatory waltzing and tidbitting, serve to orient the male to his surroundings following a fight or copulation. These reactions were found to possibly help courtship success by allowing the male to refocus his efforts after an attempted mating (Wood-Gush, 1954).

Knowing that courtship exists in most species of fowl, why is courtship less frequently observed in modern broiler breeders? Kajer and Mench (2003) detailed significant behavioral problems in production strains of chickens and turkeys due to their genetic predisposition for fertile eggs and meat. Turkeys in production facilities do not display normal mating behaviors because they are most often raised sex-separately and do not come in contact with the opposite sex. "Insemination" in commercial turkeys is accomplished almost exclusively by means of artificial insemination so any possible courtship displays would not result in a specific male producing more progeny due to his courtship behavior. The need for artificial insemination in commercial turkeys is a result of the massive size of the breeder turkey males, especially in relation to the turkey hens. Attempts at natural mating by the toms on the turkey hens can often result in injury to the hens and generally a dramatic decrease in completed mating's. However, there is more homogeneity in size of the sexes in broiler breeders than in turkeys, making it physiologically safer for broiler breeders to mate naturally. Broiler breeders are required to display high rates of gain, low feed conversion ratios, and adequate fertility and egg production. Therefore, there is an immense amount of pressure to produce breeders with the capabilities of high meat and egg yield (Emmerson, 1997). This leaves less profitable traits like courtship behaviors, with little to no selection pressure. This could be a major contributing factor to the reduced prevalence of courtship in commercial-type poultry coupled with altered management and housing practices like feed restriction and altered lighting programs.

B.   Aggressive Behaviors

Aggressive behaviors in broiler breeder males and females can cause welfare concerns for both the birds and the managers in charge of them. Aggression is not limited to fighting within one sex, males fight with males and can be overly aggressive with females, as well as females will fight with other females as well as lower ranking males. Because aggressiveness of the male seems to have more detrimental effects, it has been studied more frequently (Millman *et al*., 2000). It is also observed that in very large broiler breeders, as well as large turkeys, birds can charge and flog, injuring the humans that

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take care of them or at least deterring the caretakers from spending as much time in the payer house as they should. Forced mating, sparring, spurring, fighting, feather picking, and chasing can all be considered aggressive depending on the origin of the behavior. Females may become "bald" on their backs from aggressive mating posing a risk to their health from scratches, cuts, and subsequent infections. Competition between the males is thought to enhance these aggressive behaviors. When an established pecking order is disrupted by a change in male to female ratio, males tend to become more aggressive towards all members in the group in order to reestablish dominance (Craig, 1992). Because some birds may use aggression as a means to force mating, aggression can sometimes be considered as a factor of mating behavior.

In recent studies, aggression has been observed inside and outside of mating activity. These behaviors did not decrease with age and most forced mating did not succeed (De Jong *et al.*, 2009). In cases where aggression is associated with fertility, it has been believed that males who were less aggressive and courted less were less fertile. This hypothesis is in agreement with many growers', service technicians', and managers' observations. However, Hocking and Bernard (2009) found that one strain displayed more activities and did not maintain its flock fertility. Meaning even though the strain was more actively mating, their fertility was not necessarily better than a strain with less recorded activity. The other strain may have displayed significantly less mating activity, but maintained its fertility throughout the life of the flock. Therefore, it can be concluded that overly active flocks may actually impede their own mating efficiency when raised at male to female ratios that are used for less aggressive male breeder strains.

Contrary to De Jong and coworkers (2009), Moyle and coworkers (2012b) found that mating behavior, as well as aggression decreased with flock age. It was determined that aggression and overall mating behaviors decreased with the onset of senescence. It can be expected that with increasing age, body weight increases therefore older, larger birds spend more of their energy in physiological maintenance than for reproduction. It is to be expected that larger breeders use their expendable energy more judiciously: they focus on maintaining their bodily homeostasis and mating rather than aggression. While

Bramwell and coworkers (2010) reported that hens with increased body weight have a reduced ability for fertility as compared to hens raised at body weights close to the primary breeders recommended standard. Therefore, as bird's age, body weight becomes increasingly difficult to control and combined with the reduced fertility of hens due to age and reduced fertility of hens due to excess body weight, mating frequency becomes of utmost importance.

In a separate study, Moyle and coworkers (2012a) found that increasing age in the males did not decrease physiological semen or sperm cell count. These studies suggest that older breeder males are less aggressive and mate less frequently, but do not have a subsequent decrease in ejaculate quality. Furthermore, Bramwell and coworkers (1996) discussed the effect of age on fertility and sperm penetration values. Physiologically males can maintain acceptable fertility levels as they age when artificial insemination is utilized, while hens physiologically become less fertile even with artificial insemination. These sex-separate effects of aging on fertility give further weight to this study because most fertility problems in the commercial poultry industry are a result of lack of mating activity in naturally mating flocks.

Some studies measure frequency of mating behavior and aggression based on secondary (morphological) sexual characteristics like comb, wattle, and spur size. These characteristics, like those in primitive humans, suggest that the male may be more fertile than his flock mates. Bilcik and Estevez (2005) found that these characteristics were not a significant measure of increased fertility or increased frequency of mating behavior. In this case, the frequencies of behaviors were relatively the same. This means that size of these secondary sexual characteristics does not necessarily matter in terms of fertility. Even those males with higher instances of mating did not sire more chicks suggesting that frequently mating males had poorer mating efficiency. The study also concluded that the mating activity frequency was equal in treatment pens where one male was housed versus those where three males were housed. This is important to note because it shows male competition is not a factor of increased or decreased mating activity in this study, contrary to Kratzer and Craig (1980) where an increased frequency of

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mating was observed in pens where only one male was housed. Unfortunately, in the poultry industry it is not practical to use only one male in broiler breeder production. However, these studies suggest that space may be the more limiting factor. Thus, managers should allow adequate space for each bird, decreasing the pressure of male-male competition.

Severe feed restriction is known to cause immense stress on broiler breeders, but is necessary to ensure the birds do not become obese, lame, and unable to effectively mate or feed (Kajer and Mench, 2003). In studies where feed restriction was tested against aggression, it was found not to be a significant factor (Millman and Duncan, 2000). However, the severity of feed restriction can vary. Therefore, strict management of feed intake protocol for flocks should be implemented equally and efficiently to achieve a specific management program. Severe restriction induces severe stress and in times of stress, not only is productivity reduced, so is the welfare of the bird. Studies have tested the effects of high and low caloric value diets versus different methods of feed restriction in an effort to relieve the industry of feed restricting broiler breeders by reduced volumes of feed. According to Bilgili and Renden (1985) there may be some relationship with body fat and fertility in hens, and Bramwell and coworkers (2010) further supported this belief that increased bodyweight in hens is negatively correlated with fertility (Bakst *et al.*, 2010). Rather, it has been suggested that genetic strain is a more significant cause of aggressive behavior than is feed restriction (Millman *et al*., 2002 and *McGary et al*., 2003). Genetic strains that have been selected for higher instances of aggression can pose many concerns as previously discussed. Because there is some evidence of strain-dependency on aggressive behavior, primary breeders should consider selecting moderately aggressive strains to protect the welfare of the whole flock.

C.   Association with Fertility

High fertility has long been believed to be associated with high instances of mating behavior and aggression. This belief is held by broiler breeder managers who observe certain levels of mating behavior and associate that with the flock's fertility. However, scientific evidence suggests that frequency of

mating behavior has little relationship with fertility. Rather, the reproductive quality of the other males in the flock can have more of an effect on total flock fertility (Bilcik *et al.*, 2005). When some males are mating effectively, it may encourage other males in the flock to improve their efficiency. Birds with higher body weights were observed to display more mating behavior, although this may seem counterintuitive. However, this may be evidence that bigger males give off a sense of higher fertility and dominance. As can be expected, decreases in libido have an adverse effect on mating behavior and subsequent fertility. Older birds under severe feed restriction presented lower fertility and a drop in libido (Duncan *et al.*, 1990). Reproduction is a physiological privilege which is easily shut down in times of stress. Hunger causes an immense amount of stress on a growing bird. Therefore, the negative effects of severe feed restriction are plausible.

Bilcik and Estevez (2005) found that morphological characteristics are not a significant determinant of aggression or mating behavior. However, McGary and coworkers (2002) found that male comb area was a significantly reliable indicator of male fertility in a single strain in their study. This suggests that males with bigger combs may not mate more aggressively, but they mate more intentionally, resulting in higher fertility. When males mate with females, the females' feather covering across the back can be lost over time due to consistent wear and tear. It could be postulated that loss of back feathering in a large percentage of females in the flock could be a reliable indicator of increased mating activity and fertility. This belief holds true with most breeder managers. However, Moyle and coworkers (2009) showed that not only is the loss of female back feathering and mating activity not correlated, the loss of feathering may actually deter males. Males may see bald-backed females as sub-fertile, sick, or injured and they may choose not to breed with them. Additionally, as hens lose the feather covering on their backs, they begin to behave in a manner that makes them less available to the males because the act of mating can cause pain and or injury to the hen, thus reducing fertility. Light stimulation is a necessity used to bring about puberty and reproductive activity in broiler breeders. Light stimulating at the industry standard of 21 weeks creates uniformity of maturation in the flock. Adhering to feed restriction standards, males should

not become overweight in the growth, or rearing, phase. One study found that light stimulating flocks earlier, at 18 weeks of age, could prevent males from becoming overweight while still exhibiting the same mating behaviors as flocks aged 21 and 24 weeks. Males stimulated at 15 and 12 weeks had decreased mating activity and fertility (Moyle *et al*., 2012). While flocks that were light stimulated between 18 and 24 weeks of age mated more frequently and exhibited increased fertility over the lifetime of the flock. Therefore, it may serve some benefit to photostimulate males at 18 weeks in order to reduce the cost as stress associated with keeping males at their target weights and as productive as possible.

D.   Other Factors in Mating Behavior

Other factors that can be associated with broiler breeder mating behavior are genetic strain and feed restriction. Genetic selections effect on mating behavior and sexual characteristics is minimal. It increases after a few generations, suggesting that the genetics of mating behavior may be hidden or overshadowed by the genetics of body weight (Siegel, 1972). Severe feed restriction causes stress in broiler breeders (Brillard, 2004). This poses a welfare concern in terms of increased instance of skeletal disorders and extreme aggressiveness, as discussed previously. Birds with skeletal problems cannot move effectively, therefore impeding reproductive performance. Aggressive birds are not necessarily the most fertile, nor do they display more mating activity. Feed restriction programs have to be strictly managed to work effectively. Therefore, diligent managers should take care to evaluate their programs if high instances of skeletal problems and aggression occur (Kajer and Mench, 2003).

In conclusion, courtship, competition, aggression, genetic strain, and feed conversion are only some of the factors that play in to overall mating behavior and fertility. Aggression has been found to be more impacted by strain than feed conversion. This could impact the welfare of the flock and prohibit progress in selection for growth and yield (Brillard, 2004). Fertility cannot be determined by level or frequency of aggressive behavior. However, it may also be strain dependent or determined by some secondary sexual characteristics. Geneticists select our broiler breeders to produce cost efficient, high yielding broilers.

This selection may be at the cost of stress, physiological issues, and/or "natural" mating behaviors. Upon review, most studies reported the mating behaviors of broiler breeders in research trials not their "natural" habitat: breeder houses. Findings from research collected in breeder houses may be found to concur or differ with the current literature.

III.   Pre-incubation Embryo Integrity Modifications

Modern integrators have made strides to create a highly efficient system for hatching quality chicks. Companies employ numerous technologies to automate the hen's incubation, and each facility can produce almost a million chicks per week. Essentially, the responsibility of incubating and hatching has been alleviated from the hen and put in the hands of hatchery managers with incredible machinery. For vastly improving on the efficiency of the hen, there may be a cost. Exotic disease outbreaks, poor forecasting, and consumer demand can drive up the need for hatcheries to produce more chicks. Because eggs are a finite resource, fluctuations in demand can force hatchery managers and integrators to set any and all available eggs. To ensure there are an adequate number of eggs in each hatchery, companies can be forced to store eggs for an extended period of time. Short-term storage allows for uniformity of embryo development and a smaller hatch window. Reducing the hatch window gets chicks processed and placed in grower houses sooner. Holding chicks in the hatchery can sometimes result in dehydration from lack of immediate access to water. Growers may experience increased 7-day mortality due to some chicks dehydrating. Placing chicks in a timely manner provides a good start to their growth cycle and better performance. Recent trends in industry show a rise in the removal of some antibiotics in hatcheries. While these antibiotics are not necessarily used to treat disease, they do provide significant protection against opportunistic infections from the gastrointestinal microflora (Bedford, 2000).

Some companies may set eggs from very young and very old flocks. This is to ensure that they are producing enough chicks to meet their demand. Eggs from very young flocks may not be uniform in size, producing discontinuity in chick weight. Very small chicks can be consistently under the average or

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suggested growth curve for the integrator and may be culled out to improve average body weight and flock feed conversion ratio. Eggs from pre- and past-peak production flocks can display poor sperm penetration and increased early dead embryos, lowering the hatch of fertile for the flock. Moreover, a company may set eggs that can be contaminated by pathogens from their oviposition on the floor or slats of the broiler breeder house. When these eggs are cooled in storage, pathogens have the opportunity to pass through the porous shell and flourish inside of the nutrient-rich egg. Hatcheries typically experience, with some frequency, exploding eggs. These eggs are, usually bacterially, contaminated and due to pressure changes explode. The contents of those eggs can then contaminate other trays and baskets as well as create a sticky, smelly mess for employees to clean. These are just some examples of integrity modifications that can be made to the embryo even before they are placed in the incubator. Each instance poses a threat to the developing embryo. What factors of hatchability, chick quality, and performance are lost due to the extreme end of these modifications? Is there a threshold for pre-incubation embryo integrity modifications?

A.   Embryo Development

Embryo development begins with the sperm's penetration of the hen's ovum. Specifically, the sperm must penetrate the perivitelline layer in order to make significant changes in fertility (Bramwell, *et al*., 1995). When hens are artificially inseminated with low sperm counts, the result is lowered hatchability factors. Not only are fertility, hatch of fertile, and total number of chicks decreased, the number of early and total dead embryos are significantly negatively impacted (Eslick and McDaniel, 1992). This makes a difference when dealing with egg storage, very young or very old broiler breeders, and contaminated eggs. Males with reduced libido, lower sperm count, or decreased overall mating activity. Therefore, there may be a plausible association with lower fertility. When eggs are stored for long periods of time, the rate of embryo development is significantly slowed. This causes an increase in dead and malformed embryos (Mather and Laughlin, 1977). When sperm penetration is not most effective or embryo development is slowed, hatcheries take two hits. Not only are there less fertile eggs, the eggs that are fertile may develop

abnormally or die before hatch. It can be assumed that when a hatch experiences a challenge, whether it be delayed embryo development, bacterial contamination, or some other factor hampering embryo integrity, there will be some instance of embryo death. The resulting chicks that do hatch could experience decreased growth performance factors and increased 7-day mortality (I can't find an article that backs this up. Any ideas?). Translating to the growers, reduced profits from less birds or less live weight sent to processing. Therefore, hatcheries must produce even more chicks to cover the chance of reduced performance and increased mortality. This vicious cycle is only accelerated by egg shortages.

B.   Egg Storage

Many studies have been done to determine the effects of egg storage on embryo development, chick quality, and growth performance. We know, biologically, that fertility is not impacted by egg storage. Fertilization occurs well before the egg reaches storage; before it is even laid, or oviposited (Bramwell *et al*., 1995). Therefore, fertility cannot be affected by egg storage. However, hatch quality, hatch of fertile, embryo death, pips, cull chicks, and malformed embryos can be affected by egg storage.

The summarization of pertinent research finds a marked delay in embryo development when length of storage is increased. The result is decreased hatchability and early dead embryos (Arora and Kosin, 1966). When storage temperatures are held constant, the effects are not as severe. Fluctuation in temperature can shock the embryo into slowing and restarting embryo development. The embryo can only take so many starts and stops before it loses sufficient energy to restart growth. Thus, uncontrolled temperatures in storage or transport can negatively impact hatchability of stored eggs (Byng and Nash, 1962). The delay in embryo development may have to do with the quality of the albumen. As eggs age, albumen begins to degrade (Hurnik *et al*., 1978). This degradation can decrease the amount of detrimental nutrients and gasses that are available for exchange to and from the embryo. The albumen pH, which is most affected in the first 4 days of storage, can be reduced to unsafe levels for the embryo (Lapão *et al*., 1999). Benton and Brake (1996) found that very fresh eggs, eggs stored for less than 24 hours, might have slowed gas

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exchange and a decrease in nutrient availability due to low albumen viscosity near the germinal disc. This implies that it may be necessary to store eggs to reach an albumen viscosity that can support adequate gas exchange. Proposing that eggs must be stored comes with one big problem. How long before the effects take a negative turn?

If storage is truly necessary, there must be ways the negative effects can be combated. One study found that it was beneficial to set eggs 30-40 minutes early for each increased day of storage (Kirk, *et al.*, 1980). So, for eggs stored 4 days, they may need to be set about 2 hours (120 minutes) before eggs that were not stored. Increased turning in incubation has also been investigated. When young flocks were tested against older broiler breeder flocks, it was found that eggs stored from older flocks benefitted more from turning than did eggs stored from younger flocks (Elibol *et al.*, 2002). However, increased turning did not produce significant effects egg storage alone. Machines may be developed and programmed to turn older, stored eggs more often.

What is the optimal length of storage? Waite (1919) determined it to be 7 days. By this point, albumen viscosity, pH, and the gas/nutrient exchange rate are optimal. Although this study might seem somewhat antiquated, others (Mather and Laughlin, 1977; Elibol *et al.*, 2002; Aurora and Kosin, 1966) concur that storage past 7 days produces severe negative effects. The aforementioned studies, as well as, Mayes and Takeballi (1984) suggest that storage times greater than 7 days can have tremendous negative effects on hatchability factors. Most saw significant increases in early dead and late dead (Elibol *et al.*, 2002; Arora and Kosin, 1966; Mayes and Takeballi, 1984). Storing eggs 1 day to 7 days did not have significant negative effects on hatchability factors. Therefore, "long-term storage" can be defined as storage longer than 7 days. Most industry hatcheries do not store eggs longer than 7 days, except in extreme circumstances. Exhibition and backyard poultry producers may find it necessary to store eggs in order to have enough eggs to fill their incubator. Realizing that the hatch may be impacted by setting aged eggs, can help these types of producers in setting a hatching schedule.

C.   Broiler Breeder Flock Age

In emergency situations and times of egg shortage, integrators may be forced to set any and all available eggs. This means that eggs from pre- and post-peak production flocks may be used in order to meet chick demand. However, just like egg storage, some studies show that there may be issues associated with older flocks and fertility as well as hatchability.

As previously discussed, male fertility may or may not be impacted by age (DeJong *et al*., 2009; Hocking and Bernard, 2000). As hens age, their fertility and egg hatchability are significantly altered (Fasenko *et al*., 1992). This may be due to decreased embryo metabolism from reduced yolk quality. It takes a tremendous amount of nutrients to produce an egg. The hen must pull these nutrients from the feed or her own body. A hen can produce around 200 eggs in her lifetime leaving her body nutrient poor. Towards the end of her productive days, these eggs may not be as rich in nutrients as those from a hen in the peak of production. The hatch results in higher instances of early, mid, and late dead embryos (Hamidu *et al*., 2007). Chick quality and livability can be affected by flock age due to significant changes in yolk uptake and body composition (Peebles *et al*., 2001). Small chicks can be outperformed and outcompeted for feeder and drinker space. These chicks attribute to losses in performance and may even die as a result of starvation, dehydration, trampling, or bottom culling. Because of the changes in embryo integrity, Tomhave (1956) suggests that chick quality declines after 37 weeks of broiler breeder production. Most broiler breeder flocks can be in production for 60 weeks. Therefore, diets formulated specifically for older hens must be used. Then the hens can replace their own nutrient stocks and provide a quality egg for their embryos.

There is some opposition to the aforementioned literature regarding the effects of flock age on fertility, hatchability, and chick performance. Hocking and Bernard (2000) claim that flock age has no effect on fertility, as their data did not return significant results. When tested together, as they often are, it has been suggested that the effects of increased length of egg storage might supersede the effects of increased flock

age. Changes in incubation temperature, wet bulb measurement, and hatch cabinet environment may be more significant, and overshadow the effects of flock age in some studies (Hulet *et al*., 2007; Vick *et al*., 1993). Intuitively, altering set temperature and humidity can cause effects of a higher magnitude. Even egg weight may come into play. When eggs are similar weights, flock age does not seem to be a factor. However, when egg weights differ, the resulting chicks can be inherently challenged in growth and performance (Shanawany, 1984). These opposing findings in the literature lead us to believe that the effects of flock age may be subordinate or secondary to the effects of egg storage, incubation temperature and humidity, and/or egg shell characteristics. Even though it may be subsidiary, it is still very important to consider and account for the effects of flock age when forecasting available chicks.

D.   Location of Oviposition

Minimal studies have been conducted regarding the effects of eggs laid inside and outside of the nest box on hatchability and performance. Again, fertility cannot be affected by where the egg is laid, only by the sperm's penetration of the perivitelline layer about 25 hours before the egg is laid (Bramwell *et al*., 1995). Thus, researchers must investigate hatchability and performance factors. Studies like ones done by Dorminey (1974) attempted to determine the behavioral reasons behind hens laying eggs on the floor. It has been suggested that hens typically lay in areas in which they feel secure. Nests make good areas to lay because of the shadowed nature of the box. However, hens may lay in shadowy areas of the floor or slats because nests are occupied, or light is shining directly into the nest boxes.

Poultry litter can contain varying levels of infectious doses of pathogens. *Salmonella enterididis* and other foodborne pathogens of human clinical significance can be found in most poultry litter and the housing environments. It can be assumed that when an egg, porous in nature and with a wet, waxy cuticle, is laid on the floor of a house, the egg may come in contact with said pathogens. When laid in the nest box, the pathogen load may be lower. Then, the egg is transported to a cooler egg room where the pathogens can be potentially diffused due to low internal osmotic pressure in the egg. Those pathogens,

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usually bacteria and fungi, can then feed on the wide array of nutrients found in the albumen and yolk. Because this metabolization of energy and nutrients produces some gas and other waste, pressure builds inside the egg and can cause it to explode when the pressure and/or temperature of the egg changes. The resulting explosion can contaminate other eggs, chicks, and surfaces furthering the hatchery's problems. We know there are some levels of pathogens in the litter. However, studies to definitively confirm or deny the likelihood of contamination have yet to be conducted. Testing and improving litter quality may reduce this probability of contamination.

It has been explored that birds may gain some significant nutritive factors from pecking and ingesting feedstuffs in the litter. These factors may then be passed on to their chicks. Whether or not this increases or decreases hatchability is not determined. It has been found that animal and milk by-products in the feed can aid in providing all the dietary factors needed to significant increase hatchability (Kennard *et al.*, 1948). Diets for broiler breeders can be formulated to account for these requirements. Adding nutritive factors to the litter that might increase hatchability should also be explored.

Current literature, as outlined above, suggests that some industry practices pose threats to embryo development, hatchability, fertility, livability, and other performance factors. While these practices usually cannot be helped, it is detrimental that they are accounted for in order for the integrators to place the amount of chicks demanded. It has been established that increased length of storage over 7 days negatively impacts hatch. It is possible that the resulting chicks display impeded growth and performance. Flock age does cause some change, but may be surpassed by other embryo integrity modifications, like storage. However, it, too must be accounted for in forecasting chick orders. The effects of oviposition on the floor or in the nest box have yet to be determined. However, studies can be done to test the true pathogen load within the litter, as well as testing the hatchability of floor eggs.

IV.   Conclusion

In order to provide a hungry world with an affordable, complete protein source, the poultry industry must produce more meat and eggs with less resources. The animals, equipment, machinery, and nutrition have been engineered to be highly efficient. Some cost is associated with selecting for efficiencies in meat production. Mating behavior in broiler breeders is nearly indistinguishable from that of wild birds. Some behaviors, like aggression, remain. The primary breeders, integrators, and managers must take steps to combat the degrees of aggressiveness in flocks to improve welfare. They must also provide enough space and manage feed intake for birds to continue high levels of reproductive activity. Pre-incubation embryo integrity modifications cannot always be helped in the industry. It has been established that eggs may need to be stored in order to allow the embryo adequate media for nutrient and gas exchange. However, eggs stored longer than 7 days display higher instances of embryo mortality resulting in reduced hatchability. Flock age might be a significant factor when egg storage and other embryo integrity modifications are not also considered. Location of oviposition as a factor impacting hatchability and growth performance has yet to be conclusively determined. More studies need to be conducted on that topic. These studies reviewed can provide the industry with the knowledge to improve on the already highly efficient broiler for a more sustainable industry.

V.   References

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II.   Comparison of Male and Female Broiler Breeder Mating Behaviors across Four Different Breed Strains in a Commercial Setting.[1]

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A.   Abstract

The objective of this study was comparing differences in mating and courtship behaviors exhibited by four different breed strains of broiler breeder males and females in a commercial setting. The breed strains evaluated were Hubbard M-99 and Cobb MX males and Ross 708 and Cobb 500 females. Behaviors observed were male-hen aggression, male-male aggression, hen-hen aggression, male waltz, male wing flap, male neck flare, hen crouch, attempted hen mounts, hens mounted, attempted matings, and completed matings. These behavioral characteristics are associated with natural mating behavior and can be observed in naturally-mated breeding flocks. For this study, cameras were set up in commercial broiler breeder houses to record activity for four one-hour periods in two separate houses during mid-afternoon. Four samples were observed from a Hubbard M-99 and Ross 708 cross and three samples were observed from Cobb MX and Cobb 500 cross breeder flocks. All commercial flocks were managed by the same integrator of similar age near 50 weeks of age. The resulting videos were analyzed and behavioral characteristics were tabulated. Each category was further separated into instances per hour and significance was determined at $P < 0.05$. A significant difference was found regarding male-hen aggression ($P < 0.0072$), hen-hen aggression ($P < 0.0275$), and overall aggressive behavior ($P < 0.0331$). However, no significant difference was determined in any of the other categories. It was concluded, therefore, that aggressiveness, rather than courtship and mating behavior, was significantly different between these two strain pairings. From a behavioral perspective, general courtship behavior in commercial breed strains is not as prevalent as in wild populations of birds. However, they do exhibit different levels of courtship, which appears to be strain dependent. More studies will need to be

conducted with additional breed strains and different crosses to evaluate interactions between male and female breed strain crosses.

Key Words: mating behavior, breeder

B.   Introduction

Mating behaviors are those that directly and indirectly result in a completed mating between a male and female. These behaviors can either be appetitive or consummatory. Appetitive behaviors are those that help to satisfy bodily needs, while consummatory behaviors are those that relate directly or indirectly to the act of copulation (Wood-Gush, 1954). Some appetitive behaviors can be considered "mating behavior" because of their underlying purposes of advancement in courtship or copulation. For example, a male broiler breeder may flap his wings as a mechanism to stretch his muscles, or to draw female attention to himself for mating. The poultry industry is concerned with the mating behavior of broiler breeders because with reduced mating activity, there would be less fertile eggs to hatch, and subsequently fewer broilers sent to the processing plant. There is a strong belief that that frequency of mating behavior is directly associated with fertility. Therefore, these behaviors should be studied to determine their necessity and intricacies.

True displays of mating behavior can be found in almost all birds. However the levels of intensity and frequency vary from species to species. Some wild birds have very ornate and intricate displays where a male performs a dance, flashes his colorful feathers, or sings a song to convince a female to choose him, over other suitors, as a mate. These behaviors, while not necessarily as intricate, can also be found in exhibition and "backyard" type poultry (Wood-Gush, 1954). Both wild and domesticated turkeys (*Meleagridis galapovo*) strut to show off superior strength and body size. Peacocks (genus *Pavo*) use their colorful tail plumage in an attempt to court the peahen. McGary *et al*. (2002) found that some secondary sexual characteristics, like comb size, may indicate a quality mate in chickens. However, Bramwell

(personal communication, unpublished data) compared hen acceptance of dubbed and undubbed broiler breeder males of the same strain and found that hens showed no preference or had little choice in mating frequency due to comb size or presence. Swaffar *et al.* (2007) also found that male teste weight and volume was not correlated to comb and wattle size. Bramwell, (personal communication, unpublished data) compared hen acceptance of dubbed and undubbed broiler breeder males of the same strain and found that hens showed no preference or had little choice in mating frequency due to comb size or presence. Therefore, concluding that these characteristics are not a significant determinant of male fertility. In broiler breeders, true courtship behavior can be found, but at lower frequencies as compared to wild type fowl. In naturally-mating populations of non-commercial fowl, the female is usually in charge of choosing a mate from eligible admirers. Males are tasked to come up with the most impressive displays; however broiler breeders are much different. The choice of mate selection has been shifted from the hen to the male. When commercial broiler breeder hens are confined in a breeder house, their only escape from a forceful male is to move to the slat area or nest boxes. Instead of waiting for a male to court her, she can be chased down and forcefully mated by a male. Therefore, broiler breeders may have evolved reversed mate selection roles (Millman *et al*., 2000). This shift in mate selection, aggression, genetic selection, and severe feed restriction can all attribute to the low levels of defined courtship within breeder flocks.

Aggressive behaviors, such as fighting, pecking, and other displays of dominance, are grouped as mating behaviors because of their intent. Males may fight with other males to establish dominance or compete with other suitors. Males can be aggressive to females in order to force them to mate. When these behaviors are left uncontrolled, there can be welfare issues for the birds and their managers (Brillard, 2004). Feed restriction is widely used in broiler breeder rearing to control body weight of breeders. When breeders become too heavy, they experience decreased libido, and develop skeletal, as well as muscular issues that may impede mating frequency. It has been shown that heavy breeders are less likely to mate or even attempt to mate (Kajer and Mench, 2003). On the other hand, severe cases of feed

restriction in males can cause immense stress and thus a drop in fertility due to inactivity or decreased testicular function. This is especially the case when old breeders are severely restricted (Duncan *et al.*, 1990). Some studies show that moderate feed restriction has no effect on aggression or frequency of mating behavior (Millman and Duncan, 2000). Duncan *et al.* (2000) also concluded that aggressive behavior may be more dependent on genetic strain. This is confirmed by a genetic study by Siegel (1972) where it was found that the genetics for body weight supersede those for behavior. This is due to genetic selection pressure towards profitable factors like body weight, yield, and feed conversion ratio; therefore selection for mating behaviors, specifically courtship, can fall by the wayside. While there are relatively few strains of broiler breeders used in the commercial broiler industry, this poses an important question: are there differences in mating behavior between these broiler breeder strains?

A study was designed in continuation of the work of Moyle and colleagues (2009) to determine differences in aggression, courtship, and direct mating activity in four commercial broiler breeder strains. The study was conducted due to the beliefs held by some industry professionals that mating behavior frequency has a significant effect on fertility of the breeder flock. The goal of this study, however, was to identify significant differences in the frequency of mating behaviors in two broiler breeder crosses. The study was conducted in a commercial setting to gather realistic data from an integrator.

C.   Materials and Methods

The study was conducted at a fully-operational, commercial broiler breeder farm within Pilgrim's Pride's Nashville, Arkansas Complex. Behavioral observations were recorded by video camcorder. Two separate houses of 50 to 52 week-old sister broiler breeder flocks were used for each paired strain combination. Each recording was accompanied by a paired recording of a sister hen flock housing the same male combination. The camcorders were set on a tripod and recorded video for a 4-hour period during early and mid-afternoon. Both houses were stocked, reared, and managed by typical industry standards and owned and managed by the same farm supervisor. All observations were recorded by

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company personnel and all houses were managed to maintain the same male to female ratio, feed restriction program, and other management practices as detailed by the integrator management program. Flock 1 (F1) consisted of a cross between the Hubbard M-99 male line and the Ross 708 female line and were 50 weeks of age at the time of recording. Flock 2 (F2) was 52 weeks of age and consisted of the Cobb MX male and Cobb 500 female. A total of eight recordings were collected in paired samples from sister flocks. One recording was deemed unusable because the birds knocked over the tripod thus resulting in a recording that did not include the predetermined area of observation. Four recordings were analyzed from F1 cross and three recordings were analyzed from the F2 cross. F1 recordings were taken from March 23 to March 26 while F2 recordings were taken from April 1 to April 2 of the same year.

Instances of behavior were defined by Moyle and colleagues (2012) and Wood-Gush (1954). Instances were broken down into three categories: aggression, male behaviors, and mating activity. Aggression consists of male to male, male to hen, and hen to hen aggression. Male-male aggressive behaviors include fighting, charging, pecking the head of another male, and/or disrupting the mating activity of another male. Male-hen aggression comprises both mating and non-mating behaviors. Aggressive mating actions are those exhibited by a male that were considered excessive behavior in order to force a hen to mate. Examples of these acts are running a female down, forcing her to crouch, and forced mounting of the hen by the male. These actions can result in either a failed or successful mating. Hen-hen aggression is recorded when two or more hens fight or peck at each other in an aggressive or dominant manner that was considered abnormal or excessive.

Male behaviors are those that are considered a part of the male's courtship display. Male waltz, when a male drops one wing to the ground and "dances" toward or around a hen in order to court her, is rarely observed in broiler breeder flocks. However, some instances of male waltz are present. Male waltz is most frequently observed in wild, "free range," and exhibition and "backyard" type fowl. The behavior of a male waltzing can also be associated as an act of an expression of dominance towards either a male or female as waltzing has also been observed by one male towards another male in an attempt to establish

dominance. Wing flapping, as associated with courtship, helps a male appear bigger and more dominant to surrounding females and males. The action can also create noise and draw attention toward that male. Male neck flaring is used in the same manner. A male will raise his hackle feathers when approaching a female to appear bigger and entice her to crouch.

Mating activities are those that are directly related to the act of copulation. Hen crouch is defined as "the lowering of the body to the ground with the shoulders elevated so that the male may more easily mate with the hen" (Wood-Gush, 1954). This action can be voluntary or involuntary. In cases of forced mating, the hen may crouch as a result of fear or unequal strength. Hen crouches do not always result in a mount, nor do they always result in an attempted or completed mating. Attempted mounting consists of the male approaching the hen and attempting to climb on her back. When he successfully mounts the hen it is considered a completed mounting. Sometimes the male may grab the feathers or skin on the back of the hen's neck in order to balance himself. Not all attempted mounts result in a completed mount, but an attempted and completed mount is usually preceded by a hen crouch. Attempted and completed matings are actions that, provided the hen has crouched and the male successfully mounted, can result in copulation. Not all attempted matings are successful, but require a successful chain of events to occur.

The data were recorded using a tally sheet (Figure 1.) Because of the camcorder's capabilities and nature of movement throughout a broiler breeder house, it would have been impossible to record every instance of mating activity. Instead, an arbitrary "box" was drawn on the video screen to provide an adequate, but manageable data collection area. Each flock had nest boxes on the slat area in the house. The box was drawn to include the full view of the length of two nest box banks (Figure 2.) Only activity on the floor of the house was considered. Instances were tabulated, summed per activity, and averaged per hour of video footage.

Data were analyzed by an ANOVA using SAS® Software, Version 9.3 of the SAS System for PC (SAS Institute, 2002-2012). A Tukey HSD test was also performed. Significant differences were determined at the $P < 0.05$ level.

D.   Results and Discussion

The results of this study are shown in Tables 1-3. Data are expressed as number of behavioral instances per hour. The only category of mating behavior that showed statistically significant differences between the strain crosses was aggression. F1 males showed aggression to hens more frequently than did F2 males. F1 hens were also more aggressive toward other hens than F2 hens. A summation of male-hen (MFA), male-male (MMA), and hen-hen (FFA) aggression, or overall aggressiveness (OA) was also significantly more frequent in F1. While male-male aggression was not statistically significant ($P = 0.2568$). This indicates that males are not exclusively aggressive towards one another; rather, they are more frequently aggressive towards hens. Hens in the F1 cross showed more aggressive behavior toward each other. Their level of aggressiveness toward males was not included in this study. Testing this factor could determine whether F1 hens are exclusively aggressive to the same sex or are equally aggressive towards the opposite sex, although the expected level of hen aggression towards the male was not considered to be a prevalent problem in these flocks. The F2 cross did not display high levels of aggressive behavior. The F1 cross displayed 11.860 instances of OA per hour, compared to F2's 36.954 instances per hour. Therefore, it was concluded that when the F1 cross (Hubbard M-99 male and Ross 708 female) are paired together, they are highly aggressive. As previously mentioned, it is believed that increased aggression or activity by the males will result in higher fertility and thus more chicks per hen housed. It cannot be determined from this study which flock was more fertile without the integrator's fertility assessment, but this was not the intent of the study to correlate courtship behavior with fertility. Some studies, (Bilcik *et al*. (2005), Bilcik and Estevez (2005), Hocking and Bernard (2000), and McGary *et al*. (2003)) conclude that fertility is not associated with increased frequency of aggression or mating activity. A similar follow up study which included fertility and hatchability in association with these

courtship behaviors would provide valuable information as to the correlation between these activities and the breed strain crosses compared.

There were no statistical differences determined in male courtship or mating activity for the F1 or F2 crosses. A graph of the data (Figure 3) shows a trend that F1 males waltz (WZ), flapped their wings (WF), and flared their neck feathers (NF) more than F2 males. When these courtship behaviors are compared to F1 and F2's mating activity, (Figure 4), it is noticed that the males in the F2 cross tended to illicit hen crouches, attempt to mount, complete a mount, and attempt to mate more frequently than F1. Frequency of behavior for both crosses seems to decline steadily with each successive activity. This can be considered intuitive because not all crouches result in an attempted mount, not all attempts result in a completed mount, and so on. Mating activities are sequential in nature and cannot occur without one happening before the other. As displayed in Table 4, half of hen crouches (HC), attempted mounts (AMO), completed hen mounts (CMO), and attempted mating (AMA) resulted in completed mating (CMA). While these findings are not statistically significant, they do display a perplexing trend. F1 males had higher frequencies of courtship behavior but actually mated hens less frequently, while F2 males courted less, and but mated more frequently. Perhaps, F1 males were more effective with their mating activity meaning their courtship was less frequent, but resulted in more attempted matings than the F2 males. Perhaps F2 males displayed less courtship behavior because they are not as aggressive as F1 males. In accordance with DeJong *et al*. (2009), some attempted mating could have been forced in the highly aggressive F1 males resulting in a failed mating.

Male waltz was not a significant factor. In 27.835 hours of data collection, there were only 16 observed occasions of waltz. This supports the hypothesis that these courtship behaviors observed in wild, exhibition, and backyard birds are displayed less frequently in broiler breeders. However, because behavior of other birds was not studied in conjunction with these broiler breeder strains, it cannot be determined statistically. Selection for the male's ability to court the hen is not a priority. Therefore, when

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compared to courtship data from a domesticated, exhibition type chicken, the broiler breeder displays less overall behavior (Wood-Gush, 1954).

E.   Conclusion and Applications

1. There are significant differences in aggressive behaviors of males on hens, hens on hens, and overall aggressive behavior in the F1 cross as compared to F2. However, F1 and F2 males were not significantly more aggressive toward other males. Management strategies such as, reduced male to female ratio, to reduce the instance of injurious aggression should be implemented with the strains in F1. Subsequent studies needs to be conducted in order to determine the effect of F1's aggression on its fertility.

2. Although there were no significant differences in courtship or mating activity for the flocks, a visual trend emerges and suggests F2 courted less and mated more, while F1 courted more and mated less. This could be due to F1's significantly high levels of aggression or that their courtship displays are more effective than F2's.

3. Male waltz is more frequently observed in wild, exhibition, and backyard flocks than in broiler breeders. This study reveals that male waltz is not statistically significant across either male strain observed. Therefore, it can be concluded that this courtship behavior, while still somewhat present, is disappearing due to the shift from female mate selection to male mate selection.

4. More studies should be conducted to include more breed strains and crosses. It could be that the Hubbard M-99 male line is less aggressive when paired with a Cobb 500 female. It is also possible that some female lines do not display the same level of aggression as the male lines. Further studies could confirm or deny such hypotheses. If the results are repeated with further studies, the industry can make adjustments to management and rearing practices.

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Table 1. Instances per hour of observed aggressive behaviors (MFA = male-female aggression; MMA = male-male aggression; FFA = female-female aggression; OA = overall instances of aggression) from Flock 1 (Hubbard M-99 and Ross 708) and Flock 2 (Cobb MX and Cobb 500).

Table 1. Results from observed aggression

Flock	MFA	MMA	FFA	OA
1	7.641[a]	8.891	20.422[a]	36.954[a]
2	1.480[b]	3.970	6.410[b]	11.860[b]
P-value	0.0072	0.2568	0.0275	0.0331

[a,b]Means within a column with different superscripts within a column differ significantly ($P < 0.05$).

Table 2. Instances per hour of observed male courtship behaviors (WZ = male waltz (dropping a wing and dancing around, or up to a female in a courting manner); WF = male wing flap (standing erect and flapping wings in an effort for the male to draw attention to himself); NF = male neck flare (approaching the female and raising the hackle feathers in an attempt to make himself look bigger) in Flock 1 (Hubbard M-99 and Ross 708) and Flock 2 (Cobb MX and Cobb 500).

Table 2. Results from observed male behavior[1]

Flock	WZ	WF	NF
1	1.761	24.650	14.314
2	2.250	14.020	9.360
P -value	0.4807	0.3333	0.6218

[1]None of these means differ significantly ($P > 0.05$).

Table 3. Instances per hour of observed mating activity (HC = hen crouches (lowering to the ground to allow a male to mate); AMO = attempted hen mounts (male attempts to mount a hen for mating purposes); CMO = completed hen mounts (male obtains a position on top of hen); AMA = attempted mating (after mounting, a male tries to mate a hen); CMA = completed mating (copulation is achieved)) for Flock 1 (Hubbard M-99 and Ross 708) and Flock 2 (Cobb MX and Cobb 500).

Table 3. Results from observed mating activity[1]

Flock	HC	AMO	CMO	AMA	CMA
1	29.443	29.193	27.160	26.660	13.857
2	34.419	33.939	31.949	28.709	16.600
P -value	0.0984	0.1233	0.1291	0.2105	0.2517

[1]None of these means differ significantly ($P > 0.05$).

Table 4. Percentage of mating activities per hour (HC = hen crouches (lowering to the ground to allow a male to mate); AMO = attempted hen mounts (male attempts to mount a hen for mating purposes); CMO = completed hen mounts (male obtains a position on top of hen); AMA = attempted mating (after mounting, a male tries to mate a hen)) resulting in completed mating CMA = completed mating (copulation is achieved) for Flock 1 (Hubbard M-99 and Ross 708) and Flock 2 (Cobb MX and Cobb 500).

Table 4. Percentage of mating activity actually resulting in completed mating

Flock	HC	AMO	CMO	AMA
1	52.94	52.53	48.98	48.02
2	51.77	51.09	48.04	42.18

Figure 1. Mating behavior data collection sheet.

Broiler Breeder Mating Behavior: Pilgrims Pride, Nashville, AR

Date:			Start time:			Finish Time:		Elapsed time:		
Flock #			Male strain			Hen strain				
No. of Males/pen			No. of hens/pen				Male/female ratio:			
Male-hen aggresion	Male-male aggresion	Hen-hen aggression	Male waltz	Male wing flap	Male neck flare	Hen Crouches	Attempted mounts	Hens Mounted	Attempted matings	Completed Matings

Figure 2. Screenshot from video footage displaying the area used for data collection. The "box" is drawn to create a segment of the house from the point of camera placement to the end of the successive bank of nest boxes. The same dimensions for observations were used in every house observed in this study.

Figure 3. Graph of instances per hour of male courtship behaviors (Waltz = dropping a wing and dancing around, or up to a female in a courting manner; Wing flap = standing erect and flapping wings in an effort for the male to draw attention to himself; Neck flare = approaching the female and raising the hackle feathers in an attempt to make himself look bigger) for Flock 1 (Hubbard M-99 and Ross 708) and Flock 2 (Cobb MX and Cobb 500).
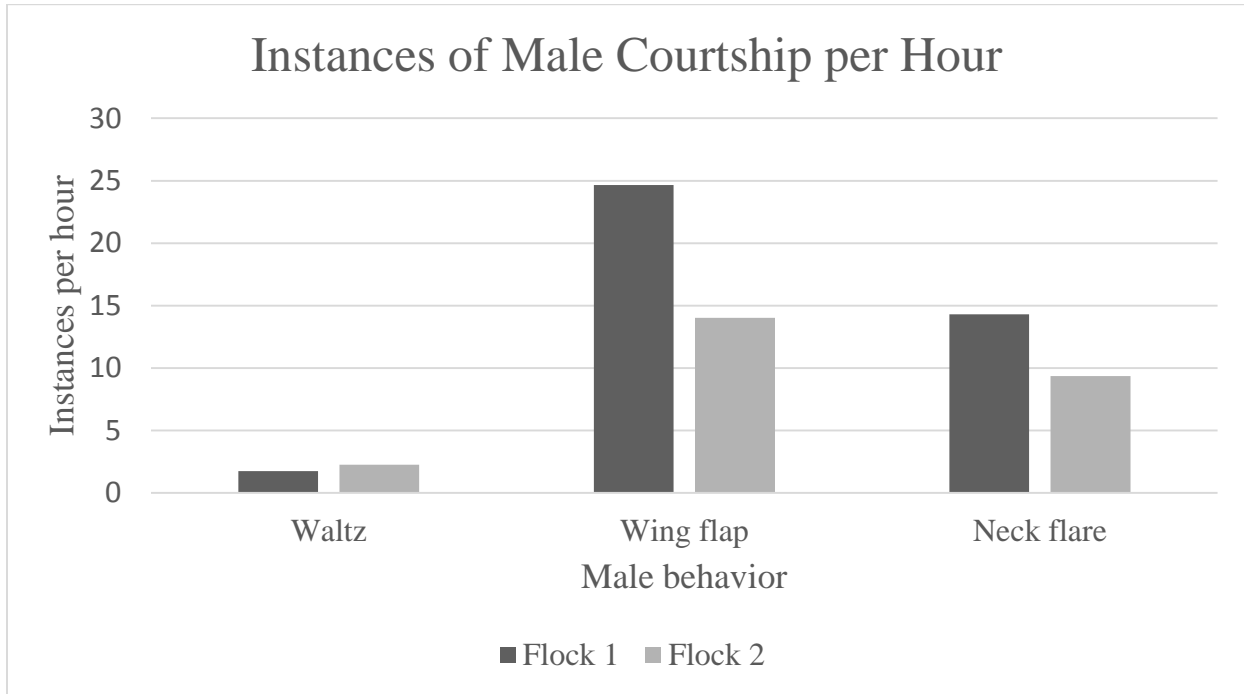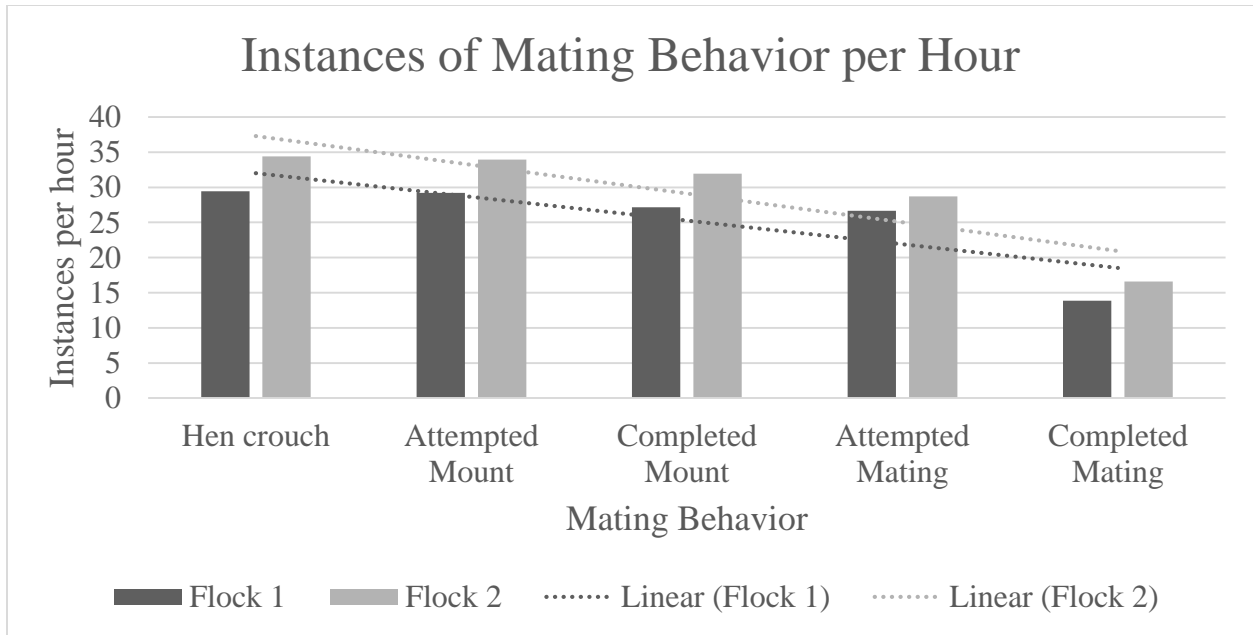
Figure 4. Graph of instances of mating behavior per hour with trend lines describing the decreasing, sequential relationship between mating behaviors for both Flock 1 (Hubbard M-99 and Ross 708) and Flock 2 (Cobb MX and Cobb 500).

III.　Comprehensive Study of the Effects of Egg Storage, Floor and Nest Eggs, and Broiler Breeder Flock Age on Hatchability and 14-day Livability, Feed Conversion Ratio, and Body Weight in Broiler Chicks.[1]

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A.　Abstract

The objective of this study was to determine the effects of egg treatment and handling on the viability and efficiency of Cobb broilers. Three experimental studies were set up: egg storage duration, broiler breeder flock age, and floor versus nest eggs. In the egg storage study (ES1 and ES2), eggs were collected from the same flock and stored for four, eight, twelve, or sixteen days. The broiler breeder flock age study (FA1), hatching eggs were obtained from four breeder flocks aged 27, 37, 46, and 56 weeks. A second flock age trial (FA2) was conducted with eggs from flocks aged 35, 39, and 46 weeks due to availability of hatching eggs. In the floor versus nest egg study (FN1 and FN2), 1000 eggs were collected from a flock forced to lay outside of the nest box and 1000 eggs were collected from inside the nest boxes. Each study was completed at the University of Arkansas Research Farm. Eggs were procured from Cobb-Vantress, Inc., set, and hatched at the University of Arkansas Hatchery. Birds were grown out in replicate pens by treatment for 14 days according to industry standards and provided feed and water *ad libitum*. An initial trial and a replicate were performed for each experiment, barring flock age; one trial was conducted with four different flock ages and one with three different flock ages. Data was collected and analyzed at weeks one and two of the broiler grow-out for: feed conversion; average bird weight (including placement weight); and mortality (livability). Hatchability was assessed via hatchery residue breakout for percent hatch, percent hatch of fertile, and percent fertility. For ES1, significant differences were found in percent hatch, percent hatch of fertile, and percent fertility, percent early dead embryos, and percent late dead

embryos. No significant differences in growth performance were determined in ES1. ES2 found significant differences in feed conversion ratio for weeks one and two, average bird weight at placement, percent hatch, percent hatch of fertile, and percent fertility, percent early dead embryos, percent late dead embryos, and percent embryos pipped. FA1 revealed significant differences in feed conversion for week one, average bird weight at placement and week one, and percent mortality for weeks one and two, percent hatch, percent hatch of fertile, and percent fertility. FA2 showed differences in percent early dead embryos (4-7 days), percent late dead embryos, and percent embryos pipped. The only criteria that displayed statistical differences for the floor and nest egg treatments (FN1 and FN2) was percent contaminated eggs in FN1. No criteria were significant for FN2. We can conclude the storage of eggs has more impact on hatchability than on grow-out performance. Very young breeder flocks may have low fertility and, therefore, lowered hatchability. Finally, where the egg was laid (floor or nest), has little significant effect on progeny performance.

Keywords: egg storage, floor and nest eggs, flock age, hatchability, broiler performance

[1]Prepared for submission to *Journal of Applied Poultry Research*

B.   Introduction

Developing embryos are highly sensitive to their environment. Fluctuation in temperature and humidity biologically alter the development process both prior to and during incubation. The subsequent effects can be detrimental to hatchability and growth performance. The poultry industry is currently experimenting with reduction or elimination of the use of antibiotics. Contract growers and hatchery managers are noticing issues with poor hatches and chick quality, high 7-day mortality, and altered growth performance. Recent disease outbreaks in other parts of the world have left the U.S. poultry industry in an egg shortage due to export of hatching eggs. These issues leave hatcheries forced to set any and all available eggs in order to meet customer demands. Eggs may be stored longer periods of time, from very young and/or very old flocks, or even contaminated to some degree. Perhaps these practices are contributing to the observed hatchability and growth performance issues.

Many factors can modify the integrity of the developing embryo. Prolonged egg storage (over seven days) is known to cause a delay in instigation of development, reduction in hatchability, and increased instance of early and late dead embryos (Mather and Laughlin, 1977; Aurora and Kosin, 1966; Byng and Nash, 1962; Lapão*et al*., 1999; Mayes and Takeballi, 1984). However, short-term storage may actually be necessary to allow for the slight degradation of albumen for gas and nutrient exchange (Benton and Brake, 1996). Waite (1919) determined that storage up to seven days has no significant effect on hatchability of the egg. So, when eggs need to be stored for longer than seven days, how should the industry account for hatch losses due to extended storage in the hatchery? Setting eggs 30 minutes earlier for each day of storage allows for optimal hatch (Kirk *et al*., 1980). The chicks that do fully develop and hatch can also show signs of delayed growth and reduced performance factors. (Whipple) Therefore, integrators will suffer from more than just lower chick numbers; the chicks that do hatch may suffer from reduced chick quality, decreased 7-day livability, and poor feed conversion ratios.

Integrators are often forced to set eggs from very young and very old flocks. Flocks outside of peak production may exhibit reduced fertility and viability (hatch of fertile) (Hocking and Bernard, 2000; Fasenko *et al.*, 1992). Age of the broiler breeder flock also causes significant differences in fertility, as well as hatchability and performance. Additionally, when stored from 3-14 days, eggs from older flocks exhibited high early and late dead embryos (Elibol *et al.*, 2002). Embryo mortality in eggs from old flocks may be due to some strain influence or decreased nutrient metabolism (Hamidu *et al.*, 2007). Other factors may actually outweigh the effects of flock age on embryo development and hatch. The environment inside the incubator and hatcher may significantly impact hatch as well as performance (Hulet *et al.*, 2007). Low wet bulb humidity can also significantly decrease hatchability in older flocks (Vice *et al.*, 1993), and dissimilar egg weights and shell characteristics can contribute to a loss in yolk uptake and altered embryo body composition (Shanawany, 1984; Peebles *et al.*, 2001). Therefore, flock age can be deemed subordinate when coupled with other factors.

In extreme situations, hatcheries may set eggs that would normally be considered "culls." There are few studies that show the effects of setting eggs cull eggs or eggs laid on the floor on hatchability and growth performance of the subsequent progeny. Floor eggs come in contact with litter and can become contaminated with the numerous pathogens found in the litter. Because of the porous nature of the egg shell, some pathogens, usually bacteria, can pass through the shell and into the egg. Setting these eggs provides the perfect environment - heat, humidity, and nutrients - for those bacteria to thrive. The buildup of gasses from the proliferation of bacteria within the egg can cause it to crack, leak, and explode, thereby exposing other eggs and the machinery to the bacteria. Contaminated eggs do not hatch as the embryo generally dies early in development. Too many exploding eggs can leave the chicks that do hatch predisposed to unusually high levels of bacteria, thus hurting growth performance. If floor eggs are definitively found to produce high levels of contamination, it should be advised to not set floor eggs.

Because of the many factors that potentially impact hatchability and growth performance of broilers, the onset of antibiotic free movements, and hatching egg shortages, it is important for the industry to

improve hatchability and chick quality issues in order to meet hatching egg demands. Therefore, a study was designed and conducted to determine the effects of long-term storage, flock age, and floor eggs on hatchability and 14-day growth performance of broiler chickens.

C.   Materials and Methods

Three studies were conducted, each having two replicate trials. The first study (ES1 and ES2) investigated the effects from length of storage of hatching eggs. The second study, (FA1 and FA2) evaluated eggs from different broiler breeder flock ages. The final study, (FN1 and FN2) tested the effects of eggs laid on the floor of the broiler breeder house versus those laid inside the next box. Cobb 700 parent stock hatching eggs were supplied by a primary breeder company from GP flocks. Eggs were then transported to the University of Arkansas Research Hatchery and Farm where they were set, hatched, and grown to 14 days of age. After each trial's hatch, the unhatched eggs were broken out and assessed for fertility and a subjected to a complete embryo diagnosis. Every hatch residue break-out was performed by the same two researchers. Birds were grown in 36, 5 x 10 foot floor pens. For ES1, ES2, and FA1 there were nine replicate pens per treatment. In FN1 and FN2 there were 18 replicate pens per treatment. For FA2, a different research house was used; pens were 6 x 6 feet and there were 12 replicate pens per treatment group. Pens were assigned randomly throughout the house with feed and water provided *ad libitum*. Birds were weighed at one, seven, and 14 days, respectively. Each bird mortality was weighed, recorded, and a post-mortem necropsy was performed to assess cause of death. Upon completion of each trial (day 14) birds were humanely euthanized and disposed of at the University of Arkansas Research Farm.

For ES1 and ES2, 2000 eggs from a 62-week old GP flock were collected. 500 eggs were collected and stored for four days in the farm's egg room. Four days later, 500 more eggs were collected and stored with the previous eggs and four days later, 500 more eggs were collected and stored with the previous

eggs then finally, 500 more eggs were collected and stored with the previous eggs in the same egg storage unit. Thus, resulting in four treatments of eggs stored four, eight, 12, and 16 days.

With FN1 and FN2 trials, a GP flock was selected which was notorious for laying eggs on the floor. As they were going out of production, the nest boxes on one side of the house were winched up toward the ceiling and made unavailable for the hens. Therefore, they were either forced to lay their eggs on the floor or in a nest box on the other side of the house. 1000 eggs were collected from the floor and 1000 were collected from nest boxes, resulting in two treatment groups.

The FA1 trial used eggs from GP flocks aged 27, 37, 46, and 56 weeks. 500 eggs were collected from each flock age resulting in four age treatment groups from one pre-peak production, two peak production, and one post-peak production flocks. Before the replicate FA2 could begin, the oldest flocks were sold. Therefore, in order to complete the trial in a timely manner, the primary breeder company selected flocks aged 35, 39, and 46 weeks. 2000 eggs were collected, 667 from each flock age.

Data was collected on hatchability and growth performance factors. The results were analyzed using SAS® software, Version 9.4 of the SAS System for PC. Copyright 2012, SAS Institute, Inc. A Tukey HSD test was also performed. Significant differences were determined at the $P < 0.05$ level.

D.   Results and Discussion

Results for each trial are shown in Tables 1-13. The same hatchability and growth performance factors were considered for each trial. Hatchability factors were: percent hatch (%Hatch), percent hatch of fertile (%HOF), percent fertility (%F), percent early dead embryos 1-3 days (%ED1-3), percent early dead embryos 4-7 days (%ED4-7), percent mid dead embryos 8-14 days (%Mid8-14), percent late dead embryos 15-21 days (%Late15-21), percent of chicks pipped in the shell (%Pip), and percent of eggs deemed contaminated (%Cont). Growth performance factors were: feed conversion ratio through week 1 of growth (FCR wk1), feed conversion through week 2 of growth (FCR wk2), average bird weight at day 1 (Avg BW d1), average bird weight through week 1 (Avg BW wk1), average bird weight through week 2

(Avg BW wk2), percent mortality through week 1 (%M wk1), and percent mortality through week 2 (%M wk2).

Table 1 shows number of eggs set per treatment in each trial and the number of chicks placed in each treatment over the six trials. In each trial, it was a priority to place every viable chick. However, due to high instances of embryo mortality, contamination, and pips, some treatments did not produce enough chicks to make all pen numbers equal.

Trial ES1 revealed significant differences in %Hatch, %HOF, %ED1-3, and %Late15-21 (Table 2). Eggs stored 12 and 16 days hatched significantly lower than eggs stored four and eight days. The %Hatch for the entire trial was only 43.10% but a low percent hatchability was expected due to the nature of the trial. ES1 had high levels of contaminated eggs, which was common from the source flock for the eggs. Overall, 5.1% of the 2000 eggs in ES1 were deemed contaminated. Contamination across the four treatments was not significant. However, by industry standards, 5% contamination is not acceptable. In hatcheries that produce a million chicks a week, 5% contamination will not only result in significant losses in hatch but will dramatically affect quality of the hatched chicks. Because other hatchability factors were significantly impacted by long-term (12 and 16 days) storage, it is possible that the high levels of contamination could also be a contributor. There were no significant differences in growth performance factors for ES1 (Table 8.). It is possible that the chicks that did hatch from 12- and 16-day stored eggs overcame the inherent challenges of egg storage and contamination and performed similarly to chicks from four and eight day stored eggs.

ES2 showed similar results in regards to hatchability factors (Table 3). Significant differences were found in stored eggs for %Hatch, %HOF, %F, %ED1-3, %Late15-21, and %Pip. In ES2, contamination was low; therefore, increased length of egg storage is the primary contributing factor for the total %Hatch being 59.3%. High instances of early dead embryos in 1-3 days of incubation can be directly attributed to egg storage length. %Late15-21 can be attributed to storage length or mechanical failure. The latter,

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however, was definitely not the case in this study. Perhaps, the 12 and 16 day stored embryos simply "ran out of steam" after having their development stagnated for such long periods of time, pre-incubation. This can hold true for the 22% of 16 day stored eggs that were pipped. This hatch was very slow moving. It is possible that these pipped eggs also "ran out of steam" and simply could not muster the energy to fully emerge from the shell in a timely manner. It is known that eggs stored for longer periods of time require longer incubation, however, this is not an exact science and is held as a general rule. For every one day of storage past seven days of storage, one hour should be added to the total incubation time. For the purposes of this study, the researchers chose to set all eggs at the same time to avoid additional variables into the study. The researchers believed that this added variable could alter the data set and subsequent results. It is important to note at this point that a significant difference in %F in ES2 is not due to the effects of egg storage. Eggs are either fertile or infertile when they leave the hen's body, therefore, storage cannot affect the fertility of the egg. Possible reasons for the significant differences in fertility for ES2 are: acute stressors like heat spikes, cold spells, severe weather, lack of feed and water, or male spiking that may have occurred during the egg collection periods. Because eggs were collected over a period of 16 days, there are many variables that could have caused differences in the flock's fertility. Unfortunately, the researchers were not provided this information so the true cause of this fluctuation in fertility cannot be conclusively determined. For ES2, FCRwk1, FCRwk2, and Avg BW d1 were found to be statistically significant ($P< 0.0001$, $P<0.0182$, and $P< 0.0002$, respectively). In each case, the 16-day treatment had higher FCR and average body weight at placement.

For FN1, no significant differences were found in any hatchability or growth performance factors except %Cont (Table 4 and Table 10). In this case, floor eggs displayed a higher instance of contamination ($P< 0.0207$) than nest eggs. However, the contaminated eggs only made up 0.5% of the floor eggs that were set. That percentage is very low, but still, statistically significant when compared to 0.1% in eggs collected from the nest boxes. No significant effects were determined from FN2 in either hatchability or growth performance (Table 5 and Table 11). These birds were forced to lay their eggs on

the floor due to the created lack of the proper number of nest boxes per hen. Therefore, the data may not be representative of hens that truly choose to lay their eggs on the floor. It is also possible that the floor of the house is not as dirty and contaminated as previously thought. Because only 0.3% of eggs from FN1 and 1.3% of eggs from FN2 were deemed contaminated, and only FN1 was statistically significant, it cannot be definitively concluded that floor eggs display higher levels of contamination. Additionally, it cannot be concluded that floor eggs negatively or positively impacted hatchability and performance because nearly all factors were statistically insignificant. It is possible that more studies with eggs from breeders that are more notorious for laying on the floor will provide different results.

FA1 and FA2 should be considered as two separate trials because of their discontinuity in flock ages tested. Therefore, data from FA1 (Table 6 and Table 12) should be compared independently, as should the data from FA2 (Table 7 and Table 13). FA1 showed significant differences in the 27-week-old treatment for %Hatch ($P< 0.0001$), while %HOF was also significantly lower ($P < 0.0002$) in both the 27- and 37-week-old treatments. %F was lowest in the 27-week-old treatment and highest in the 46-week-old treatment ($P< 0.0004$) as would be expected. This can be attributed to the flock's mating activity or level of sexual maturity. However, being in the pre-peak stage of production, it can be expected that the difference in onset of sexual maturity is the driving force. No significant differences were determined from the other hatchability factors. Therefore, for FA1, only flock age significantly impacted %Hatch, %HOF, and %F. In terms of growth performance, the 27-week-old flock's chicks had significantly higher FCR wk1 ($P < 0.0001$). However, FCR in wk2 data were not significant. Avg BW d1 and Avg BW wk1 were significantly lower in the 27- and 37-week-old treatments. %M wk1 and %M wk2 were significantly different in the 27 and 37 week treatments, as well. These differences among treatments in hatchability and growth performance factors concludes that seven-day growth performance and 14 day livability are both negatively impacted by the 27- and 37-week-old treatments. Based on this study, it might be suggested to integrators to expect lower fertility and hatchability in these younger flocks as well as reduced growth performance from chicks from these younger flocks.

FA2 (Table 7 and Table 13) showed significant differences in %Early4-7, %Late15-21, and %Pip. The 39-week-old treatment had a slightly higher instance of early dead embryos 4-7 days of incubation. %Late15-21 was increased in the 35- and 46-week-old treatments with 7.37% and 9.47%, respectively, of these eggs contained embryos that were recorded as late dead. This explains why the 35-week-old treatment had a higher instance of pipped eggs. It should also be mentioned that %Early4-7 and %Pip were also statistically significant ($P < 0.0412$ and $P < 0.0424$, respectively). The significant factors in growth performance were Avg BW d1, Avg BW wk1, and Avg BW wk2 ($P < 0.0001$, $P < 0.0019$, and $P < 0.0008$, respectively). Chicks from the 46-week-old flock were significantly larger, on average, at hatch (d1) than those from the 35- or 39-week-old flocks. However, chicks from the 39-week-old flock were larger at weeks one and two than the chicks from the other age treatments. Because this data does not compare congruently to FA1, it is difficult to draw conclusions on the true effects of flock age. The presence of a very young or very old flock in FA2 would make the differences scientifically reportable.

E.   Conclusion

1.   Egg storage for 16 days has significant effects on some factors of hatchability and growth performance. However, the presence of high levels of contamination may also be a contributing factor in these negative effects. Only ES2 showed significant differences in growth performance, while both ES1 and ES2 showed detrimental effects on hatchability.

2.   Floor eggs do not seem to have significant effects on hatchability or growth performance.

3.   Hatchability and growth performance seem to only be affected by pre-peak production or very young flocks, rather than peak- and post-peak production flocks. Due to heterogeneity of sexual maturity in young breeder flocks, fertility is impacted significantly. Therefore, percent hatch and percent hatch of fertile are also affected. In terms of growth performance, the 27-week old flock exhibited a significantly higher feed conversion ratio at week one as well as significantly lower body weights at placement and week one. Mortality for the very young flocks was also higher at weeks one and two.

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Table 1. Table describing the number of eggs set per trial[1] (egg storage, floor versus nest eggs, and flock age), per treatment, as well as number of chicks placed per trial, per treatment, per pen. For ES1, ES2, FN1, FN2, and FA1, there were 9 pens per treatment. For FA2, there were 12 pens per treatment.

Table 1. Number of eggs set and chicks placed per trial, per treatment.

Trial[1]	Treatment	Eggs set	Chicks placed per pen
ES1	4 day	496	27
	8 day	498	26
	12 day	499	19
	16 day	499	17
ES2	4 day	499	43
	8 day	500	38
	12 day	479	30
	16 day	500	17
FN1	Floor	998	29
	Nest	999	29
FN2	Floor	1000	41
	Nest	993	31
FA1	27 weeks	497	28
	37 weeks	496	39
	46 weeks	485	44
	56 weeks	495	42
FA2	35 weeks	665	40
	39 weeks	665	40
	46 weeks	664	40

[1]ES1 = egg storage trial one, ES2 = egg storage trial two, FN1 = floor versus nest egg trial one, FN2 = floor versus nest egg trial two, FA1 = flock age trial one, FA2 = flock age trial two.

Table 2. Analysis of hatchability factors[1] per treatment for the egg storage trial one.

Table 2. Egg Storage #1 – Hatchability[1,2,3]

Treatment	%H	%HOF	%F	%ED1-3	%ED4-7	%Mid	%Late	%Pip	%Cont
4	49.8[a]	61.30[a]	81.24[a]	8.44[a]	0.41	0.20	10.70[ab]	2.42	4.24
8	54.60[a]	65.05[a]	83.94[b]	7.64[a]	0.41	0.00	8.05[a]	3.62	5.62
12	34.25[b]	42.30[b]	80.97[a]	11.44[a]	0.99	0.80	13.85[ab]	4.23	6.42
16	33.81[b]	41.95[b]	80.54[a]	17.03[b]	0.60	0.40	14.62[b]	6.22	4.20
P - value	0.0001	0.0001	0.0460	0.0011	0.6600	0.0694	0.0226	0.2110	0.0817

[1]%H = percent hatch, %HOF = percent hatch of fertile, %F = fertility, %ED1-3 = percent of embryos dead between 1 and 3 days of incubation, %ED4-7 = percent of embryos dead between 4 and 7 days of incubation, %Mid = percent of embryos dead between 8 and 14 days of incubation, %Late = percent of embryos dead between 15 and 21 days of incubation, %Pip = percent of chicks pipped through the shell, but unhatched, %Cont = percent of eggs deemed contaminated.
[2]All factors were assessed by hatchery residue breakout and embryo diagnosis.
[3]Statistical significance was held at the *P* < 0.05 level.

Table 3. Analysis of hatchability factors[1] per treatment for the egg storage trial two.

Table 3. Egg Storage #2 – Hatchability[1,2,3]

Treatment	%Hatch	%HOF	%F	%ED1-3	%ED4-7	%Mid	%Late	%Pip	%Cont
4	78.73[a]	84.49[a]	93.19[ab]	4.40[a]	0.00	0.00	5.02[a]	1.80[a]	0.00
8	69.98[a]	75.55[a]	92.60[ab]	5.59[a]	0.00	0.00	6.80[a]	4.99[a]	0.00
12	56.71[b]	59.70[b]	94.96[a]	7.46[ab]	0.00	0.00	12.84[b]	9.88[b]	0.20
16	31.57[c]	34.53[c]	91.39[b]	11.20[b]	0.00	0.20	19.42[b]	21.62[b]	0.40
P - value	0.0001	0.0001	0.0209	0.0119	0.0000	0.4411	0.0011	0.0001	0.2172

[1]%H = percent hatch, %HOF = percent hatch of fertile, %F = fertility, %ED1-3 = percent of embryos dead between 1 and 3 days of incubation, %ED4-7 = percent of embryos dead between 4 and 7 days of incubation, %Mid = percent of embryos dead between 8 and 14 days of incubation, %Late = percent of embryos dead between 15 and 21 days of incubation, %Pip = percent of chicks pipped through the shell, but unhatched, %Cont = percent of eggs deemed contaminated.
[2]All factors were assessed by hatchery residue breakout and embryo diagnosis.
[3]Statistical significance was held at the $P < 0.05$ level.

Table 4. Analysis of hatchability factors[1] per treatment for the floor versus nest egg trial one.

Table 4. Floor vs Nest Eggs #1 – Hatchability[1,2,3]

Treatment	%Hatch	%HOF	%F	%ED1-3	%ED4-7	%Mid	%Late	%Pip	%Cont
Floor	54.01	63.29	85.33	7.93	0.10	0.00	10.91	9.70	0.50[a]
Nest	54.82	63.20	86.77	5.88	0.61	0.10	11.61	8.63	0.10[b]
P - value	0.8400	0.9855	0.2801	0.0910	0.2576	0.3409	0.7662	0.6485	0.0207

[1]%H = percent hatch, %HOF = percent hatch of fertile, %F = fertility, %ED1-3 = percent of embryos dead between 1 and 3 days of incubation, %ED4-7 = percent of embryos dead between 4 and 7 days of incubation, %Mid = percent of embryos dead between 8 and 14 days of incubation, %Late = percent of embryos dead between 15 and 21 days of incubation, %Pip = percent of chicks pipped through the shell, but unhatched, %Cont = percent of eggs deemed contaminated.
[2]All factors were assessed by hatchery residue breakout and embryo diagnosis.
[3]Statistical significance was held at the *P* < 0.05 level.

Table 5. Analysis of hatchability factors1 per treatment for the floor versus nest egg trial two.

Table 5. Floor vs Nest Eggs #2 – Hatchability[1,2,3]

Treatment	%Hatch	%HOF	%F	%ED1-3	%ED4-7	%Mid	%Late	%Pip	%Cont
Floor	78.87	84.83	93.01	3.60	0.10	0.20	5.74	2.19	1.52
Nest	75.33	79.52	94.73	5.05	0.20	0.11	6.35	2.04	1.41
P - value	0.3482	0.2018	0.0969	0.1009	0.5490	0.6988	0.6353	0.8882	0.8633

[1]%H = percent hatch, %HOF = percent hatch of fertile, %F = fertility, %ED1-3 = percent of embryos dead between 1 and 3 days of incubation, %ED4-7 = percent of embryos dead between 4 and 7 days of incubation, %Mid = percent of embryos dead between 8 and 14 days of incubation, %Late = percent of embryos dead between 15 and 21 days of incubation, %Pip = percent of chicks pipped through the shell, but unhatched, %Cont = percent of eggs deemed contaminated.
[2]All factors were assessed by hatchery residue breakout and embryo diagnosis.
[3]Statistical significance was held at the *P* < 0.05 level.

Table 6. Analysis of hatchability factors[1] per treatment for the flock age trial one.

Table 6. Flock Age #1 – Hatchability[1,2,3]

Flock Age	%Hatch	%HOF	%F	%ED1-3	%ED4-7	%Mid	%Late	%Pip	%Cont
27	48.31[a]	68.82[a]	70.44[a]	12.01	0.00	0.21	7.9	1.41	0.00
37	62.94[b]	76.90[a]	81.86[b]	7.46	0.00	0.21	8.64	1.39	0.00
46	79.01[c]	86.33[b]	91.49[c]	6.35	0.20	0.20	4.89	0.42	0.00
56	81.04[c]	90.94[b]	89.13[b]	5.87	0.00	0.00	5.83	0.41	0.20
P - value	0.0001	0.0002	0.0004	0.0586	0.4411	0.8015	0.1256	0.1395	0.4411

[1]%H = percent hatch, %HOF = percent hatch of fertile, %F = fertility, %ED1-3 = percent of embryos dead between 1 and 3 days of incubation, %ED4-7 = percent of embryos dead between 4 and 7 days of incubation, %Mid = percent of embryos dead between 8 and 14 days of incubation, %Late = percent of embryos dead between 15 and 21 days of incubation, %Pip = percent of chicks pipped through the shell, but unhatched, %Cont = percent of eggs deemed contaminated.
[2]All factors were assessed by hatchery residue breakout and embryo diagnosis.
[3]Statistical significance was held at the *P* < 0.05 level.

Table 7. Analysis of hatchability factors[1] per treatment for the flock age trial two.

Table 7. Flock Age #2 – Hatchability[1,2,3]

Flock Age	%Hatch	%HOF	%F	%ED1-3	%ED4-7	%Mid	%Late	%Pip	%Cont
35	76.82	99.45	90.95	4.35	0.15[a]	0.30	7.37[ab]	2.11[a]	0.74
39	78.19	99.44	93.06	5.54	0.60[b]	0.45	4.68[a]	0.76[b]	1.49
46	76.22	99.44	92.33	4.52	0.15[a]	0.15	9.47[b]	0.77[b]	0.60
P - value	0.5082	0.274	0.1912	0.5167	0.0421	0.4360	0.0036	0.0424	0.5447

[1]%H = percent hatch, %HOF = percent hatch of fertile, %F = fertility, %ED1-3 = percent of embryos dead between 1 and 3 days of incubation, %ED4-7 = percent of embryos dead between 4 and 7 days of incubation, %Mid = percent of embryos dead between 8 and 14 days of incubation, %Late = percent of embryos dead between 15 and 21 days of incubation, %Pip = percent of chicks pipped through the shell, but unhatched, %Cont = percent of eggs deemed contaminated.
[2]All factors were assessed by hatchery residue breakout and embryo diagnosis.
[3]Statistical significance was held at the *P* < 0.05 level.

Table 8. Analysis of growth performance factors[1] per treatment for the egg storage trial one.

Table 8. Egg Storage #1 - Growth performance[1,2]

Treatment	FCRwk1	FCRwk2	AvgBWd1*	AvgBWwk1*	AvgBWwk2*	%M1	%M2
4	1.2120	1.3257	0.0495	0.1563	0.3933	3.29	4.94
8	1.1879	1.3199	0.0502	0.1576	0.4041	2.71	4.19
12	1.2119	1.3269	0.0498	0.1590	0.4103	5.26	6.43
16	1.2336	1.3400	0.0495	0.1545	0.3909	4.36	6.88
P - value	0.3359	0.6469	0.1126	0.1842	0.1561	0.5952	0.6462

[1]FCRwk1 = feed conversion ratio through week 1, FCRwk2 = feed conversion ratio through week 2, AvgBWd1 = average body weight per bird at placement or day 1, AvgBWwk1 = average body weight per bird at week 1, AvgBWwk2 = average body weight per bird at week 2, %M1 = percent mortality at week 1, %M2 = percent mortality at week 2.
[2]Statistical significance was held at the $P < 0.05$ level.
*Units measured in kilograms.

Table 9. Analysis of growth performance factors[1] per treatment for the egg storage trial two.

Table 9. Egg Storage #2 - Growth performance[1,2]

Treatment	FCRwk1	FCRwk2	AvgBWd1*	AvgBWwk1*	AvgBWwk2*	%M1	%M2
4	1.3817[a]	1.3537[ab]	0.0481[a]	0.1359	0.3317	0.51	2.57
8	1.3545[a]	1.3586[ab]	0.0473[a]	0.1366	0.3305	1.17	1.75
12	1.4194[a]	1.3191[a]	0.0475[a]	0.1342	0.3352	0.74	2.22
16	1.6717[b]	1.6371[b]	0.0496[b]	0.1365	0.3387	1.13	2.61
P - value	0.0001	0.0182	0.0002	0.8718	0.7085	0.7799	0.8911

[1]FCRwk1 = feed conversion ratio through week 1, FCRwk2 = feed conversion ratio through week 2, AvgBWd1 = average body weight per bird at placement or day 1, AvgBWwk1 = average body weight per bird at week 1, AvgBWwk2 = average body weight per bird at week 2, %M1 = percent mortality at week 1, %M2 = percent mortality at week 2.
[2]Statistical significance was held at the $P < 0.05$ level.
*Units measured in kilograms.

Table 10. Analysis of growth performance factors[1] per treatment for the floor versus nest egg trial one.

Table 10. Floor vs Nest Eggs #1 - Growth performance[1,2]

Treatment	FCRwk1	FCRwk2	AvgBWd1*	AvgBWwk1*	AvgBWwk2*	%M1	%M2
Floor	1.211	1.311	0.0385	0.1210	0.3333	0.048	0.057
Nest	1.187	1.308	0.0380	0.1197	0.3336	0.054	0.073
P - value	0.3347	0.4686	0.2892	0.3807	0.2214	0.8162	0.7429

[1]FCRwk1 = feed conversion ratio through week 1, FCRwk2 = feed conversion ratio through week 2, AvgBWd1 = average body weight per bird at placement or day 1, AvgBWwk1 = average body weight per bird at week 1, AvgBWwk2 = average body weight per bird at week 2, %M1 = percent mortality at week 1, %M2 = percent mortality at week 2.
[2]Statistical significance was held at the $P < 0.05$ level.
*Units measured in kilograms.

Table 11. Analysis of growth performance factors[1] per treatment for the floor versus nest egg trial two.

Table 11. Floor vs Nest Eggs #2 - Growth performance[1,2]

Treatment	FCRwk1	FCRwk2	AvgBWd1*	AvgBWwk1*	AvgBWwk2*	%M1	%M2
Floor	1.242	1.441	0.0436	0.1288	0.3373	0.005	0.012
Nest	1.252	1.429	0.0429	0.1272	0.3313	0.006	0.01
P - value	0.4998	0.3941	0.2952	0.4615	0.2185	0.7574	0.8294

[1]FCRwk1 = feed conversion ratio through week 1, FCRwk2 = feed conversion ratio through week 2, AvgBWd1 = average body weight per bird at placement or day 1, AvgBWwk1 = average body weight per bird at week 1, AvgBWwk2 = average body weight per bird at week 2, %M1 = percent mortality at week 1, %M2 = percent mortality at week 2.
[2]Statistical significance was held at the $P < 0.05$ level.
*Units measured in kilograms.

Table 12. Analysis of growth performance factors[1] per treatment for the flock age trial one.

Table 12. Flock Age #1 - Growth performance[1,2]

BB Flock Age	FCRwk1	FCRwk2	AvgBWd1*	AvgBWwk1*	AvgBWwk2*	%M1	%M2
27	1.7466[a]	1.3785	0.0350[a]	0.0933[a]	0.4486	3.22[a]	3.62[a]
37	1.3946[b]	1.4553	0.0450[b]	0.1177[b]	0.3600	0.28[b]	0.57[b]
46	1.3432[b]	1.3982	0.0462[c]	0.1226[c]	0.3749	1.04[ab]	2.05[ab]
56	1.4222[b]	1.4356	0.0453[c]	0.1209[c]	0.3772	1.06[ab]	1.06[ab]
P - value	0.0001	0.8968	0.0001	0.0001	0.8418	0.0421	0.0388

[1]FCRwk1 = feed conversion ratio through week 1, FCRwk2 = feed conversion ratio through week 2, AvgBWd1 = average body weight per bird at placement or day 1, AvgBWwk1 = average body weight per bird at week 1, AvgBWwk2 = average body weight per bird at week 2, %M1 = percent mortality at week 1, %M2 = percent mortality at week 2.
[2]Statistical significance was held at the *P* < 0.05 level.
*Units measured in kilograms.

Table 13. Analysis of growth performance factors[1] per treatment for the flock age trial two.

Table 13. Flock Age #2 - Growth performance[1,2]

BB Flock Age	FCRwk1	FCRwk2	AvgBWd1*	AvgBWwk1*	AvgBWwk2*	%M1	%M2
35	0.8818	1.2363	0.0445[a]	0.1228[a]	0.3407[a]	1.46	2.50
39	0.8763	1.2097	0.0461[a]	0.1298[ab]	0.3626[ab]	1.25	1.88
46	0.9568	1.2443	0.0463[b]	0.1266[b]	0.3508[b]	1.04	2.29
P - value	0.2139	0.0904	0.0001	0.0019	0.0008	0.8768	0.8422

[1]FCRwk1 = feed conversion ratio through week 1, FCRwk2 = feed conversion ratio through week 2, AvgBWd1 = average body weight per bird at placement or day 1, AvgBWwk1 = average body weight per bird at week 1, AvgBWwk2 = average body weight per bird at week 2, %M1 = percent mortality at week 1, %M2 = percent mortality at week 2.
[2]Statistical significance was held at the $P < 0.05$ level.
*Units measured in kilograms.

IV.   Conclusion

The poultry industry has, indeed, made incredible advances to provide affordable protein to a hungry world. In doing so, it has also revealed some self-limitations in selection for growth rate, feed conversion ratio, and reproductive efficacy. These studies set a foundation for further understanding of two problems facing the industry today: strain-dependent mating behavior and egg treatment's effect on hatchability and performance.

Data presented reflects some belief that aggression is strain-dependent in broiler breeders. It is also noted that while some behaviors are not significantly different across strains, they are reduced to rare occurrences in the flocks studied. More studies with different crossed strains may reveal more evidence for or against these arguments. In accordance with some current literature, it is also concluded that egg storage and flock age can significantly impact hatchability and growth performance. However, some parts of this study need to be modified and repeated to provide true statistical and scientific evidence to the industry.

As the world's population grows and the wealth disparity gap decreases, poultry products will be in high demand. In order for the industry to continue their efforts, studies such as these will provide a basis of knowledge for problem solving in the future.