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Effect of double interspiking on fertility, behavior, and blood parameters in broiler breeder males reared under heat stress conditions

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To the Graduate Council:

I am submitting herewith a thesis written by Karianne Mei-Ying Chung entitled "Effect of double interspiking on fertility, behavior, and blood parameters in broiler breeder males reared under heat stress conditions." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Michael O. Smith, Major Professor

We have read this thesis and recommend its acceptance:

Henry G. Kattesh, Kelly R. Robbins

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A Thesis presented for the

Master of Science Degree

The University of Tennessee, Knoxville

Karianne Mei-Ying Chung

August 2010

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Dedication

This thesis is dedicated to my grandfather

who taught me the importance of lifelong learning.

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I would like to express my sincerest appreciation and gratitude to my major professor, Dr. Mike Smith for the opportunity to pursue a master's degree in Animal Science at the University of Tennessee. Through his constant support and exemplary mentorship, I have had the most amazing opportunities allowing me to grow both professionally and personally. I will always be grateful for the knowledge I have gained through his guidance.

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Abstract

Broiler breeders experience a natural decline in fertility levels as a flock ages. A male management practice such as double interspiking could be applied to counteract this decline. Our objective was to investigate the effects of double interspiking on flock fertility, testosterone concentrations, stress responses, and behavioral responses of broiler breeders in heat stressed environments. Two hundred and eighty-eight broiler breeder pullets and thirty-six broiler breeder roosters (Ross 708) were assigned to three groups at 21 weeks of age (WOA). All three groups were housed in slatted floor pens in which room temperature cycled from 23.8 to 30 C to mimic heat stressed environments. Double interspiking was carried out between two pens at 42 and 52 WOA. Beginning at 32 WOA, eggs were set every two weeks, candled at day 12, and percent fertility calculated. Concentrations of the hormones testosterone and corticosterone (CS) were measured by radioimmunoassays at specific time points in the experiment. Lymphocytes and heterophils were counted in order to calculate heterophil:lymphocyte (H:L) ratios. Behavior was monitored with Panasonic video cameras which recorded video footage using the Noldus MPEG Recorder from 1900 hr to 2100 hr at specific time points. Recordings were analyzed, and frequency of specific behavioral responses of individual (preening, feather ruffling, wing flapping), male-to-male (fighting, pecking, retreating), and male-to-female (attempted and completed mating) behaviors were assessed. The introduction of unfamiliar roosters resulted in a fertility increase ($P<0.01$) between control and spiked birds after the first interspike and a sustainment of fertility levels after the second interspike. Testosterone concentrations declined ($P<0.0001$)

from 1.95ng/mL to 0.11ng/mL as the birds aged. Corticosterone concentrations differed among bird groups and was elevated (P=.0333) for both groups that were interspiked. H:L ratios were also different among bird groups with all birds housed in the interspiked rooms showing higher ratios. Double interspiking increased the occurrence of male-to-male interactions, but there were no significant differences in male-to-female or individual behaviors. Regardless of treatment, there was a decrease in male-to-female behaviors as the birds aged. Double interspiking does show promise as a tool to increase fertility levels in a broiler breeder flock.

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Literature Review

Introduction

The poultry industry is mainly focused on the end products of two types of birds: meat yielding broilers and egg yielding layers. Much research has been done in order to obtain the highest yield of product possible - in these cases broiler meat and layer eggs. Broiler breeders, however, are unique in that they are both meat yielding and egg laying type birds. The main role for broiler breeders is to be an optimal producer of fertile broiler eggs while having large size and weight which can be passed on to their offspring. Due to this selection for higher body weight and the large musculoskeletal frame, broiler breeders have been affected by greater fertility problems (McDaniel et al., 1981; Hocking, 1990; McGary et al., 2003; Bilcik et al., 2005). When this intrinsic fertility problem is combined with the fertility decline that is associated with an aging flock (Sexton et al. 1989; McGary et al., 2002), it is easy to see that steps must be taken to counteract this fertility decline that affects broiler breeders throughout the world.

A common practice in industry is to apply a male management practice to an already established flock in order to counteract the decline in fertility levels. One practice that is most commonly used in industry today is known as spiking. This practice requires the introduction of young, unfamiliar, inexperienced birds into an already established flock. Not only does spiking involve the costly expense of maintaining and running a separate stud farm to house the young roosters, but also this practice could possibly cause larger biosecurity risks by introducing new disease-

causing organisms such as viruses into an already established poultry house. A second, less commonly researched male management practice is the use of double interspiking. This practice requires the exchange of experienced males between two different poultry houses located on the same farm at two different time points during the life of the flocks. This practice is not heavily investigated, but the concept is that the introduction of any new male, regardless of its age, will affect fertility levels of an established flock. Double interspiking would alleviate some of the problems that are associated with spiking such as eliminating the need for stud farms and preventing the introduction of immune-challenges into a poultry house.

The male management practice of double interspiking will likely improve fertility levels of a flock. One possible reason for this disruption is that the introduction of unfamiliar males into a flock will disrupt the pecking order causing an increase in sexual behavior. The abrupt change of a new environment to some roosters, and new individuals to a stable social group, will increase sexual behavior and compensate for loss in libido and mating interest of the older roosters (Casanovas, 2002). This experiment was conducted in order to investigate the use of double interspiking as a possible male management technique to use among broiler breeder houses located within a farm. The current experiment focused on monitoring changes in fertility levels in a flock, and testosterone concentrations, stress responses as measured by corticosterone concentrations and H:L ratios, and behavioral responses in broiler breeder roosters undergoing double interspiking in heat stressed environments.

Broiler Breeders

In the poultry industry, broiler breeders are selected for many different and diverse animal traits. Whereas broilers are selected for fast weight gain and layers are selected for large egg production, broilers breeders are selected to be both a high egg laying and a high meat yielding chicken. Therefore, genetic selection of broiler breeders is directed towards particular breeder traits which would involve not only excellent production of durable, sound, fertile eggs, but also efficient growth, feed conversion, and muscle growth. This balance between high weight gain and optimal reproductive success is very difficult to maintain as generally one trait always negatively impacts the other. Many researchers have found strong negative correlations between body weight gain and reproductive potential (Siegel, 1963; Wilson et al., 1979; Hocking and Duff, 1989). Even with this knowledge, the common practice has been to select for larger body size and faster weight gain regardless of its impact on the reproductive capabilities of these birds.

This selection for larger body size and faster gain have resulted in broiler breeders experiencing growing fertility problems (McDaniel et al., 1981; Hocking and Duff, 1989; Hocking, 1990; Dunnington et al., 1990; McGary et al., 2003; Bilcik et al., 2005) and lower duration of fertility (Dunnington et al., 1990), because of the large size and muscular composition of the breeder's body. As a result of this body composition, heavy roosters quite often experience low sperm mobility and the inability to perform completed matings that achieve cloacal contact with the breeder hens (Wolanski et al., 2004; Bilcik et al., 2005). In addition, heavier breeder roosters show low mating frequencies (Bilcik et al., 2005) and inefficient matings (Hocking, 1990) due to the large

breast muscle deposition (Hocking and Duff, 1989; Wilson, 2002; Wolanski et al., 2004) which negatively affects mating ability. Not only does the increased body size affect sexual activity of the broiler breeders, but heavier breeder parents also pass on poor quality traits to their offspring. In heavy broiler breeders, there was a higher frequency of embryonic death during the incubation period (Dunnington et al., 1990). Solely due to the nature of bird's body, broiler breeders experience detrimental effects on their reproductive successes regardless of management practices.

Production, Fertility, and Hatchability

With the continued genetic selection towards fast growth and large body size, a major problem that affects broiler breeder producers all over the world is fertility decline. It is widely known that fertility levels will naturally decline with age in a normal broiler breeder flock's production (Wood-Gush, 1958; Novikov, 1979; Kirk et al., 1980; Sexton et al., 1989; Weil et al., 1999; McGary et al., 2002). This decline poses a large economical threat to the producer of the breeder flocks. In a typical timeline of events, most birds become sexually mature at 22 weeks of age (WOA) due to the light stimulation and the transfer of birds from a pullet farm to a breeder farm. Breeder hens begin to lay eggs with relatively low fertility levels around 65-75% (Wilson, 2002). At this point, low fertility levels are believed to be a result of the sexual inexperience of the hens and the roosters. Eventually, when birds are sexually mature and sexually experienced, they will reach peak production and peak fertility and will produce a higher number of viable, fertile eggs. Some researchers state that optimal fertility is around 95-98% at 25 to 27 WOA (Wilson, 2002) while others suggest that fertility will peak at

90-96% at 32 to 37 WOA (Hocking, 1990; Weil et al., 1999; Casanovas, 2002; Végi et al., 2006). This apparent discrepancy could be due to strain and management differences. As flocks approach 40 WOA, fertility levels begin to decline, and this decline is usually attributed to a problem with the roosters (Casanovas, 2002; Wilson, 2002; Wolanski et al., 2004). The theory behind blaming the roosters for the infertility issue is found in the normal mating ratio between males and females in a breeder flock. In a normal flock setting, production guides recommend that one rooster is necessary for every eight to twelve hens. Therefore, the effects of only one infertile rooster will extend to a large population of the flock (eight to twelve hens) and could cause a greater detrimental impact on fertility levels (Wilson et al., 1979).

Several studies have been done investigating the main male reproductive hormone testosterone and its effects on fertility. Unexpectedly, in these studies, testosterone levels did not correlate well with fertility percentages (Jones and Mench, 1991; Brougher et al., 2005). However, just as fertility levels declined with age, testosterone levels were also seen to decline with age (Sexton et al., 1989; Weil et al., 1999; Hocking and Bernard, 2000). In these studies, plasma testosterone concentration was low prior to sexual maturity, increased and peaked during sexual maturity, and decreased as the birds aged. Concentrations were shown to peak at 30 WOA to 37 WOA (Weil et al., 1999) and fertility seemed to peak at similar times. Plasma testosterone is believed to decline as a result of testicular regression (Wolanski et al., 2004) and could also mimic the decline in libido that affects roosters (Duncan et al., 1990). This decline in libido was expected since levels of testosterone showed a slight correlation with sexual behavior (Brougher et al., 2005), the courtship behavior waltzing,

and the number of completed matings (Jones and Mench, 1991), which are essential for increased fertility. Although testosterone values did not differ significantly between two different breeds, it was found that within the same breed, testosterone values were quite variable among individuals (Jones and Mench, 1991). Within a flock, irrespective of breed, testosterone concentration was likely variable due to the different genetics of the individual birds.

Similar to testosterone, sperm concentration (Lake and Wood-Gush, 1956; Fiser and Chambers, 1981; Hocking and Bernard, 1997) and semen volume (Wood-Gush and Osborne, 1956; Fiser and Chambers, 1981) also showed a large variation between individuals within the same strain. This individual difference was likely caused by a genetic difference. Sperm concentration was shown to differ during the day with the greatest concentration found later in the day (Lake and Wood-Gush, 1956). Some studies showed that there were no correlations between bird age and volume of semen (Wilson et al., 1979; Hocking and Bernard, 1997), sperm motility (Bilcik et al., 2005), sperm concentration (Wilson et al., 1979; Hocking and Bernard, 1997), or dead sperm percentage (Wilson et al., 1979; Sexton et al., 1989). Other studies found that age had an effect on sperm concentration (Sexton et al., 1989; Hocking and Bernard, 1997; Casanovas, 2002), sperm production (Wilson et al., 1979; Hocking and Bernard, 1997), as well as on semen quality (Hocking, 1990; Casanovas, 2002). More importantly though, semen volume, sperm motility, and dead sperm percentage did not correlate well with fertility level (Wilson et al., 1979), but sperm concentration (Wilson et al., 1979; Bilcik et al., 2005) negatively affected fertility levels. These factors within the rooster are important to investigate since the males are blamed for the fertility problem that affects

broiler breeders. Another parameter that has been studied is sperm penetration ability, but this factor must be evaluated by inspection of a laid egg. Studies have found that fertility levels are highly positively correlated with the number of sperm penetrations or the inner perivitellin layer (IPVL) holes around the germinal disk (Bramwell et al., 1995; McDaniel et al., 1995; Bramwell et al., 1996; McGary et al., 2002; Barna et al., 2007). These sperm-egg penetration sites have proven to be very important since a low number of holes is related to low fertility levels. Sperm penetration has been noted as a better indicator of sperm viability, and fertility problems can be blamed on the inability of a sperm to fertilize the egg (Bramwell et al., 1995). In addition, age has been shown to be negatively correlated with sperm-egg penetrations (Bramwell et al., 1996; McGary et al., 2002; Barna et al., 2007). Sperm penetrations declined after peak production and the possibility of no sperm penetration increased greatly during the fertility decline (McGary et al., 2002).

Just like fertility levels, hatchability decreases towards the end of the life cycle (Fiser and Chambers, 1981; Creel et al., 1998; Hocking and Bernard, 2000; Barna et al., 2007). Although hatchability and fertility both decline and are low at the end of the production, there is no correlation between the two parameters (Hocking and Bernard, 2000). Hatchability is low initially around 27 to 29 WOA, peaks around 33 to 37 WOA, and will decline steadily until about 55 to 57 WOA (Creel et al. 1998; Hocking and Bernard, 2000). Kirk et al. (1980) reported declining hatchability levels as early as 44 WOA. Either way, it was recorded that hatchability is highest during peak production and the highest number of early deads are found at the beginning and end of a production cycle.

Unlike fertility levels, the age of roosters does not affect percent hatchability (Hocking and Bernard, 2000). Therefore, roosters are not blamed for low hatchability levels. Low hatchability in broiler breeders has been attributed to the interaction of the age of the hen and the storage time before incubation (Reis et al., 1997). For instance, it has been shown that eggs from older flocks had higher levels of hatchability when incubated right away. However, eggs from younger flocks showed similar levels of hatchability regardless of storage time. Lastly, the body composition of the hen could also affect hatchability, since Hocking (1990) found that body weight was negatively correlated with hatchability levels and early dead embryos.

Male Management Practices

Steps can be taken to positively influence fertility levels as birds age. Practices like artificial insemination and other male management techniques can be used to provide opportunities to maintain optimal fertility levels. Even though broiler breeders are challenged by declining fertility levels based on their genetic composition and age, actions can be taken to increase or maintain high fertility levels. Factors that could possibly affect the fertility decline in broiler breeders are male management techniques such as spiking and double interspiking.

Spiking

Roosters are usually blamed for the fertility decline generally seen in broiler breeders. Therefore, management practices have been directed towards fixing this male problem. The most popular male management practice of spiking aims to maintain optimal fertility percentages throughout the life of the flock. This practice

involves the replacement of male broiler breeders at a particular point in the growing cycle with younger, unfamiliar roosters. The objective of spiking is to introduce young roosters into an already established flock in order to achieve higher fertility levels and to increase mating activity among birds within a flock. Spiking is applied when fertility levels begin to decline due to old age of a flock. This usually occurs around 40-45 WOA when normal fertility decline begins to affect all poultry flocks. The young roosters are approximately 25-28 WOA and are raised on a separate stud farm (Casanovas, 2002; Wilson, 2002). In the stud farm, birds are reared and housed among other young males. The number of birds introduced into the flock replaces a percentage of the older roosters from the flock. Dependent on the conditions of the older roosters and the number of roosters that need to be culled due to illness or poor physical condition, approximately 2-33% of the population of older roosters are replaced by younger males during spiking (Casanovas, 2002). The flock is only spiked once throughout the lifetime of the flock since fertility levels should show improvement after one spiking. There should be a 5-10% increase in fertility for five to ten weeks following the spiking with fertility levels as high as 90% until 60 WOA (Wilson, 1999).

As a result of spiking, total sexual and mating activity increases among all males within a flock. Not only do the spiked roosters mate frequently, but also the older roosters increase mating frequency for approximately four to eight weeks (Casanovas, 2002). The increase in mating activity of the older roosters causes the total fertility levels of the flock to increase. Mating frequency for the younger roosters is also high. The difference is that younger roosters require four to six weeks to learn how to mate efficiently due to their sexual inexperience dealing with hens. When the mating

frequency of the older males begin to decline, the younger males will have gained the experience necessary to mate effectively with the hens and to continue in maintaining optimal fertility levels of the flock.

There are a few problems that are associated with the male management practice of spiking. With the use of birds from a stud farm, the most important drawback is the increase in the biosecurity risk for the already established flock. A stud house rooster could transfer parasites, viruses, or diseases to individuals within the established flock. Since animals are being brought into an already established group, the biosecurity risk is highly increased. In order to combat this security risk, blood tests can be analyzed in order to check for problems such as Salmonella and Campylobacter, but this does not protect the existing flock completely. Also, it is important to inspect the spike males for parasites such as mites, fleas, and ticks before introducing them to an established flock. This will increase labor needs and subsequently costs. A second problem for broiler breeder producers is the extra economic cost of maintaining and operating a separate stud farm in a separate facility to house the young roosters. In order to have individuals that are of the correct age to introduce into a flock, stud farms house all roosters of different ages within the same facility. It is important to maintain proper body weight, prevent injuries among the males, and familiarize roosters to breeder house equipment in order to introduce the most optimal birds into the breeder flock (Wilson, 1999). Thirdly, a stud farm is not an ideal location to house and rear young roosters. The stud farm does not provide roosters the opportunity to learn how to mate effectively with the hens. When young birds are first introduced into a breeder flock, they are sexually inexperienced and require a steep learning curve in which they

learn how to mate effectively and gain sexual experience. In addition, when sexually inexperienced roosters are introduced to a hen, roosters act aggressively towards the hen and do not engage in mating behavior. A certain period of time is required to replace the aggressive behavior with normal courtship and mating behaviors upon introduction (Wood-Gush, 1958). For young roosters, about four to six weeks is required in order to gain the experience they need to mate more efficiently with the hens resulting in successful sperm transfer and completed matings (Wilson, 1999; Végi et al., 2006).

A final problem with spiking is that there is a large increase in aggressive behavior between all roosters upon introduction of strangers to a flock. Not only did aggressive behavior increase in flocks that were spiked, but sexual interference increased the first week following spiking (Casanovas, 2002). Aggressive behavior should decline one week following the spike, but the degree of aggression is a cause for concern for breeder house owners (Casanovas, 2002). If aggressive behavior between males does not decline or if it is excessively violent, breeder farms could lose a large percentage of roosters in the breeder house as a result of death or injury since mortality among roosters increased three weeks following the spike (Casanovas, 2002). Although spiking has been noted to improve fertility levels in a flock, the disadvantages might outweigh the benefits to some broiler breeder producers.

Not all studies have shown as impressive results in fertility increases due to spiking the flocks. In a study that involved a 100% and a 50% spiking of a flock, the number of IPVL holes around the germinal disk decreased significantly regardless of

spiking (Barna et al., 2007). Although researchers reported a higher presence of IPVL holes during 100% spiking, fertility levels only increased slightly in two weeks and did not rise to peak fertility levels. For the 50% spike, the number of IPVL holes slightly increased, but two months were required in order to see any effect on fertility levels. It was concluded that spiking was not capable of improving the mating efficiency in either the 100% or 50% spikes (Barna et al., 2007). Other researchers saw a slight increase in sperm transfer with 17%, 20%, 25%, and 50% spiking (Végi et al., 2006). However, sperm transport increases were not significant from the control flock, and the different percentages of birds that were spiked did not affect sperm transport. Although this practice is used most often, it is possible that spiking does not provide as many benefits as producers expect.

Double Interspiking

Current industry practice recommends the use of spiking at 40 to 45 weeks of age to counteract fertility problems as the flock ages. A different male management practice that is not commonly used in the poultry industry today but one that could alleviate the problems caused by spiking, is double interspiking. This practice is fairly new and has not been investigated thoroughly, but double interspiking has been proposed as a possible alternative way to improve fertility levels of a flock. The objective of double interspiking is to swap roosters from one existing flock into another existing flock of the same age on the same farm at two different time periods in order to maintain optimal fertility levels in a breeder flock. The first interspike of roosters is performed around 40 to 45 WOA because, at that time, normal fertility declines as a

result of age. A second interspike (hence the name double interspiking) is recommended once fertility levels and mating activity begin to decline again. Double interspiking should improve fertility levels fairly quickly due to the roosters' familiarity to a similar housing system as well as because of their previous mating experience.

As a result of double interspiking, just like when spiking is used, sexual activity should increase among males in a flock and an improvement in fertility levels should occur. Due to the first interspike and the transfer of males between the two houses, mating frequency will increase for four to eight weeks among all males (Wilson, 2002). All roosters – those newly introduced into the flock and those resident birds that were not moved between flocks – are experienced breeders and do not require extra time to learn how to mate effectively. They also do not require a large acclimation period since houses within a farm are fairly similar in set-up and design. However, fertility levels will decline again due to the bird's age and lowered interest levels; when this happens, another interspike must occur in order to increase fertility levels until the end of the life of the flock. This second transfer occurs around 50 weeks of age when fertility and mating levels begin to decline a second time, and different males should be used in order to better disrupt the established social hierarchies (Casanovas, 2002). This study showed that frequency of mating behavior increased for five to six weeks. Hatchability levels also increased following interspikings and lasted for a total of 15 weeks. Lastly, higher levels of aggressive behavior and higher levels of sexual interferences were expressed the two weeks following both interspikings (Casanovas, 2002).

The theory behind the male management practice of double interspiking is that fertility levels will improve as the introduction of males will disrupt the established pecking order causing an increase in sexual behavior. Initial mating activity will increase when birds are transferred and introduced into a new social hierarchy. This change in location and social group will cause an increase in mating behavior thereby compensating for the loss in libido and mating interest of older roosters (Casanovas, 2002). Also, biosecurity risks should be eliminated because the males are transferred within two houses on the same farm and not recruited from a separate stud farm. Double interspiking eliminates the need to obtain males from a different farm and this eliminates the introduction of outside disease causing organisms into an already established breeder house.

Poultry Behavior

With the newfound interest on animal welfare, much attention has been directed towards the behavioral responses of individual animals and the possible use of these responses to explain emotional states of a chicken. Normal chicken behavior is believed to be directed by internal factors within the chicken's body which are explained in terms of behavioral needs (Duncan, 1998). These behavioral needs must be expressed despite the presence of any harsh environmental factors or external stimuli.

One of the most important characteristics of a flock of chickens is that they are social animals. Chickens are housed together in big groups and are able to establish a fairly stable social hierarchy within a flock. This hierarchy is called a pecking order and results in dominant and subordinate individuals within a flock. These hierarchy tiers are

gender independent resulting in separate social hierarchies. Roosters are passively dominant over the hens (Allee et al., 1939; Guhl, 1949; Kruijt, 1964; Ylander and Craig, 1980; Rushen, 1983; Queiroz and Cromberg, 2006) with the lowest ranking rooster having a higher ranking status than the highest ranking hen (Kruijt, 1964). The rooster's higher social dominance allows for them to passively dominate the hens in normal social environments without the use of aggressive acts like pecking. The pecking order requires about a week to be established, and once established, it is very important in dictating all social interactions among individuals within a flock (Gross and Siegel, 1973). Instead of performing aggressive acts, demonstrations of threatening by dominant birds and submission and avoidance by subordinate birds are expressed (Allee et al., 1939; Kruijt, 1964; Queiroz and Cromberg, 2006).

In order for these social hierarchies to be maintained, individual recognition is required and it is important for birds to be able to recognize their flock mates and decipher dominance statuses. In a study by Guhl and Ortman (1953), recognition among birds was tested in order to see what types of changes and patterns would affect identification of individuals. With the use of young White Leghorn pullets, researchers changed the contour and color of some of the feathers on the bird's body in order to see the other bird's reactions upon reentry of the altered bird. Whether adding plumage to the body, placing colors on the animal, denuding feathers, or changing contours, there was not a loss in recognition of the individual. This ability to recognize and identify a flock mate even with an altered appearance is what helps to maintain the stable pecking order within a flock. Individual recognition among chickens is believed to be a learned ability rather than an inherited characteristic (Ramsay, 1951). Secondly, regardless of

any changes to their appearance, a chicken will act based on habit. The altered chicken will know its place within the pecking order. If unrecognized when introduced back into a flock with an altered appearance, the individual will instinctively avoid any dominant birds and fight with any inferior bird that attempts to challenge its position within the pecking order (Guhl and Ortman, 1953). Lastly, chickens have the ability to determine dominant or submissive statuses for individuals without engaging in conflict themselves. For instance, after seeing a dominant flock mate being defeated by a stranger, it is very unlikely that a subordinate individual will fight the stranger, and if a stranger is defeated by the dominant member, the subordinate individual will initiate attack 50% of the time and will dominate the stranger 50% of the time (Hogue et al., 1996). Upon seeing this conflict settlement, a stranger bird will avoid the dominant individual and will confront the submissive individual in order to establish its place in the pecking order. Chickens have been recorded to have a strong pecking order and this is a result of the individual's ability to identify their flock mates and to learn and remember their social statuses within the social hierarchy.

Male-to-Male Interactions

In order to limit death and injury of birds within a flock due to fighting and aggression, the pecking order established among males is the most important in preventing unnecessary fights among individuals. Aggressive behavior begins to develop as early as one WOA in chicks (Kruijt, 1964), but once the pecking order is established, unnecessary male-to-male interactions and conflicts among roosters in a flock are prevented as a result of the pecking order. Dominant individuals play an

important role in the pecking order since less aggressive behavior will occur if a dominant bird is within close proximity (Bshary and Lamprecht, 1994). Therefore, in a normal flock, antagonistic behaviors like fighting and pecking will decline. However, broiler breeder males have been reported to display higher levels of aggressive behavior in comparison to laying strain males (Millman et al., 2000) with a higher frequency of antagonistic interactions between roosters within a flock (Bilcik et al., 2005). This type of behavior shown by broiler breeders towards individuals of the same sex occurs even though the pecking order has already been established. The highest level of aggressive behavior has been expressed from the dominant males towards the subordinate roosters in the flock (Rushen, 1983). One of the challenges associated with increased aggression among roosters in a flock is that more time is spent on male-to-male interactions instead of male-to-female interactions. With this increase in aggressive behavior among roosters, there is a decline in all expression of reproductive behavior which is important to maintain optimal fertility levels in broiler breeders (McGary et al., 2003). Certain male-to-male behaviors like chasing, pecking, fighting, or jumping on another rooster have been reported in broiler breeder roosters (Millman et al., 2000).

Certain aggressive behaviors occur more frequently within male-to-male interactions. Especially when a new individual is introduced into the same pen, both fighting and pecking will occur until the dominance and subordinance statuses have been determined and the pecking order established (Guhl, 1968). Fighting behavior occurs when individuals stand opposite of each other with feathers raised in a threatening manner. Fighting generally begins when one individual leaps at the other

opponent with feet raised. The bird seems to kick the opponent with wings flapping. This action usually provokes the other individual to engage in a forward lunge with its feet raised or to attempt to retreat from the fight. Pecking is described as a movement of the beak towards the head or body of the opponent individual. This behavior is first displayed in chicks at around ten days old, but does not become a part of the aggressive behavior displays until after three weeks of age (Kruijt, 1964). There are different degrees of severity to the pecks. Pecks could be so severe that blood is drawn from the comb or feathers plucked, or pecks could be light and not harm the opponent (Allee et al., 1939). These behaviors are expressed at high frequencies and high intensities until newly introduced individuals have found their position within the social order. The introduced individual is very disadvantaged in terms of gaining in social status. They are likely to be attacked immediately by dominant individuals upon introduction (Kruijt, 1964), and regardless of their size, the stranger bird is less likely to win a fight against a bird in its home pen (Wood-Gush, 1958). Pecking orders can even be skewed if a group of birds attack the newcomer together thereby each individual in the group establishing dominance over the newcomer.

Antagonistic behavior is believed to be influenced by the androgen testosterone. In one study, injections of testosterone propionate, a hormone with similar properties as the male androgen testosterone, resulted in an increase in the level of aggressive behavior of an individual. This increase in aggressive behavior can in turn allow for a rise in social status within the pecking order as subordinate individuals were able to gain a higher social status within the flock (Allee et al., 1939; Queiroz and Cromberg, 2006). This also is possible when hens are injected with testosterone. After injection, the hens'

demeanor was much more aggressive allowing them to revolt against the dominant hens (Guhl, 1968). This revolt allowed the hens to move up in rankings and move up on the social hierarchy. In both cases, when testosterone was circulating at higher levels, the behavior of the individuals was more aggressive.

Male-to-Female Interactions

The male-to-female interactions are the most important behaviors expressed, because this sexual behavior is what allows for reproductive success. Because hierarchy tiers are gender independent and roosters only passively dominate over hens, aggressive behavior from roosters towards hens is normally infrequent (Craig and Bhagwat, 1974; Ylander and Craig, 1980; Bshary and Lamprecht, 1994). In mature domestic chickens, this type of behavior is considered aberrant behavior, since male-to-female interactions are usually classified as sexually motivated or sexual interactions (Millman et al., 2000). Aberrant behavior includes sexual aggression and aggressive pecking of the hen among others. According to reported observations, broiler breeder roosters exhibit higher levels of aggressive behavior towards hens than are seen in broiler stock or laying strains (Rushen, 1983; Millman et al., 2000; Millman and Duncan, 2000; McGary et al., 2003; Bilcik et al., 2005; Moyle et al., 2010). Due to this aberrant behavior, hens seem fearful of the roosters in the pens because of the constant pecking and chasing by the roosters. Their reaction is then one of avoidance of the males, and because of this, the male-to-female aggression increases forced matings with females. Broiler breeder males attempt to mount hens (willing or not) and were usually unsuccessful in completing a mounting of a hen. In one study, at time of mixing at

approximately 20 WOA, roosters displayed a high expression of aggressive behavior and forced sexual behavior from males towards the females (De Jong et al., 2009). However, even when mating more frequently, roosters were incapable of completing a mating due to the hen escaping or the inability to achieve cloacal contact. The social hierarchy of the hens can also indirectly disrupt the males' mating behavior. Although the hierarchy of hens is less stable, if a hen is highly dominant within the group, she will not display the sexual crouch as often as subordinate hens. This was concluded due to the negative correlation that occurred between dominance status and mating behavior of hens (Guhl, 1950). Lastly, broiler breeder roosters displayed very little courtship behavior to the hens in comparison to a laying stain (Bilcik et al., 2005). This generally results in the absence of courtship behavior and broiler breeder hens rarely approaching roosters for mating.

To explain this unusual male to female antagonistic behavior, the possible frustration of being sexually rejected by hens could cause an increase in rooster's aggressive behavior towards hens. This display of aggression could be the attempt of roosters to intimidate hens into being more receptive to mating with the roosters (Moyle et al., 2010). This behavior could also be a result of the inability for broiler breeder roosters to mate effectively with hens since unsuccessful mounts were seen more frequently in large broiler breeders (Millman and Duncan, 2000). Due to the large body size, some roosters might be unable to achieve successful cloacal contact during mating. Reports of the frequency of expression of sexual displays of heavier roosters have been inconsistent. Duncan et al. (1990) observed that heavier roosters showed higher expression of sexual displays and more mating attempts than their lighter flock

mates whereas, in contrast, McGary et al. (2003) found a lower expression of reproductive behavior frequencies by the heavier breeders.

The status within the pecking order that the birds occupy affects their subsequent fertility and mating activity. Dominance status can be positively correlated to fertility levels (Jones and Mench, 1991) and higher expression of mating activity and sexual behavior (Guhl and Warren, 1946; Craig and Bhagwat, 1974; Jones and Mench, 1991). The number of offspring that the rooster parented was also correlated to the dominance status of the rooster (Wood-Gush and Osborne, 1956; Guhl, 1968; Jones and Mench, 1991). Dominant males were seen to have more successful matings, more egg fertilization, and a larger number of offspring sired. Between a dominant individual and his subordinate, it was sometimes seen that the dominant individual would interfere when his subordinate was mating possibly causing many incomplete matings without sperm transfer (Guhl and Warren, 1946). Sometimes aggressive acts were even performed causing comb lesions on the subordinate individual. However, it was also seen that dominant males were likely to have a larger body size due to their dominant status during feeding. These heavier dominant males were unable to mate effectively which caused a possible decline in fertility levels (Hocking, 1990). Wood-Gush (1960) and Rushen (1983) did not find any strong relationships between the dominance status of an individual and the mating behavior expressed, but it was found that the subordinate roosters showed higher levels of aggressive behavior towards hens. Frequency of matings and cloacal contacts were displayed higher in groups with one rooster than those with three roosters as a result of the male-to-male competition for hens (Bilcik and Estevez, 2005). When a rooster was moved into a group with a lower

number of males, their expression of mating activity increased due to the lack of competition in the new group. When a rooster was transferred into a group with a higher number of males, their expression of mating activities decreased. In this situation, individuals did not show a high mating frequency mainly due to the fact that there was more male-to-male competition over the hens.

Since some studies have reported that the sperm produced by aged roosters still have a high potential to fertilize a hen's egg, male mating behavior is believed to be one of the most important factors that affect fertility levels (Casanovas, 2002). Behavioral factors could possibly correlate with the reproductive success of the roosters in a flock. McGary et al. (2003) found a positive correlation of total reproductive behavior and fertility levels in one strain that they investigated which indicates that the expression of this type of behavior can indicate fertility levels. Guhl and Warren (1946) also found a slight relationship with the number of eggs fertilized to the number of times a rooster mounted a hen. The expression of all types of sexual behavior decline as the rooster ages (Craig and Bhagwat, 1974; Duncan et al., 1990; Hocking and Bernard, 2000; McGary et al., 2003; Wolanski et al., 2004). Younger broiler breeders show high levels of mating activity, but activity still declined with age. The frequency of younger roosters' mating activity was observed to be as much as twice as frequent as in older chickens (Craig and Bhagwat, 1974; Hocking and Bernard, 2000). Although frequency is decreased, there were no differences detected in completed matings by the two age groups. Other researchers have not been able to find a correlation between sexual behavior and fertility levels (Bilcik et al., 2005; Duncan et al., 1990). Fertility levels do decline around 38 to 58 WOA, but a relationship between fertility and sexual behavior

was not found. It has been noted that the majority of all sexual activity and mating frequency occur at the end of the light phase (Lake and Wood-Gush, 1956; Pizzari and Birkhead, 2001; Bilcik et al., 2005; Bilcik and Estevez, 2005). A study found that 70-75% of all mating activity occurred during the last four hours of light in a pen (Casanovas, 2002). This is in agreement with Craig and Bhagwat (1974) who noted that mating activity occurred later in the day. More specifically, reproductive behaviors (matings, forced mating, and cloacal contacts) peaked from 19:00 hr to 21:00 hr and decreased until lights were out at 22:00 hr (Bilcik and Estevez, 2005).

Individual Behavior

In terms of individual type behaviors, chickens will express certain behaviors as a means of maintenance of self. These behaviors are not normally motivated by social relationships, and these behavioral needs will occur regardless of external stimuli.

Wing flapping is a behavior that was characterized under the activity of stretching (Kruijt, 1964). This behavior occurs when one or both wings are outstretched, lifted, and flapped above the back. Wing flapping is also believed to be a type of behavior that announces and displays the bird's presence to the flock mates. Feather ruffling is a maintenance type behavior that is an individual's response to peripheral stimulation (Duncan, 1998). To express this behavior, feathers of the neck, head, and body are raised and moved in a shaking manner. The combination of shaking neck, head, and body feathers is recorded as early at two day of age, but the entire body combination of neck, head, body, and tail feather ruffling is not seen until five weeks of age in chickens (Kruijt, 1964). Feather ruffling is sometimes seen in combination with wing flapping with

wing flapping occurring after feather ruffling. Preening behavior is associated with rearranging feather plumage that are located at the breast or wing area. This behavior is recognized as pecking or stroking of the feathers with the individual's beak (Kruijt, 1964). This behavior is designated as comfort behavior because it is done when birds are mildly frustrated or are involved in conflict (Kruijt, 1964; Bshary and Lamprecht, 1994; Duncan, 1998). When birds were frustrated with a particular situation like feed withdrawal, birds' preens were shorter in time duration, and were preformed towards a location that was closer to the bird's beak (Duncan and Wood-Gush, 1972). However, although duration and location were different, expression frequency of preens did not differ regardless of the frustration of this external stimulus. In comparison to other activities, there is usually very little space between birds when individuals are engaged in preening (Keeling and Duncan, 1991).

Secondary Sexual Characteristics

Secondary sexual characteristics have been studied because it is possible that they are indicators of the overall health or reproductive status of a rooster (Hamilton and Zuk, 1982). The theory behind this is that size, color, and asymmetry of these secondary characteristics can imply either good or poor male quality. In terms of the practical use for the rooster, the comb is important in terms of heat loss. In heat stress conditions, the use of the comb to dissipate heat from the bird's body is of utter importance. However, the comb can provide other benefits as well.

Secondary sexual characteristics are believed to be used by females in order to select their mates. This is a result of the effects of testosterone level on the size of the

comb of the rooster. When birds were injected with testosterone propionate, the comb will begin to grow immediately. Therefore, comb size can be used as an indicator of the amount of androgens circulating within a rooster's body (Allee et al., 1939; Verhulst et al., 1999). Studies by McGary et al. (2003) and Bilcik et al. (2005) found that the expression of reproductive behavior can be positively correlated with a large comb size. It is also the case that comb area can be correlated with the fertility of a strain (McGary et al., 2002). The larger comb size correlated with fertile males in addition to sperm penetration and testis weight. Therefore, it is not surprising that when initially paired, hens find roosters with larger combs to be more attractive (Bilcik et al., 2005). This might also extend to the wattle as well since Bilcik and Estevez (2005) found a correlation with wattle width and sperm motility in roosters.

It has been noted that comb size plays a role within the initial establishment of the pecking order among chickens. Since identification is so important in a pecking order, Guhl and Ortman (1953) researched the impact of modifications of an individual's external appearance on their dominance status. Their study concluded that modifications to the head and neck regions resulted in a greater loss in recognition. Therefore, all characteristics from the head and neck like the comb and wattle are very important in terms of maintenance of the social hierarchy. For instance, individuals with larger combs expressed higher levels of aggressive behavior and were more dominant than smaller combed roosters (Allee et al., 1939; Bilcik et al., 2005). Larger combs have become an indication of a high ranking individual. If an individual is confronted by a stranger, roosters with the large combs were usually more likely to win during a conflict against a rooster with a smaller comb (Allee et al., 1939; Guhl and Ortman,

1953). The larger comb gave the individual an added advantage in the initial encounter. When a rooster's comb was increased or decreased in size, reactions to that individual with a larger comb mimicked that if they were a dominant individual (Guhl and Ortman, 1953).

These external features are sometimes referred to as a burden. The down side to the large comb is that not only does it requires greater energy expenditure for the bird to carry this weight, but also it is the area of the body most prone to injury or disease (Verhulst et al., 1999). In addition, a few researchers have not found any beneficial effects due to the secondary sexual characteristics. The frequency of sexual activity was generally not correlated to the size of either the comb (Wood-Gush and Osborne, 1956) or the wattle (Bilcik and Estevez, 2005). When a rooster was dubbed and the entire comb removed, mating activity did not appear to change in frequency either. Fertility was not seen to be correlated with the wattle width (Bilcik and Estevez, 2005), and secondary sexual characteristics didn't seem to play a role in terms of reproductive success either (Wolanski et al., 2004; Bilcik et al., 2005).

Stress

General Stress

Stress is normally defined as a non-specific response to any demand on the body (Selye, 1976). This stress can either be a result of an external or internal stressor. The effects of the stressor on the individual are also different dependent on duration of exposure (i.e. acute or chronic stress). Stress is usually believed to negatively affect an animal (distress) and cause detrimental effects on its health and well-being. However,

some researchers have concluded that a certain amount of stress is important (eustress) and even necessary in order to get optimal performance from birds (Gross and Siegel, 1981; Zulkifli and Siegel, 1995). This stress is important in terms of immunity defense against certain bacterial infections that affect chickens (Gross and Colmano, 1971; Gross and Siegel, 1973). However, too many stressors will have a detrimental effect on the overall health, development, growth, lifespan, and reproduction of the individual with chronically elevated stress hormones being even more harmful to the individual (Gross and Siegel, 1973; Mumma et al., 2006). Researchers have found that in addition to body weight decline, weight of lymphoid organs like the thymus, spleen, and bursa of Fabricius decreased at a rate that was inversely proportional to the amount of stress hormone circulating in the body (Siegel, 1980; Post et al., 2003; Vahdatpour et al., 2009). Due to these effects, research has been conducted on the main stress hormone of avian species corticosterone (CS), and it has been investigated in order to measure stress levels of an animal.

Upon detection of a stressor stimulus, certain physiological changes occur within a chicken's body which indicates stress levels in chickens. When a stressor stimulus is detected, the hypothalamic-pituitary-adrenal (HPA) axis is stimulated. At the onset of the stress response, the hypothalamus secretes corticotrophin-releasing factor (CRF) which then stimulates the anterior pituitary gland to release adrenocorticotropic hormone (ACTH). ACTH stimulates the adrenal cortex to secrete the main glucocorticoid in avian species CS. This adrenocortical hormone is released for circulation in the blood and signals a stressor. The stress response allows the bird to benefit with an increase in glucose availability through the use of body reserves.

Although this is the normal pattern for CS secretion, avian species are unlike mammals in that the hypothalamus does not have as much control over the pituitary gland and the anterior pituitary does not have as much control over the adrenal gland (Siegel, 1980). Regardless of the reduced control of the hypothalamus and pituitary gland, the adrenal gland will still release CS when stimulated by a stressor.

To determine if the HPA axis is relevant in avian species, studies were performed in which chickens were treated with ACTH. With the addition of ACTH, plasma CS levels did increase which follows the HPA axis mechanism (Gross and Colmano, 1971; Beuving and Vonder, 1978; Guémené et al., 2006; Mumma et al., 2006). Upon infusion of ACTH, corticosterone levels were shown to become elevated at different rates. Puvadolpirod and Thaxton (2000) found an increase in CS levels six hours post-implant and the concentration increased until six days post-implant while Beuving and Vonder (1978) found CS concentrations to increase within a few minutes of the ACTH injection. When CS was added to the feed, plasma CS concentrations were elevated one hour post treatment and remained elevated for 24 hours (Shini et al., 2008). Corticosterone levels did revert back to baseline levels when birds were measured ten days later (Shini et al., 2008). Being the final stress hormone of the HPA axis, corticosterone has been the parameter studied when considering stress in chickens.

Without the effect of stress on an animal, the plasma CS concentration follows a circadian rhythm with higher concentrations measured during light periods and lower concentrations measured during dark periods (De Jong et al., 2001). When cannulas were placed in broiler breeder birds, the researchers measured the concentration

differences every four hours in birds housed in 8 hr light: 16 hr dark day environments. During the dark periods, CS concentrations remained relatively low and there were no differences in CS concentrations. During periods of light, CS concentrations increased for the first four hours, but this change was not significant. Right before lights went out, plasma CS significantly declined to a lower concentration. During periods when an external stressor is applied to the animal, the rise in CS concentration is a good indicator to explain and measure acute stress (Gross and Siegel, 1983; Davis et al., 2000; Turkyilmaz, 2008; Bedanova et al., 2007). Chickens, in the presence of an acute stressors such as beak trimming (Davis et al., 2004), shackling (Guemene et al., 2006; Bedanova et al., 2007), manual restraint (Korte et al., 1997), or feed withdrawal (Braw-Tal et al., 2004), will show an increase in CS levels over time. Levels of these detrimental effects are dependent on the level and the time duration of the stressor.

Whereas corticosterone is believed to be a better indicator for chickens undergoing acute stress, the heterophil:lymphocyte (H:L) ratio is seen to be a less variable measurement of acute stress and a better indicator of chronic stress conditions (Gross and Siegel, 1983; Maxwell, 1993; Siegel, 1995; Davis et al., 2000; Vakili and Akbarogli, 2006; Bedanova et al., 2007). The H:L ratio has shown to be a heritable trait in chickens (Al-Murrani et al., 1997; Campo and Davila, 2002a) with lymphocytes having a higher count than heterophils normally. The H:L ratio usually ranges around 0.4, but this ratio can increase to as high as 8 among birds in great stress (Bedanova et al., 2007). This ratio is a calculation of the leukocytes heterophils and lymphocytes which circulate in the blood. An increase in the ratio equates to the presence of a stressor. This parameter was studied by Gross and Siegel (1983) in which chickens were fed

different levels of CS in their feed. Both H:L ratios and CS levels increased as a result of the concentrations of CS in the feed, but the H:L ratio was the parameter that was able to explain differences between the different groups of birds fed different levels of CS. The H:L ratio was also capable of explaining the differences between the two strains of chickens that were genetically designed to have a high or low CS response. In a different study by McFarlane and Curtis (1989), the researchers put chicks through multiple stressors such as beak trimming, electric shock, heat stress, and aerial ammonia. Corticosterone levels were sometimes undetectable at the end of the trial period, but H:L ratios were always elevated. The changes in leukocyte numbers in response to many types of stressors were less variable than the glucocorticoid CS. The H:L ratios showed more capability in explaining long-term changes. Even though H:L has been indicated as the better measurement for chronic stress, H:L ratio is affected by acute stressors too. When an acute stressor like shackling was applied to chickens, researchers saw an increase in the heterophil count, a constant lymphocyte count, and an elevated H:L ratio in the shackled animals in comparison to the control birds when birds were sampled 20 hours later (Bedanova et al., 2007). Regardless, the H:L ratio has been a measurement that has been used to study chronic stressors and the ratio increases in the presence of these stressors.

Variations in the H:L ratio in chickens is seen relative to sex, age, housing ratio, and total flock size. In regards to sex, the H:L ratio is variable since roosters had a higher number of heterophils and a lower numbers of lymphocytes circulating in the blood than the hens did. This would mean that roosters have a higher H:L ratio than hens regardless if a stressor is applied or not. With age, H:L ratio increased throughout

the life of the flock. The H:L ratio of the rooster was low at 20 WOA but steadily increased as the birds aged and was significantly higher at the last sampling at 36 WOA (Campo and Davila, 2002a). In hens, mean H:L ratios increased with age and peaked at 64 WOA during molting (Davis et al., 2000). Another factor that affected the H:L ratio is the housing ratio of hens to roosters in a flock. Hens housed in one rooster to one hen mating-ratio groups showed higher levels of H:L in comparison to groups that were housed one rooster to 11 hens (Campo and Davila, 2002b). The hens showed signs of both increased heterophil and decreased lymphocyte counts in the one to one mating ratio groups resulting in a higher H:L ratio. Interestingly, these changes in mating ratios did not have any significant effects on the H:L ratio, heterophil counts, or lymphocyte counts of the roosters. There was a trend for increased heterophil and decreased lymphocyte counts, but this change was not significant. When group sizes were tested, hens showed increased heterophils and decreased lymphocytes if birds were housed in a group size of 60 instead of a group size of 12 (Campo and Davila, 2002b). Roosters did not show significant changes in heterophil counts, lymphocyte counts, or the H:L ratio when housed in the larger group of 60 in comparison to 16. Although not significant, the trend was towards heterophilia and lymphopenia.

In response to stressors or to CS circulating levels, heterophils will increase result in heterophilia (Siegel, 1980; Gross and Siegel, 1983; Siegel, 1995; Davis et al., 2000; Campo and Davila, 2002b; Post et al., 2003; Shini et al. 2008) and lymphocytes will decrease due to lymphopenia (Siegel, 1980; Gross and Siegel, 1983; Campo and Davila, 2002b; Shini et al., 2008). In addition to mature heterophils, there is an increase in circulation of immature heterophils which are due to the increased release of these

cells from the bone marrow (Shini et al., 2008). Some researchers have found a sole increase in heterophil frequency without any effects on other leukocytes and have proposed that heterophil frequency be used as a better indicator of stress levels due to the rapid increase of heterophil counts (Post et al., 2003). However, the general trend is to look at the less variable parameter of H:L ratios instead of just heterophil counts. The amount of time necessary to see changes in H:L ratios have been fairly variable. Some studies have shown that H:L ratios are not affected within the first 24 hours, but will show an elevation on the second day and remain elevated for eight days post-stressor (Puvadolpirod and Thaxton, 2000). Other studies have seen a rapid increase in H:L ratios which continued to rise for 24 hours and did not return to baseline values until ten days later (Shini et al., 2008).

Heat Stress

The housing of broiler breeders in heat stressed environments has many detrimental effects on the health and fertility of the flock. Broiler breeder producers in the Southeast United States and those in tropical climates need to address this issue due to the large costs associated with keeping birds healthy and productive during times of extreme heat. The normal body temperature for a chicken is 41 C. The zone of thermoneutrality for chickens is between 21 to 24 C, and this temperature range is where birds show optimal health and performance. Variations to this temperature will cause a decline in health and welfare of the bird. If body temperatures reach 47 C, there is a high mortality rate among the flock as this is a lethal temperature for birds. With high temperatures, poultry producers see reduced food consumption, decreased

body weight gain, lowered egg production, fertility and hatchability problems, poor sperm-egg penetration, reduced semen quality, increased water consumption, decrease in physical activity, increased mortality, and higher disease susceptibility in broiler breeders (Sands and Smith, 1999; Singh, 1999; Bartlett and Smith, 2003).

Chickens are highly susceptible to heat stress. With any rise in temperature, the chicken's body reacts as if it is exposed to a heat stressor. Chickens that are exposed to acute heat stress show an increase in their blood CS concentration (Beuving and Vonder, 1978; Siegel, 1980; Guémené et al., 2006; Turkyilmaz, 2008). When birds were sampled after being exposed to 2.5 hours of high heat (45 C), plasma CS levels rose quickly with a peak occurring only after one hour of exposure (Siegel, 1980). High CS levels did not remain elevated and did begin to decline. In addition to the rise in CS levels, the H:L ratio also increases during times of heat stress as long as stress levels are not extreme (Maxwell, 1993). During heat stress, the increased body temperature leads to rapid changes in the leukocytes composition in avian blood. When chickens are exposed to heat stressed conditions, the calculated H:L ratios are increased which denotes that the birds were environmental stressed (Al-Murrani et al., 1997; Altan et al., 2000; Zulkifli et al., 2003; Turkyilmaz, 2008). An increase in body temperature resulted in an increase in heterophil counts and a decrease in lymphocyte count which led to a final elevated H:L ratio. The H:L ratio was shown to rise within three hours when exposed to elevated temperatures of 32 C for a one hour period (Zulkifli et al., 2003)

In addition to the stress parameters, heat stress plays a major role on the fertility issue of broiler breeders. Already fertility declines with age, but fertility problems are

only increased in heat-stressed birds. When heat is involved, fertility was significantly lowered (McDaniel et al., 1995; McDaniel et al., 1996; Singh, 1999). An analysis of semen from heat stressed roosters in comparison to control roosters showed that semen volume, sperm packed cell volume, and dead sperm were not significantly different when exposed to heat stress conditions (McDaniel et al., 1995). However, sperm penetration was decreased by 48% when sperm was used from males housed in 27 C instead of 21 C (McDaniel et al., 1996). When semen was exposed to higher temperatures of 32 C, sperm penetration, uterovaginal sperm storage, and fertility decreased even further. When the sperm from these roosters housed at 32 C was inseminated into broiler breeder hens, the sperm penetration was decreased by about 67% whether the hen was in a heat stressed environment or not (McDaniel et al., 1995). This decline in IPVL holes due to the lower sperm penetration numbers could explain the increased fertility decline in the summer months since IPVL holes are highly correlated with fertility. With the removal of heat, levels of sperm penetration began to return to control levels. However, when sperm from males exposed to heat stress were used to inseminate hens, 60% less sperm were stored in the sperm storage tubules of the uterovaginal junction in comparison to sperm from control males (McDaniel et al., 1996). This decrease was recovered within two weeks once heat was removed from the pens. Researchers found a positive correlation of sperm storage and sperm motility and a negative correlation of these two traits with male rectal body temperature. Regardless of the duration or temperature of the heat stress, exposure to a heat stressed environment exacerbated the decline in fertility levels. In contrast, when

broiler breeder roosters are exposed to elevated heat conditions, hatchability levels are not affected (McDaniel et al., 1996).

Conclusion

Fertility issues affect all broiler breeders around the world. Whether due to age or to the breeder's propensity towards body growth, it is important to study the cause of the fertility decline among broiler breeders. Instead of applying the male management practice of spiking to the flock, a second, less-frequently investigated, male management practice of double interspiking will be studied in hopes that it can provide a way to increase the fertility levels that decline when birds age and remove the problems that are associated with spiking. The experiment described in the following pages investigated the effects of double interspiking on fertility levels of the flock and on endocrine functions, stress responses, and behavior of broiler breeder roosters that are reared under heat stress conditions.

Materials and Methods

Birds and Housing

All animal procedures were reviewed and approved by the University of Tennessee Animal Care and Use Committee. In this study, a total of 324 Ross 708 broiler breeder chickens (288 females, 36 males) were obtained from a Pilgrim's Pride Corporation farm in Alabama. All chickens were 21 weeks old upon arrival. Chickens were separated into three different pens each containing 96 females and 12 males in order to maintain an eight to one female to male ratio. Each pen was a 12X20 ft enclosure area and was housed in individual temperature controlled rooms in the Johnson Animal Research and Teaching Unit (JARTU) at the East Tennessee Research and Education Center. Each room was on a 16 hour light: 8 hour dark schedule. Lights were set on an automatic timer and were turned on at 0600 hr and were turned off at 2200 hr. The environmental temperature cycled from a low of 23.9 C to a high of 30 C in order to simulate a heat-stressed environment. Birds were exposed to nine hours of 23.9 C, four hours of 23.9 to 30 C, two hours of 30 C, and nine hours of 30 to 23.9 C (Figure 1). Chickens were raised in accordance with the Ross 708 Parent Stock Performance Objectives commercial management program guide. Males and females were fed age-appropriate feed in sex separate feeders. The hens' feeders had metal bars across the top and openings too small for the entry of a rooster's head. The roosters were fed in a separate feeder that hung approximately 20" above the ground which was too high for the hens to access. This height was adjusted as the roosters grew. A representative number of birds (ten males and ten females) in each pen were

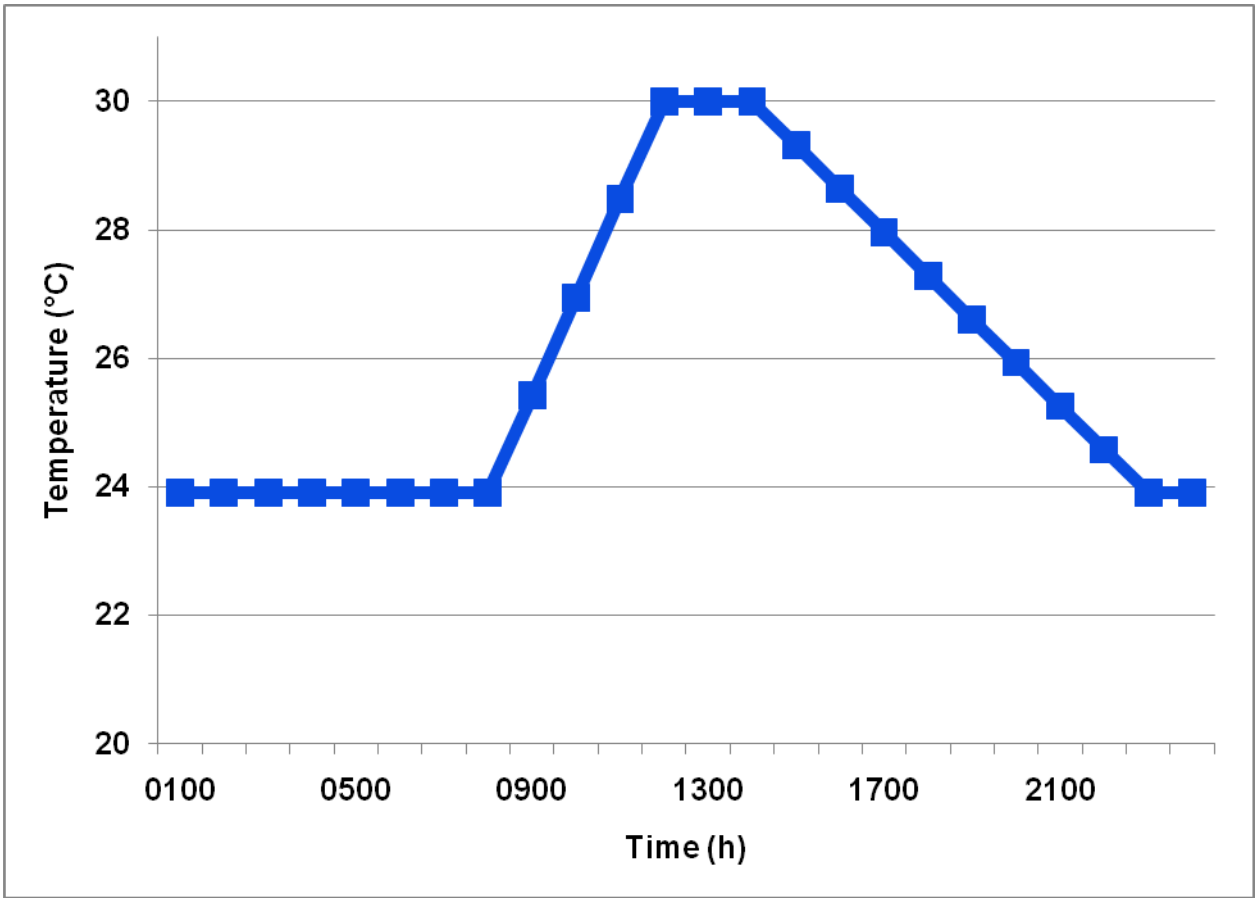

Figure 1. The daily temperature cycle for all pens.

Room temperature cycled from 23.8 to 30 C to mimic heat stressed environments.

weighed weekly in order to assess and record body weight and size. Feed amounts were adjusted weekly in order to achieve target body weight and optimal production as published in the breeding company manual. Feed amounts were calculated weekly based on the commercial management guide. Cup drinkers were used to provide water ad libitum.

All roosters were identified with numbered colored bird identification tags (Heartland Tag, LLC, Fair Play, MO, USA). Birds were tagged in two locations: one on the back of the neck and one on the wing's web in order to prevent misidentification of the birds if one tag was lost. An applicator tool, nylon fasteners, and tags were used to apply the identification tags. To attach the tag, ½ inch of skin on the specified area on the rooster was pinched between two fingers. The needle from the applicator tool was inserted through the skin, and the tool's handle was squeezed. Once the tag was secure, the needle was removed from the bird's skin. This procedure left a tag along with a nylon fastener attached to the rooster. Birds were also identified by color in order to distinguish individuals on the video footage. Randomly selected, birds were painted with colored lines along the dorsal side of the bird. Pink, blue, orange, and green Prima Tech Twist-up Livestock markers (Prima Tech, Kenansville, NC, USA) were used to draw the horizontal lines across the chicken's back. All roosters had either one line located at the back of the neck, two lines located at the back of the neck and shoulders, or three lines located at the back of the neck, shoulders, and tail regions. Both identification procedures were applied to all roosters in all three rooms.

Double Interspiking

Double interspiking involves the transfer of roosters between two pens at two different time points. In this experiment double interspiking was applied when birds were 42 and 52 weeks of age. Pen one and pen two were double interspiked, while pen three served as the control pen and therefore was not disturbed. At both 42 and 52 weeks of age, twenty-five percent of the rooster population was transferred between pen one and pen two. Of the total 12 roosters in the pens, three roosters were transferred from pen one into pen two, and three roosters were transferred from pen two into pen one (Figure 2). At 52 weeks of age, three roosters that were not previously transferred in the first interspike were transferred from pen one into pen two and three roosters with corresponding colors were transferred from pen two into pen one. Birds that were identified with the same color pattern were transferred in order to allow for easy identification of the birds within each pen.

Fertility and Hatchability

At the start of lay, total counts of eggs laid per pen were recorded in order to calculate percent egg production with the formula % Egg Production = (Total Eggs Laid/Number of Hens)*100. Of these eggs, a representative number of eggs were set every two weeks in order to track fertility levels throughout the experiment. Since each pen had a population of 96 hens, 72 eggs were set from each of the rooms every two weeks. Eggs were cleaned with emery paper and candled to check for internal cracks, blood spots, or floating air cells so that only good quality, sound eggs were set. Eggs were set into labeled plastic incubator egg flats, and all egg flats from all three pens

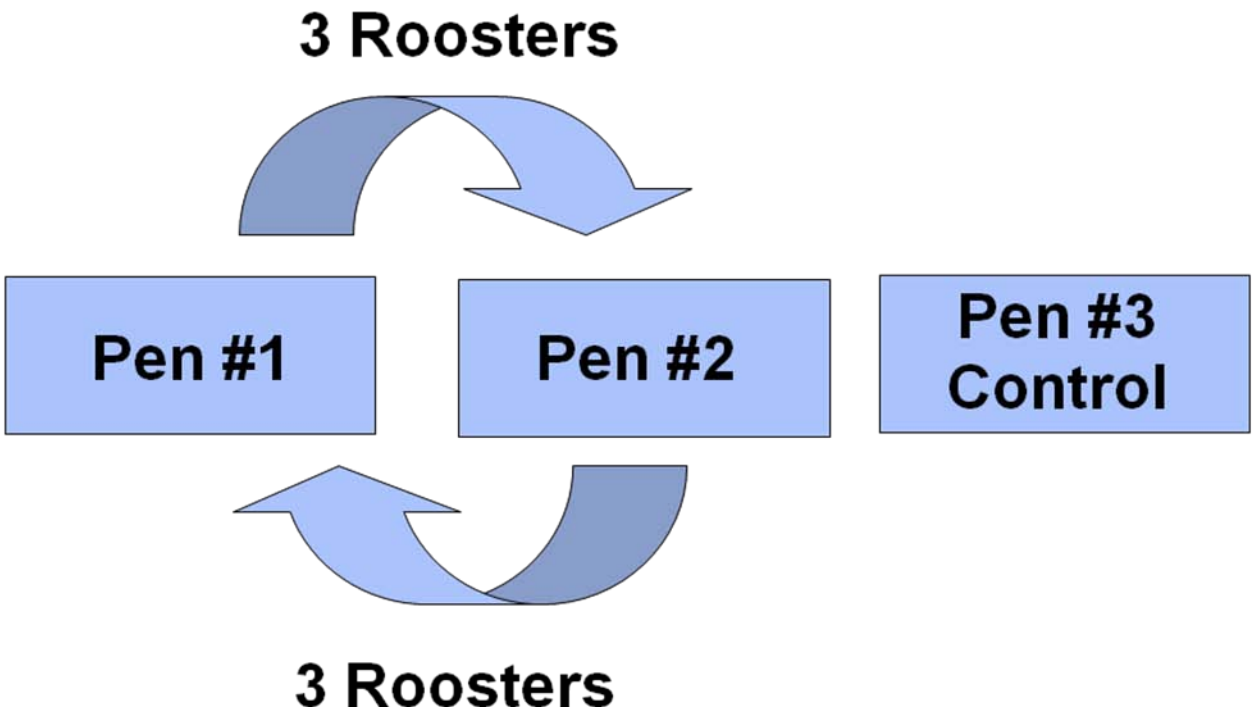

Figure 2. The male management technique of double interspiking performed at 42 and 52 weeks of age.

Twenty-five percent of the rooster population was transferred during interspiked weeks.

placed into a Jamesway single-stage incubator (Jamesway Incubator Company Inc., Cambridge, Ontario, Canada). The setter was equipped to turn eggs and maintained proper temperature and humidity levels. On the 12th day of the incubation cycle, eggs were candled in order to determine if the egg was fertile or not. This number was then used to calculate the percent fertility of the flock with the equation % Fertility = ((Total Eggs Set-Infertile Eggs)/Total Eggs Set)*100. Hatchability was determined at specific time points in the experiment: Pre-interspike 1 (32 WOA), Week of Interspike 1 (42 WOA), Post Interspike 1 /Pre Interspike 2 (44 WOA), Week of Interspike 2 (52 WOA), Post Interspike 2 (54 WOA), and End of Productive Life (58 WOA). The eggs that were set during these weeks to test for fertility levels were incubated for the remainder of the 21 day period. At day 21, chicks were counted, and those eggs that did not hatch were broken out to determine early or late deads. Hatchability was calculated using the equation % Hatchability = (Number of Hatched Chicks/Fertile Eggs)*100.

Behavior

In order to test for and monitor behavior, a Panasonic Color CCTV video camera was installed in each of the rooms. Cameras were mounted to the wall and were set to capture all areas of the enclosed pen. When the cameras were connected to the computer, the Noldus MPEG Recorder program v. 2.1 (Noldus, Wageningen, the Netherlands) previewed the video footage that was captured by the video cameras for all three pens simultaneously. Video was captured from 1900 hr to 2100 hr at specific time points in the experiment: Pre-interspike 1 (41 WOA), Day of Interspike 1 (42 WOA), Post Interspike 1 (44 WOA), Pre Interspike 2 (51 WOA), Day of Interspike 2 (52 WOA),

Post Interspike 2 (54 WOA), End of Productive Life (62 WOA). This duration of time for video recording was chosen because studies have shown that 70% of all mating activity occurs within the last four hours of light in a day with a peak in mating behavior occurring later in the day (Duncan et al., 1990) and more specifically one to two hours prior to when lights go out (Bilcik and Estevez, 2005). The software was programmed to save each recording of the rooms into its own individual file on the computer for future analysis.

The Noldus Observer XT 8.0 program was used to analyze all of the behavior interactions of the roosters by viewing each individual pen one at a time in order to capture each individual rooster's behaviors. The Noldus Observer XT program allowed for replay of the video files captured from the Noldus MPEG Recorder. Each video file was first uploaded into the computer program, and coding schemes created for subjects and behaviors. Behaviors were mutually exclusive since different behaviors could not occur at the same time for a specific individual. As videos were observed, behaviors were scored on the program. Most attention was paid to the frequency of interactions. These were categorized as male-to-male, male-to-female, and individual behaviors. Male-to-male behaviors represented antagonistic and aggressive behaviors between two roosters. These behaviors included fighting, pecking, and retreating. Pecking represented the action of one male pecking another male with his beak with a clear reaction from the other rooster. This usually was done as a downward stroke towards the head or dorsal region of the attacked bird. Fighting represented the action of one rooster lunging or flying towards another rooster with his claws with the intention to fight and with a clear reaction from the attacked rooster. Retreating represents the sudden

avoidance of a rooster eliciting a reaction of the aggressor rooster to chase or attack. Behaviors were only recorded if these actions elicited a response from both the aggressor and the attacked roosters. This was done in order to avoid possible investigatory behaviors like feather pecking. Male-to-female behaviors represented the sexual behavior that occurred between a rooster and a hen. Completed and attempted matings were recorded. Completed mating was recorded if a male correctly mounted a hen for a sufficient period of time in order to allow for cloacal contact between the two individuals. Attempted mating was recorded when males showed interest in mating with the hens but did not achieve cloacal contact. This could result if a rooster forcefully pecked the hen's head with an unsuccessful attempt to mount due to the hen's escape, or if mounting did not lead to cloacal contact due to another male's interference or due to the individual's inability to mate effectively. Individual behaviors represented maintenance type behaviors and included preening, wing flapping, and feather ruffling. Preening behavior was associated with any contact with the bird's beak to its own feathers on any part of its body resulting in a rearrangement of the bird's feathers. Wing flapping required outstretch of one or both of the rooster's wings associated with a hearty flapping of the outstretched wing(s). Feather ruffling represented the raising of feathers from the bird's body with a type of rocking motion. Frequencies of each of the individual behaviors were marked per individual, and frequency of each interaction was analyzed. Behavior was reported as frequency of behavioral events/rooster/two hour period.

Secondary Sexual Characteristics

The width and height of the combs and the wattles were measured on all roosters at specific time points of the experiment: Measurements were taken close to peak production (38 WOA), around fertility decline (42 WOA), and at the end of the life cycle (62 WOA). Value were recorded, and comb area and wattle area were calculated by the formula area=width*height.

Blood Sampling

All roosters were bled at specific time points of the experiment: Pre-interspike 1 (32 WOA), Day of Interspike 1 (42 WOA), Post Interspike 1 /Pre-Interspike 2 (44 WOA), Day of Interspike 2 (52 WOA), Post Interspike 2 (54 WOA), End of Productive Life (62 WOA). On day of blood sampling, all roosters from each pen were placed in a small portable pen. Individual roosters were randomly selected and restrained. Each bird was placed on its side on a table with one wing outstretched. Feathers surrounding the brachial vein were plucked for clear visibility of the vein. Heparin-coated syringes were used to collect blood samples from the brachial vein. Blood was transferred from the syringe into sodium – heparinized coated tubes, and the samples then packed on ice until all blood samples were collected. Samples were centrifuged using a Beckman Model TF6 centrifuge (Beckman Coulter, Inc., Brea, CA, USA) for 20 minutes at 6X1000 rpm. After centrifugation, plasma was pipetted into polypropylene tubes in two aliquots and stored for later analysis of CS and testosterone concentration. Plasma samples were stored at -20 C until time for analysis. Additionally, blood smears were completed for cell differentiation on day of sample collection. Two 3”X1”X1mm Fisherbrand

microscope glass slides (Fisher Scientific, Inc., Pittsburgh, PA, USA) were labeled per individual rooster. Ten uL of blood was pipetted onto one end of each of the two glass slides. One end of a separate slide was placed on the blood, and a smear was made by sliding the edge along the entire length of the slide. Duplicate blood smears were made per individual and were left overnight to dry. Slides were stored at room temperature until staining and analysis. Blood samples were analyzed for plasma CS and testosterone concentrations, and blood smears were analyzed for H:L ratios.

Radioimmunoassays

Total plasma testosterone concentrations were measured using a Coat-a-Count™ assay kit (Diagnostic Products Corp., Los Angeles, CA, USA). Frozen plasma samples for the testosterone analyses were thawed at room temperature and were vortexed with the Eppendorf Centrifuge (Eppendorf AG, Hamburg, Germany) for eight minutes at 7X1000 rpm. Tubes were consecutively numbered. Samples were analyzed in duplicates within each assay. Radioimmunoassay kit instructions were followed directly. Tubes were counted on the Perkin Elmer 1470 Automatic Gamma Counter (PerkinElmer, Hebron, KY, USA). Intra- and inter- assay CV was 22.3 and 9.9% respectively for low (0.2 ng/mL) and 4.0 and 3.9% respectively for high (4.0 ng/mL) testosterone standards.

Total Plasma CS concentrations were analyzed in duplicate with the commercial ImmuChem™ Double Antibody Corticosterone ^{125}I Corticosterone RIA kit (MP Biomedicals, LLC, Orangeburg, NY, USA) as described by Cheng et al. (2001) for use with chicken plasma. Frozen plasma samples were thawed at room temperature and

vortexed with the Eppendorf Centrifuge (Eppendorf AG, Hamburg, Germany) for eight minutes at 7X1000 rpm. All other radioimmunoassay kit procedures were followed directly. Precipitates were counted on the Perkin Elmer 1470 Automatic Gamma Counter (PerkinElmer, Hebron, KY, USA). Intra- and inter-assay CVs were 17.4 and 11.6% respectively for low (5.2ng/mL) and 10.6 and 24.7% respectively for high (31.2ng/mL) corticosterone standards.

H:L Ratio

In order to prepare the slides for cell differentiation, smears were stained with the PROTOCOL Hema 3 Staining System (Thermo Fisher Scientific Inc., Kalamazoo, MI, USA). Slides were placed on plastic racks and submerged into different solutions (fixation and staining) which stain slides comparable to the Wright-Giemsa method. Cells were then counted using a light microscope, and heterophils and lymphocytes identified (Gross and Siegel, 1983). One hundred granular (heterophils, eosinophils, and basophils) and nongranular (lymphocytes and monocytes) leukocytes were counted on two slides per bird. Values were recorded, and H: L ratios were calculated by dividing the heterophil count by the lymphocyte count in order to determine the H:L ratio.

Experimental Design and Statistical Analysis

Egg production, fertility and hatchability data was conducted as a completely randomized design with the number of roosters housed within the pen as a covariate. The treatment was either the spiked or the control flock.

Behavioral data was analyzed as a completely randomized design. Treatments were control, resident, 1st interspiked, and 2nd interspiked groups. Behaviors were separated by male-to-male dominance, male-to-female sexual, and individual maintenance behavior.

Secondary sex characteristics, testosterone, corticosterone, and H:L ratio data were analyzed as a completely randomized design with repeated measures over time. Treatments were the four different groups of birds: control, resident, 1st interspiked, and 2nd interspiked. Control represents those birds that were housed in the control pen. Resident represents those birds housed in the interspiked pens that were not switched due to the double interspiking. First interspiked represents those birds that were switched at 42 WOA for the first swap. Second interspiking represents those birds that were switched at 52 WOA for the second swap. The initial testosterone and initial corticosterone values were used as covariates for the testosterone and CS analyses respectively.

Correlations were run with the behavior types, secondary sex characteristics, testosterone levels, CS concentrations, and H:L ratio values.

All data were analyzed using the mixed model analysis of variance (ANOVA) procedure of SAS 9.1 (SAS Institute, Cary, NC, USA). The least square means were compared and the treatment differences evaluated in order to determine significance which was set at a value of $P<0.05$.

Results

Egg Production, Fertility, and Hatchability

Egg production declined towards the end of the production life cycle (Figure 3). At 32 WOA, egg production was 84.4%, and this was the highest level of egg production achieved in these flocks (Table 1). Production declined gradually until the end of the life of the flock at 62 WOA. At this time point, egg production was measured at 39.3%.

Fertility levels decreased as the flocks aged regardless of male management practice (Figure 4). This decline was expected and was consistent with common observations in poultry production. Fertility which was 88.2% at 32 WOA, reached a peak of 93.9% at 34 WOA, and then declined to approximately 38.9% at 62 WOA (Table 1). Fertility levels declined (P<.0001) gradually but showed significant differences from the start of lay to the end of the life of the flock. Double interspiking was applied to two of the pens at 42 and 52 WOA. Following the first interspike at 42 WOA, there was an increase (P<.0001) in the fertility levels of the interspiked flocks for the four following weeks with an increased trend towards higher levels of fertility until the second interspike at 52 WOA (Figure 5). At 44 and 46 WOA, fertility was higher (P=.0085) in the interspiked flocks in comparison to the control flock. After the second interspike at 52 WOA, there was a sustainment of the fertility levels of the spiked flock in comparison to the control flock when the flocks were 54, 56, and 58 WOA as differences in fertility measurement between the interspiked flocks and the control flock were not significant. Towards the end of the flock's productive life around60 and 62 WOA, fertility levels of the control flock were higher (P=.0085) than that of the spiked

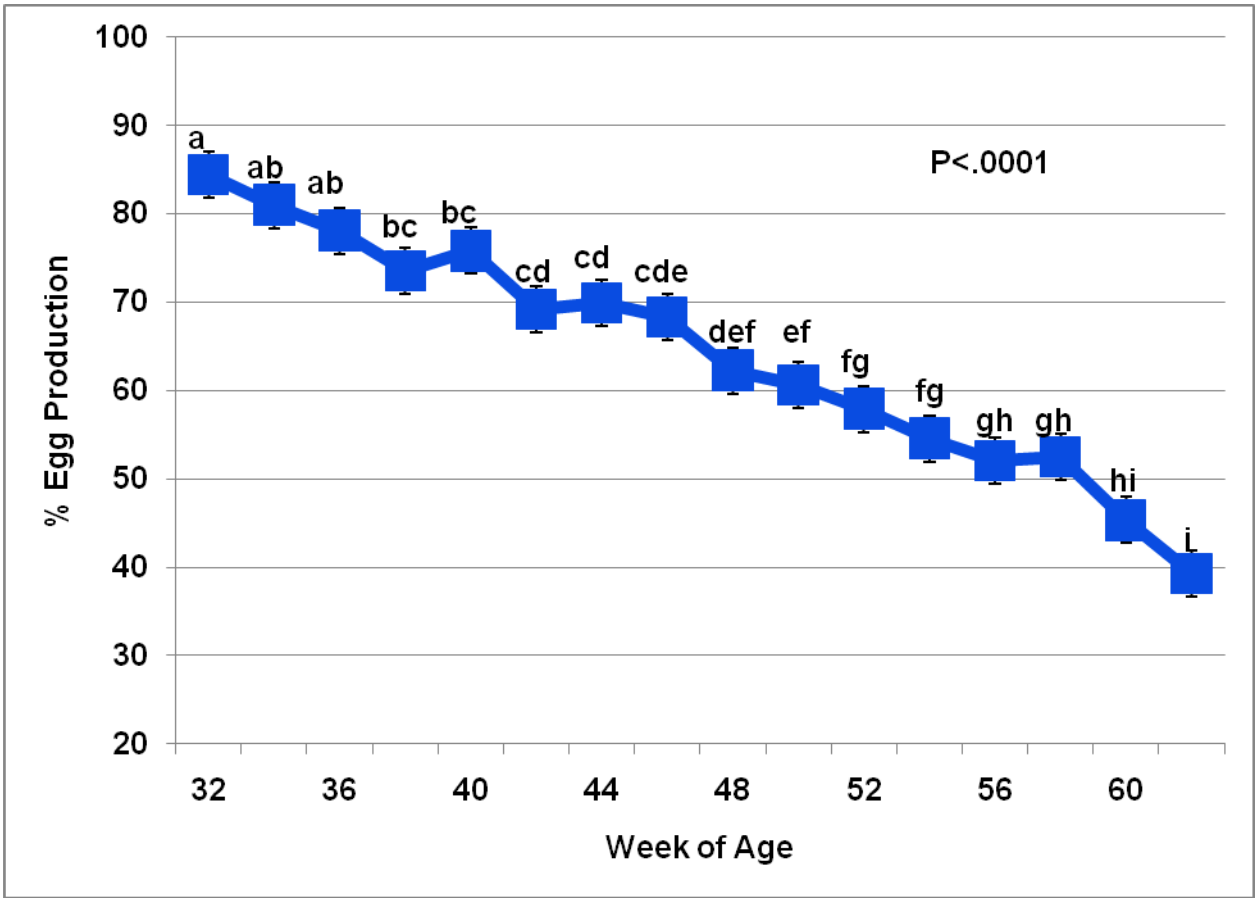

Figure 3. Hen day egg production.

Mean percentages of egg production across sixteen observation periods for all flocks. Values are expressed as mean ± SE. Weekly percent egg production means with different letters differ (P<.05).

Table 1. Least squares means for egg production, fertility, and hatchability.[1]

Week of Age	% Egg Production	% Fertility	% Hatchability
32	84.4[a]	88.2[ab]	98.3[ab]
34	81.0[ab]	93.9[a]	-
36	78.1[ab]	89.4[a]	-
38	73.5[bc]	92.7[a]	-
40	75.8[bc]	92.7[a]	-
42	69.1[cd]	85.3[ab]	98.2[a]
44	69.9[cd]	76.2[bc]	90.1[b]
46	68.3[cde]	72.6[c]	-
48	62.3[def]	65.7[c]	-
50	60.6[ef]	66.0[c]	-
52	57.9[fg]	67.7[c]	97.4[a]
54	54.6[fg]	53.1[d]	98.3[a]
56	52.1[gh]	43.1[de]	-
58	52.4[gh]	38.7[e]	94.9[ab]
60	45.3[hi]	47.5[de]	-
62	39.3[i]	38.9[e]	-

[1]Means within a column with different letters differ (P<.05)

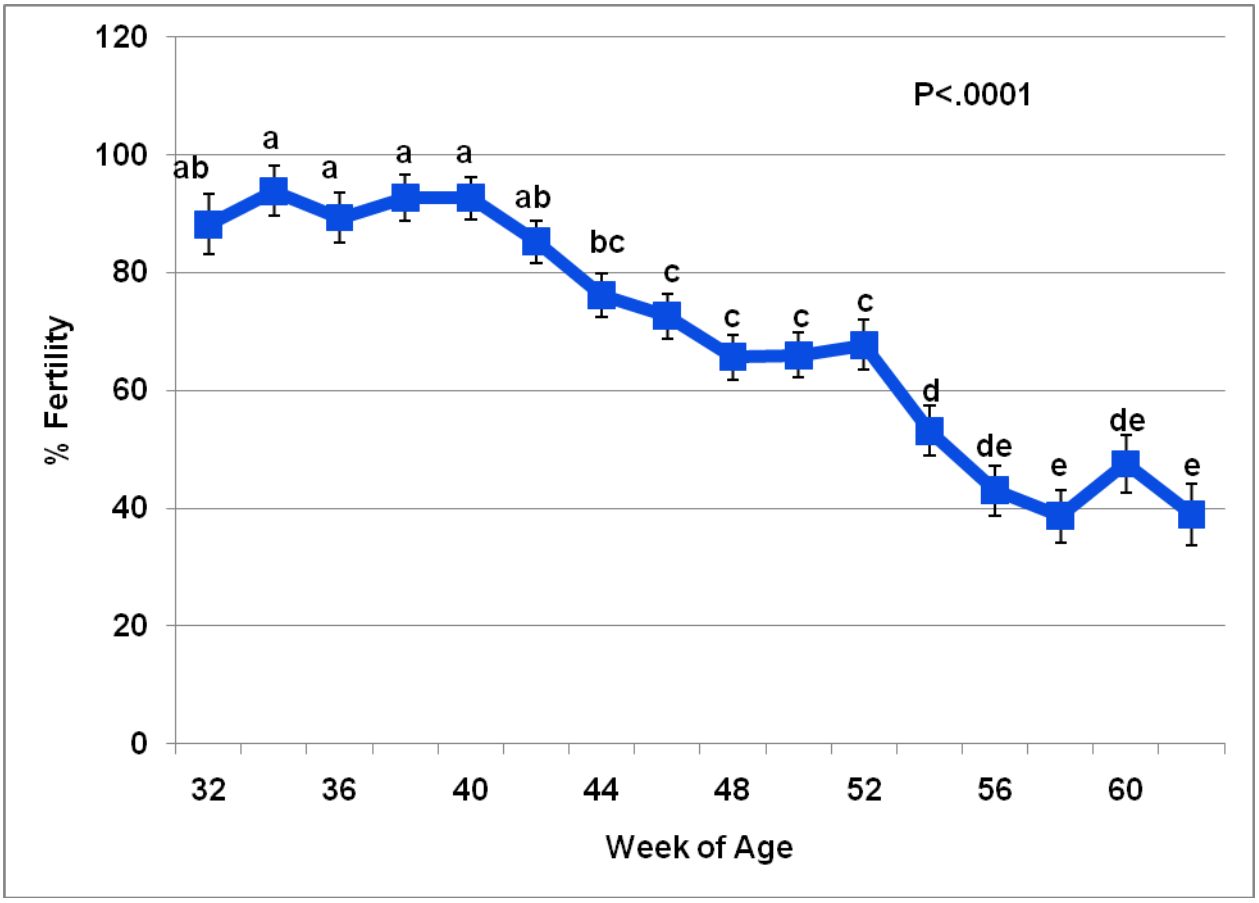

Figure 4. Age related changes in fertility.

Mean percentage fertility of eggs that were set across sixteen observation periods for all flocks. Values are expressed as mean ± SE. Weekly fertility means with different letters differ (P<.05).

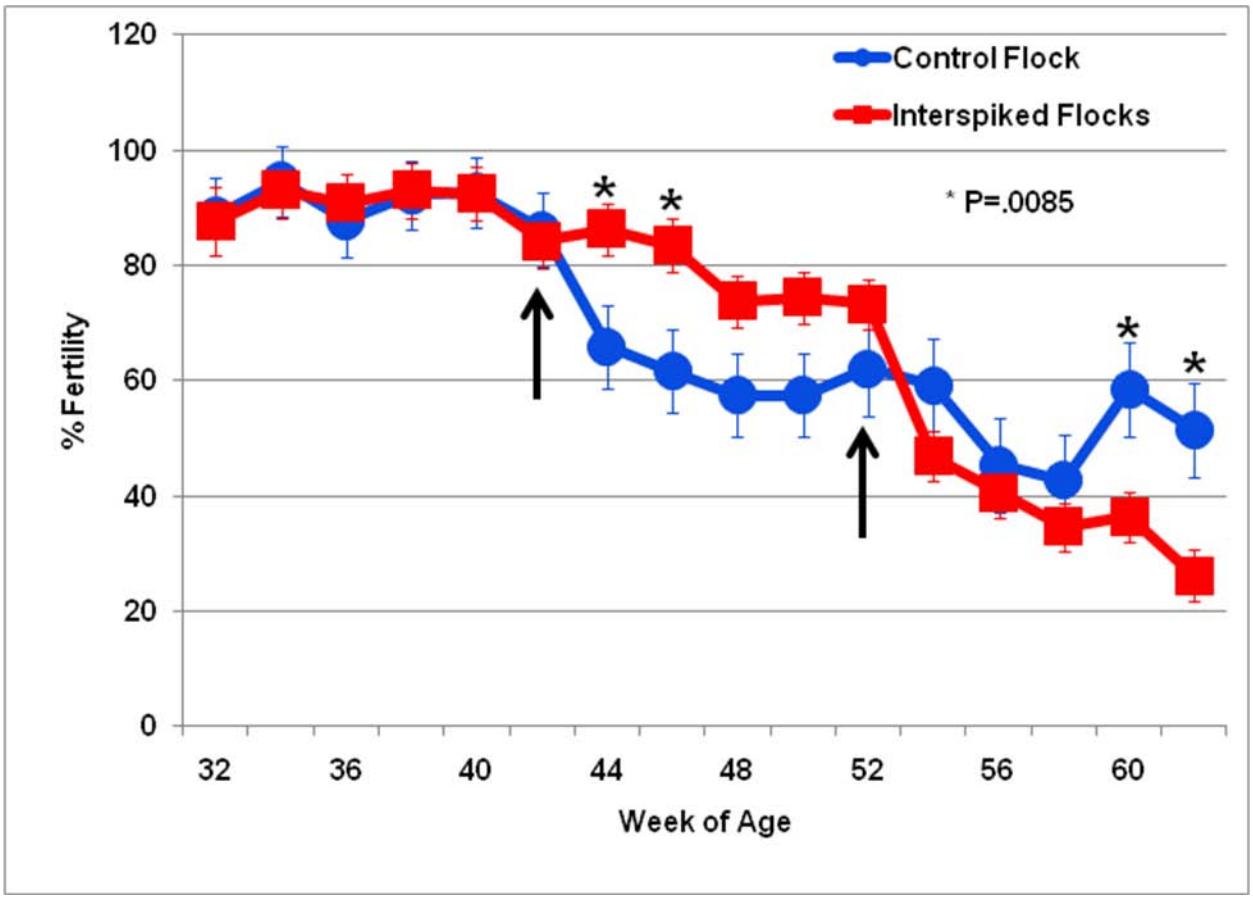

Figure 5. Age related changes in fertility between interspiked and control groups. Mean percentage fertility of eggs that were set across sixteen observation periods for all flocks. Values are expressed as mean ± SE. Arrows represent dates when double interspiking occurred. Within a graph, (*) denotes differences between the control flock and the interspiked flock at a given age (P<.05).

flock.

Hatchability fluctuated during the life of the flock (Figure 6). At peak production (32 WOA), hatchability of fertile eggs was highest at 98.3% (Table 1). Hatchability remained consistent at this level until 44WOA when it declined to approximately 90.1% (P<.0001). However, at 52 WOA, hatchability levels increased to 97.4% which was significantly elevated from the previous measurement at 44WOA. This level remained elevated until the end of the productive life of the flock. There were no significant differences between the different groups of birds in terms of hatchability.

Behavior

During the two weeks that the flocks were interspiked, there was a significant increase in total male-to-male dominance (fighting, pecking, and retreating) behaviors at 42 and 52 WOA (Figure 7). At weeks 42 and 52, there was a higher frequency (P<.0001) of male-to-male behavior expressed in comparison to this type of behavior at 41, 44, 51, 54, and 62 WOA (Table 2). When male-to-male behavior was analyzed for differences between bird groups, the second group of birds that were interspiked displayed significantly different (P=.0015) behavioral patterns than the other three groups (Figure 8). The birds interspiked at 52 WOA also showed greater expression (P=.0015) of male-to-male behaviors in comparison to all of the other groups with birds experiencing a higher level of aggression at this time. The groups of birds that were interspiked at 42 WOA and those that were not moved (termed Resident) during either interspike showed a trend for increased expression of aggressive behavior in comparison to the control group (Figure 8).

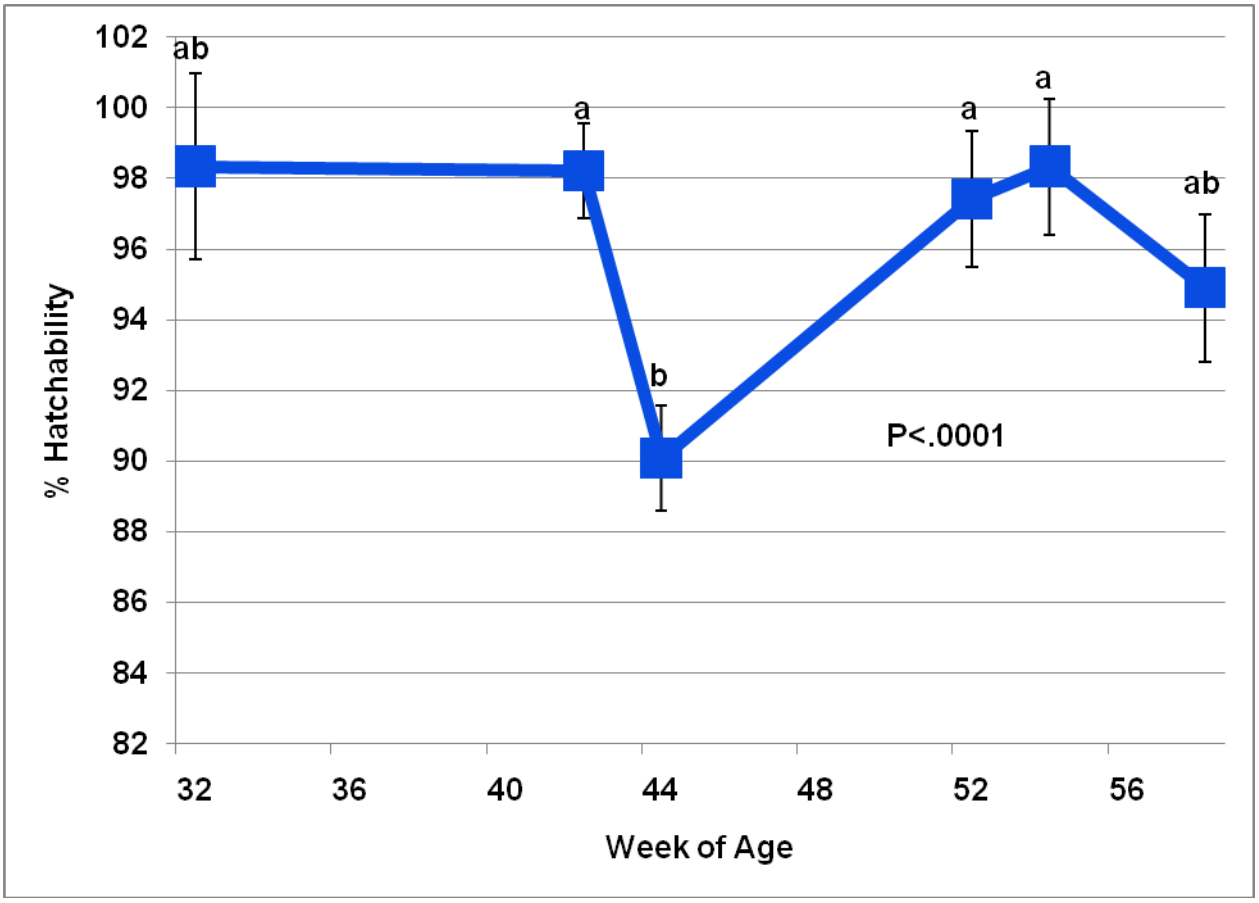

Figure 6. Age related changes in hatchability.

Mean percentage hatchability of eggs that were set across six observation periods for all flocks. Values are expressed as mean ± SE. Weekly hatchability means with different letters differ (P<.05).

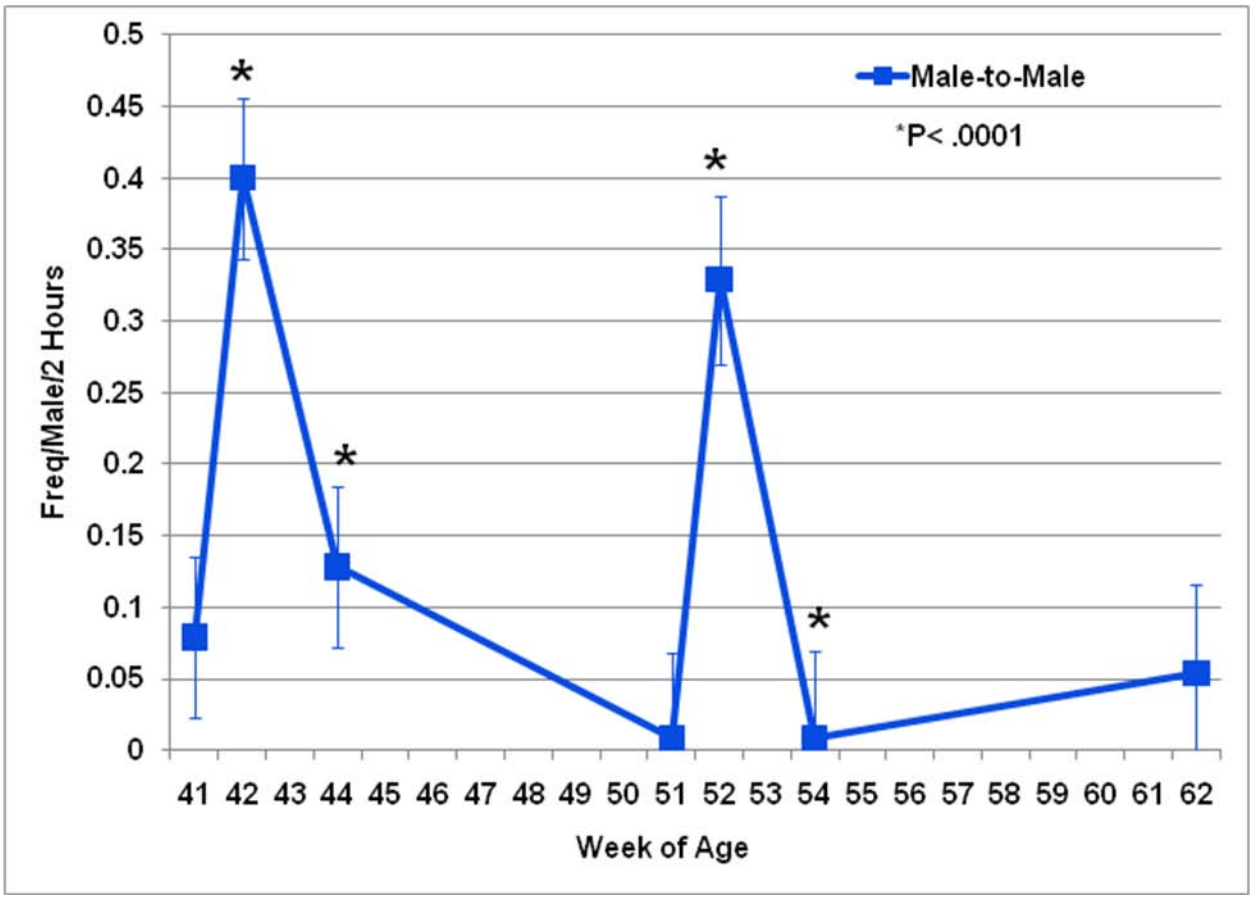

Figure 7. Age related changes in male-to-male behaviors (fighting, pecking, and retreating).

Mean frequencies per male within the two hours of recording were measured across seven observation periods for all flocks. Values are expressed as mean ± SE. Differences (P<.05) between a given age period and the previous interval are denoted by (*).

Table 2. Least squares means for behavior expression changes related to age.[1, 2]

Week of Age	Male-to-Male	Male-to-Female	Individual
41	0.08[a]	2.47[a]	4.08[a]
42	0.40[b]	2.05[a]	4.12[a]
44	0.13[a]	1.36[b]	2.87[ab]
51	0.01[a]	0.48[c]	2.73[b]
52	0.33[b]	0.68[c]	3.85[ab]
54	0.01[a]	0.29[c]	2.93[ab]
62	0.05[a]	0.17[c]	2.95[ab]

[1]All behaviors are expressed as frequency of behavior per male within two hours

[2]Means within a column with different superscripts differ (P<.05)

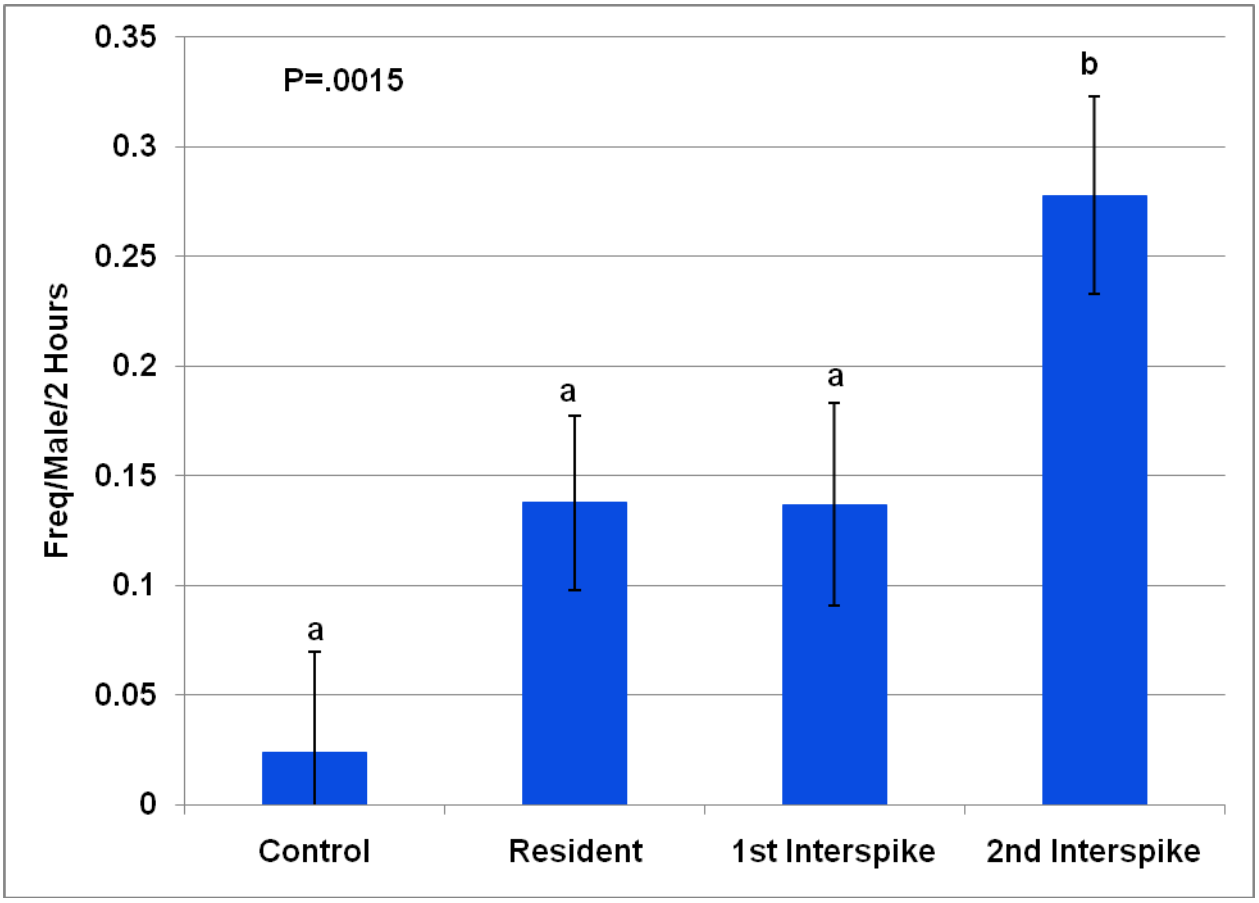

Figure 8. Expression of male-to-male behaviors among control, resident, 1st interspike, and 2nd interspike groups.

Mean frequencies per male within the two hours of recording were measured. Values are expressed as mean ± SE. Means with different letters differ (P<.05).

As expected, male-to-female behavior (completed mating and attempted mating) declined as birds aged (Figure 9). Regardless if matings were completed or only attempted, roosters displayed approximately 2.47 mating attempts at 41 WOA per male within the two hours of video footage, but only 0.17 times at 62 WOA (Table 2). In comparison to the previous interval, sexual behavior was lower ($P<.0001$) at 44 WOA than when birds were 41 WOA and lower at 51 WOA in comparison to 44WOA. No significant differences were found between the different bird groups for male-to-female behavior (Figure 10).

The expression of individual type behaviors which represented maintenance behaviors did not differ in number throughout any of the video monitoring periods (Table 2). These included preening, wing flapping, and feather ruffling. There were also no significant differences between the different groups of birds in terms of the frequency of the types of maintenance behavior (Figure 11).

Secondary Sex Characteristics

Both the comb and wattle area did not differ by date or group. Comb area was slightly positively correlated with the expression of sexual behavior ($r=0.13$, $P=0.0270$) while wattle area showed a trend for a slight correlation ($r=0.11$, $P=0.0619$). Wattle area showed a trend for a possible relationship with aggressive behavior ($r=0.08$, $P=0.0769$).

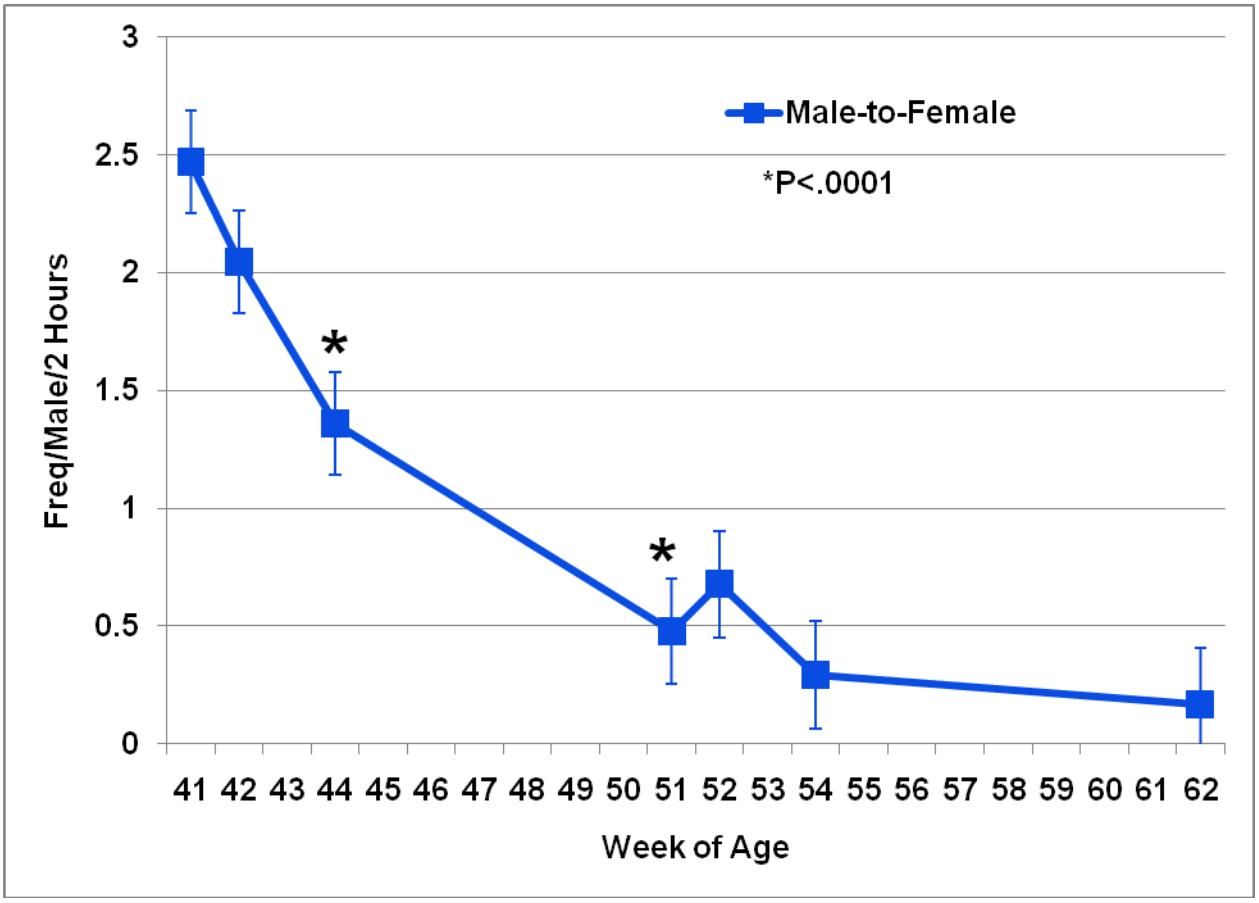

Figure 9. Age related changes in male-to-female behaviors (attempted and completed matings).

Mean frequencies per male within the two hours of recording were measured across seven observation periods for all flocks. Values are expressed as mean ± SE. Differences (P<.05) between a given age period and the previous interval are denoted by (*).

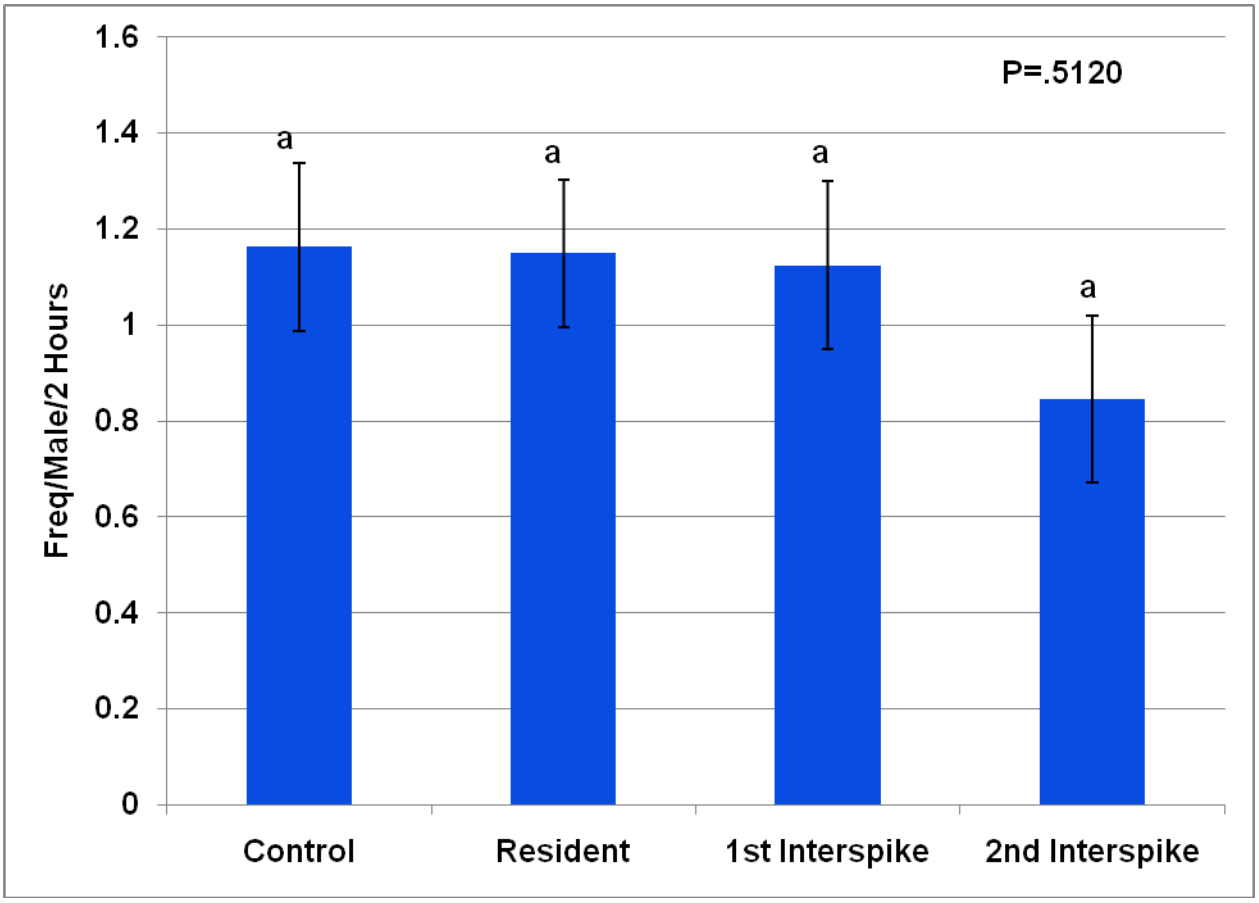

Figure 10. Expression of male-to-female behaviors among control, resident, 1st interspike, and 2nd interspike groups.

Mean frequencies per male within the two hours of recording were measured. Values are expressed as mean ± SE. Means with different letters differ (P<.05).

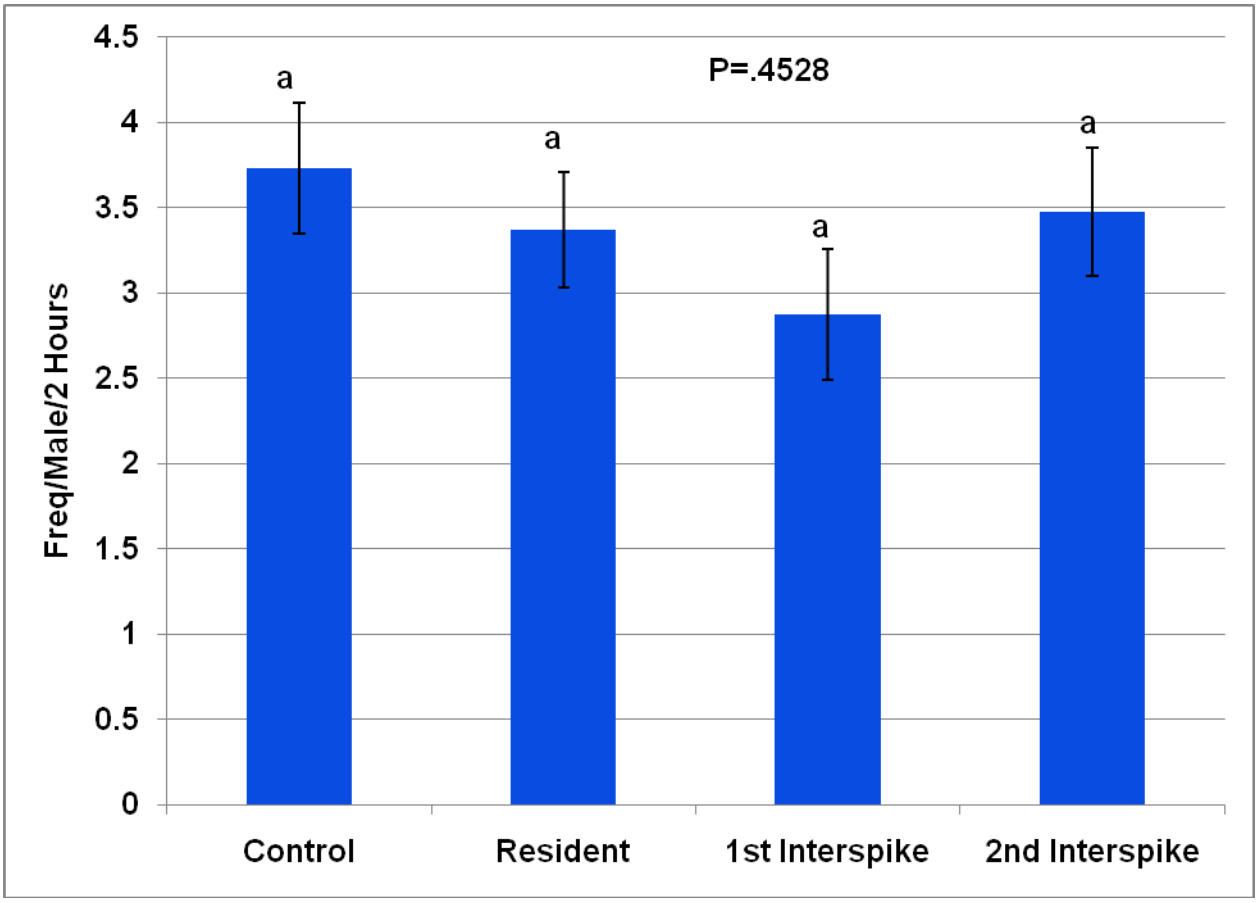

Figure 11. Expression of individual behaviors among control, resident, 1st interspike, and 2nd interspike groups.

Mean frequencies per male within the two hours of recording were measured. Values are expressed as mean ± SE. Means with different letters differ (P<.05).

Hormones

Testosterone concentrations (ng/mL) declined significantly as roosters aged regardless of treatment (Figure 12). When all bird groups were examined together at different time periods, testosterone concentration levels started at 1.95 ng/mL at 32 WOA but gradually declined until it reached 0.11 ng/mL at 62 WOA (P<.001) (Table 3). The testosterone concentration at 42 WOA was significantly lower than the previous measurement at 32 WOA. Also, at 52 WOA, testosterone concentrations declined in comparison to the previous measurement at 44 WOA. Testosterone levels also differed significantly by groups (Figure 13). The group of birds that were interspiked at 52 WOA showed higher levels of testosterone than the control group (P=.0243). The resident birds housed in the interspiked pens showed a trend for increased testosterone concentration in comparison to the control group. The group that was interspiked at 42 WOA did not show any differences in testosterone levels when compared to the control group. As expected, sexual behavior was positively correlated with testosterone levels of the roosters (r=0.43, P<.0001), and there was a trend for slightly positive correlations between comb area and testosterone concentrations (r=0.07, P=0.0670).

Corticosterone concentrations (ng/mL) fluctuated throughout the life of the flock (Figure 14). At 32 WOA, concentration levels were recorded at 5.0 ng/mL (Table 3), but by the end of the life of the flock at 62 WOA, CS concentrations reached a peak of 7.0 ng/mL. The concentration at 42 WOA was higher (P<.0001) in comparison to the previous CS concentration evaluated at 32 WOA. The CS levels declined in the weeks following the first interspike with the concentrations measured at 44 WOA being similar

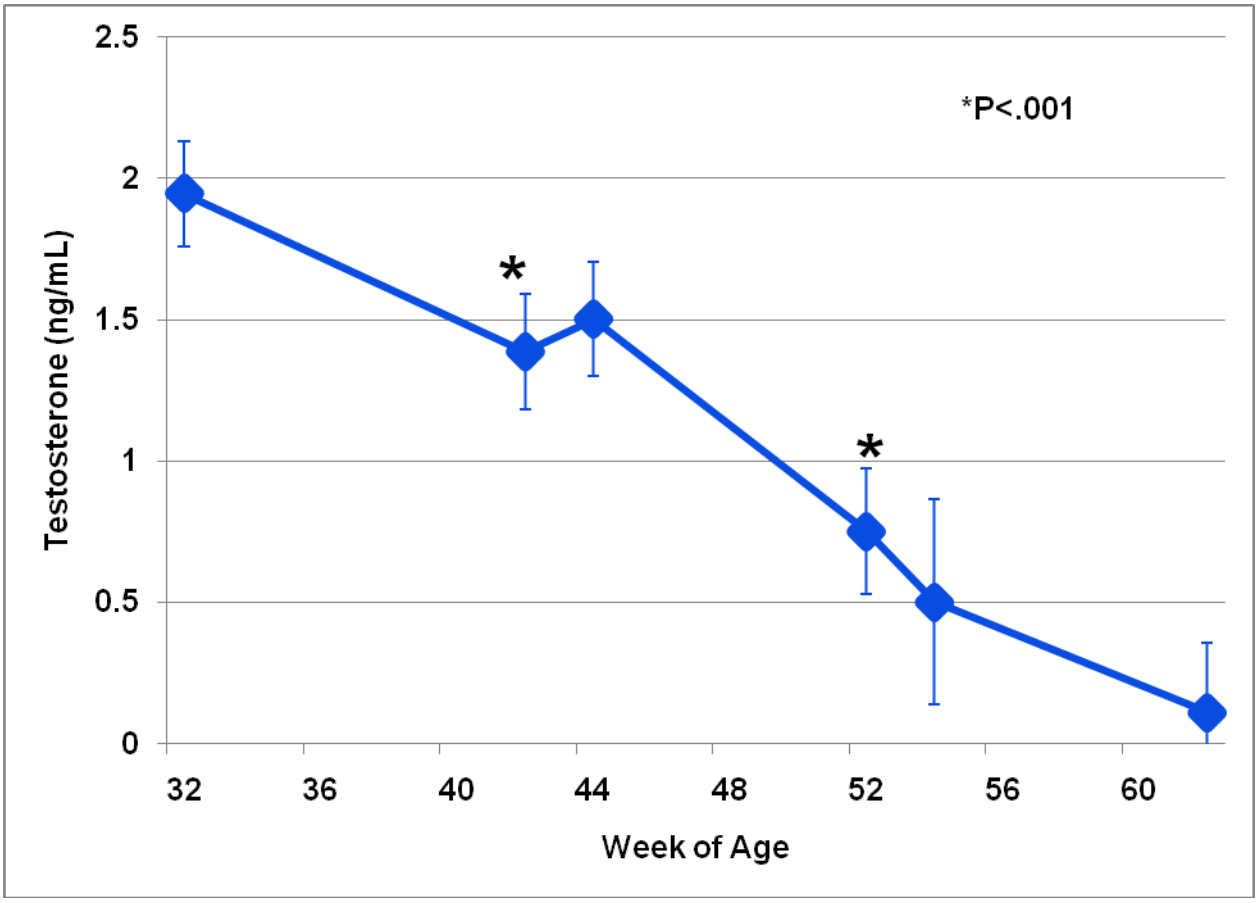

Figure 12. Plasma concentration of testosterone (ng/mL) in male broiler breeders from 32 to 62 weeks of age.

Values are expressed as mean ± SE. Differences (P<.05) between a given age period and the previous interval are denoted by (*).

Table 3. Least squares means ±SE for hormone concentrations (ng/mL) at different ages.[1]

Week of Age	Testosterone	Corticosterone (CS)
32	1.95±0.19[a]	4.97±0.55[a]
42	1.39±0.20[b]	7.66±0.59[b]
44	1.50±0.20[ab]	4.44±0.59[a]
52	0.75±0.22[c]	4.92±0.63[a]
54	0.50±0.36[c]	7.06±0.64[b]
62	0.11±0.25[c]	7.00±0.67[b]

[1]Means within a column with different superscripts differ (P<.05)

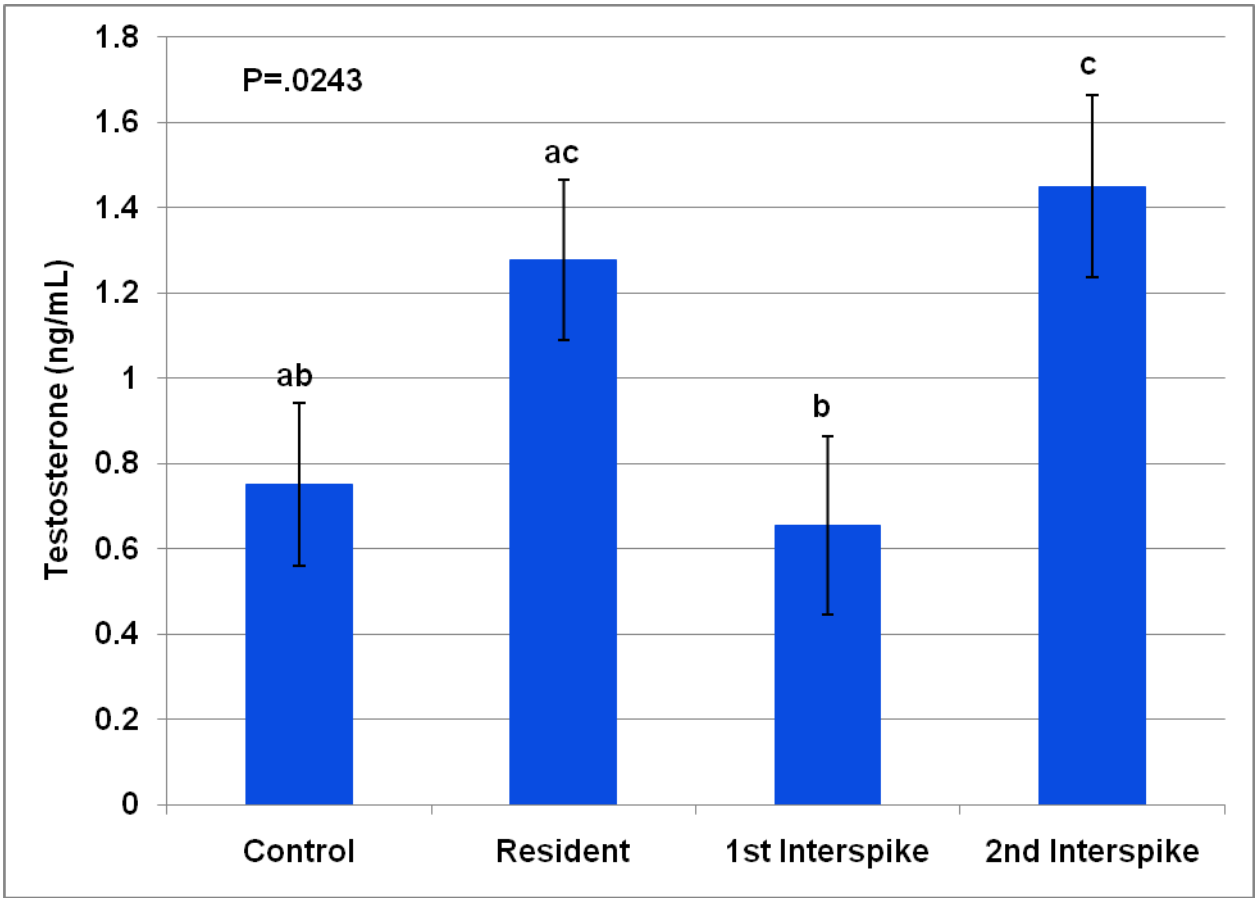

Figure 13. Overall plasma concentration of testosterone (ng/mL) among control, resident, 1st interspike, and 2nd interspike groups.

Values are expressed as mean ± SE. Means with different letters differ (P<.05).

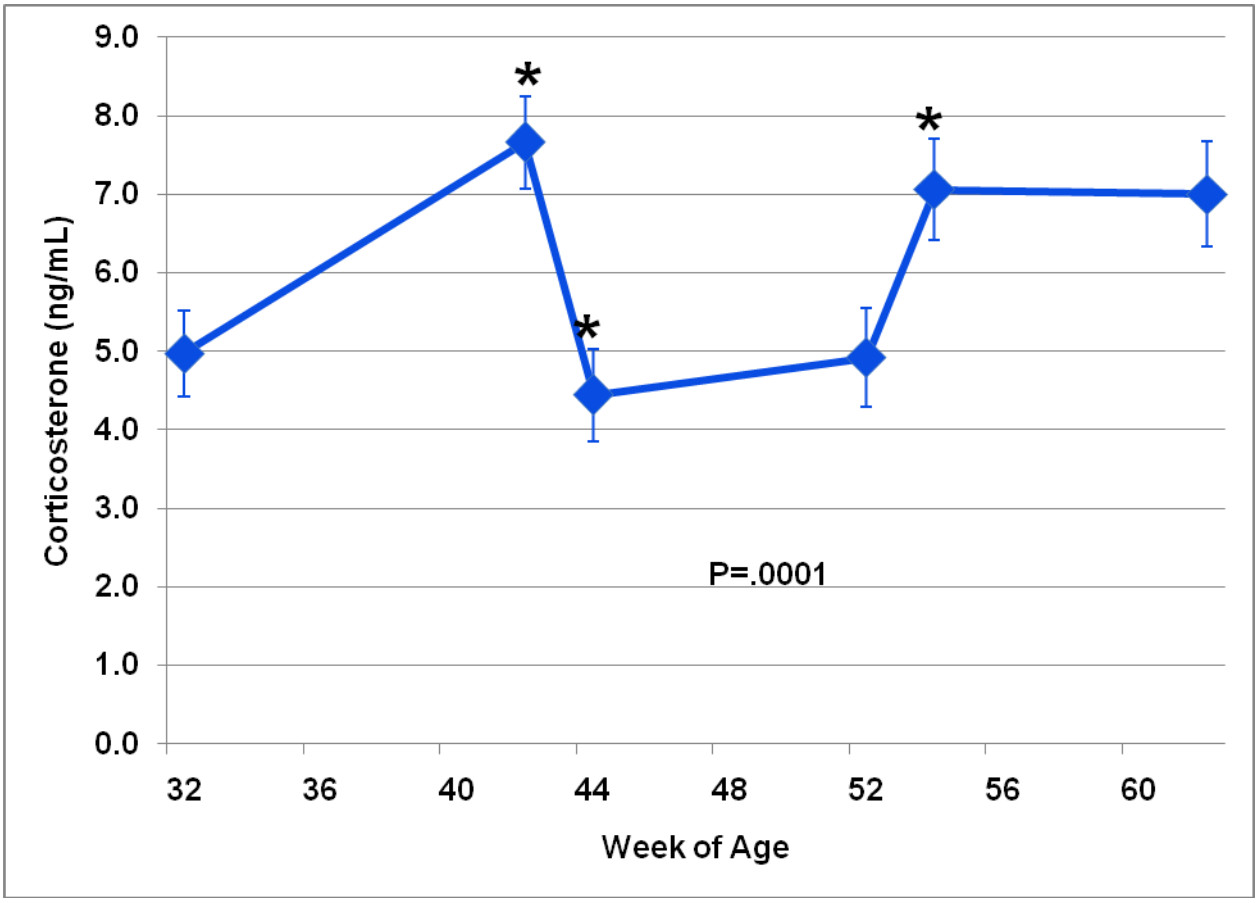

Figure 14. Plasma concentrations of corticosterone (ng/mL) in male broiler breeders from 32 to 62 weeks of age.

Values are expressed as mean ± SE. Differences (P<.05) between a given age period and the previous interval are denoted by (*).

to the 32 WOA baseline levels. After the second interspike at 52 WOA, there were no significant differences in CS levels from the previous CS measurement. However, by week 54 and continuing to the end of the life of the flock at 62 WOA, there was a significant increase in the CS levels. Corticosterone concentrations were not significant by groups but were significant in the group by date interaction. The treatment by date interaction was significant at 42 weeks of age among different groups of birds (Figure 15). In response to the first interspiking that was applied at 42 WOA, CS levels for the groups of birds that were interspiked at 42 WOA and resident birds that were housed within the interspiked room showed higher levels (P=0.0194) of CS concentration than the control group of birds.

Heterophil:Lymphocyte Ratio

The heterophil:lymphocyte ratio (H:L ratio) fluctuated throughout the life of the flock (Figure 16). At 32 WOA, H:L ratios were observed at 0.44. At the end of the life of the flock at 62 WOA, H:L ratios were observed around 0.38 (Table 4). The ratio at 42 WOA was higher (P<.0001) in comparison to the previous H:L ratio evaluated at 32 WOA with a value of 0.50. This was likely a result of the first interspiking affecting the heterophil and lymphocyte percents. The H:L ratio declined in the weeks following the first interspike with the ratio at 44 WOA lower than both time points taken previously. After the second interspike at 52 WOA, there was a significant increase in H:L ratio which was a value similar to that measured from the first interspiking at 42WOA. Following the second interspike, H:L ratios decreased and remained low until the end of the life of the flock. H:L ratios were significant by groups as well (Figure 17). Roosters

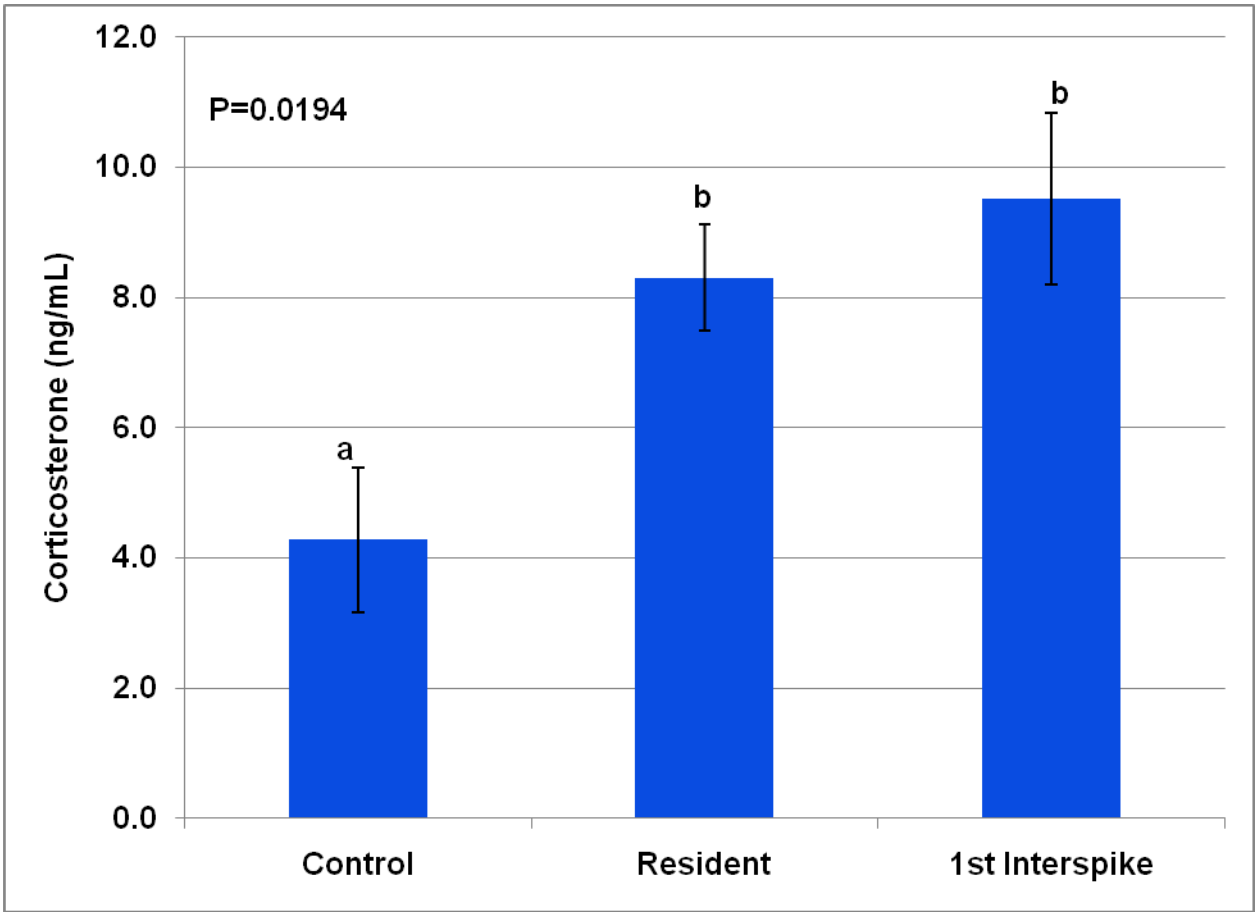

Figure 15. Overall plasma concentration of corticosterone (ng/mL) among control, resident, and 1st interspike groups at 42 weeks of age.

Values are expressed as mean ± SE. Means with different letters differ (P<.05).

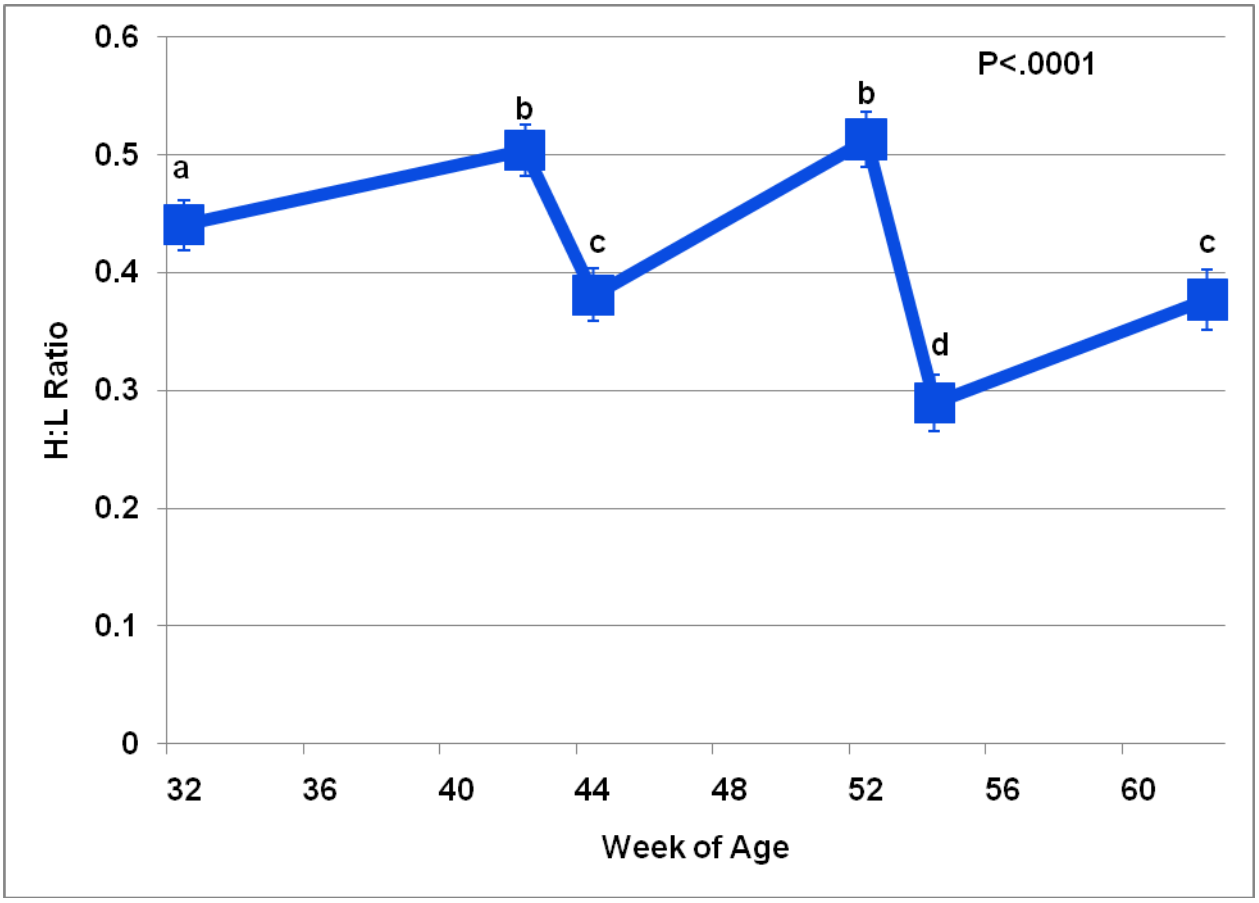

Figure 16. H:L ratio in male broiler breeders from 32 to 62 weeks of age.

Values are expressed as mean ± SE. Means with different letters differ (P<.05).

Table 4. Least squares means for total leukocytes counts related to age.[1]

Week of Age	H:L Ratio	Heterophil	Lymphocyte	Basophil
32	0.441[a]	29.15[ab]	68.75[a]	1.51[ab]
42	0.504[b]	31.60[bc]	66.30[ab]	1.92[bc]
44	0.381[c]	26.58[ad]	71.83[c]	1.54[ab]
52	0.513[b]	31.81[c]	65.97[b]	2.19[c]
54	0.289[d]	21.68[e]	76.91[d]	1.31[ab]
62	0.377[c]	26.06[d]	72.63[c]	1.22[a]

[1]Means within a column with different superscripts differ (P<.05)

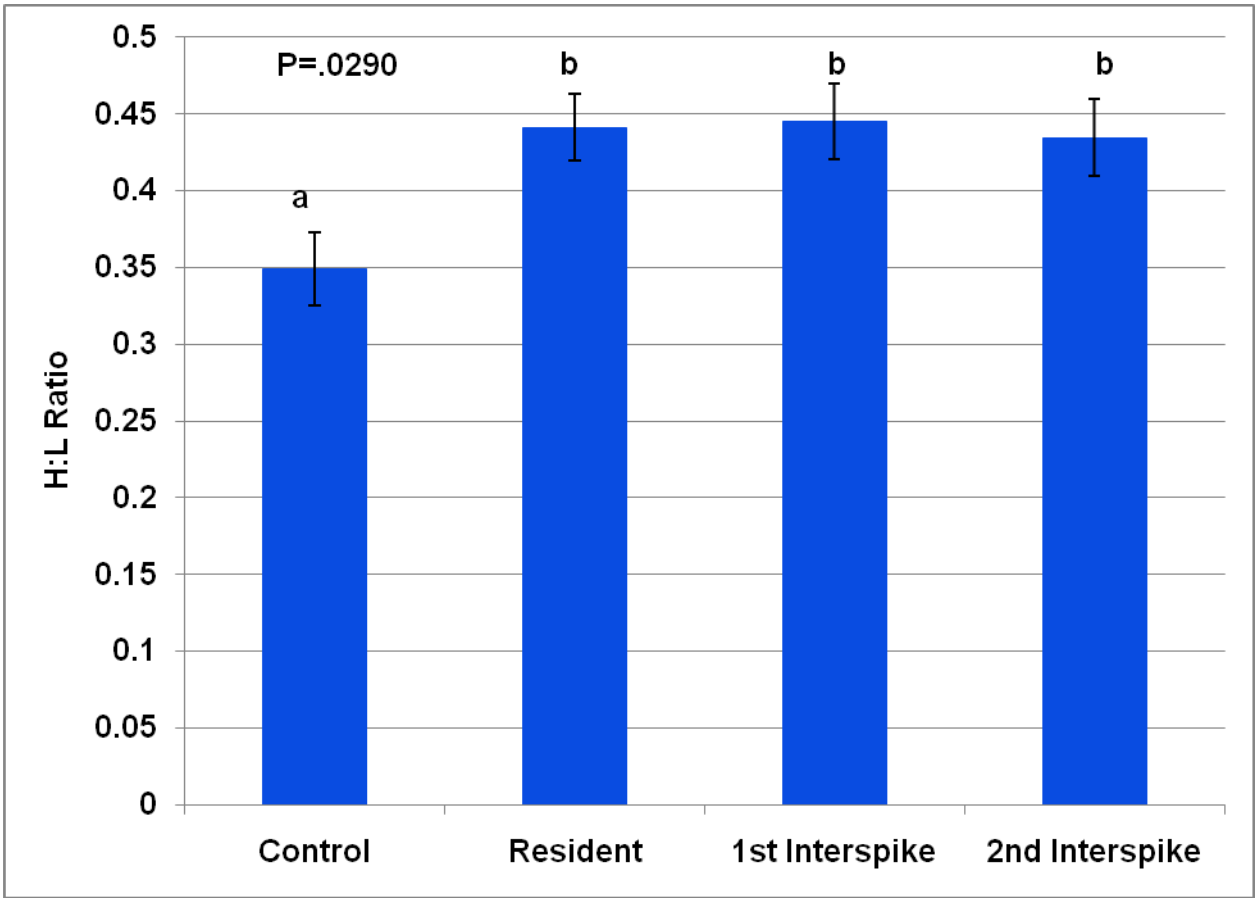

Figure 17. Overall H:L ratios among control, resident, 1st interspike, and 2nd interspike groups.

Values are expressed as mean ± SE. Means with different letters differ (P<.05).

housed in the control room showed lower H:L ratios than all three other groups. All three groups of roosters housed in the interspiked rooms showed higher H:L ratios and all of these groups were similar in values. Lastly, there was a treatment by date interaction that was significant at 42 and 52 WOA. At 42 WOA, all groups that were housed in the interspiked rooms showed increased H:L ratios in comparison to the control roosters (Figure 18). At 52 WOA, both groups of birds that were interspiked (either during the first or second switch) showed higher H:L ratios (Figure 19). The last group of birds within the interspiked rooms, the resident birds showed a trend for increased H:L ratios in comparison to the control birds. Finally, aggressive behavior was found to be slightly correlated with H:L ratios ($r=0.15$, $P=0.0280$) indicating that aggression may be related to the degree of stress.

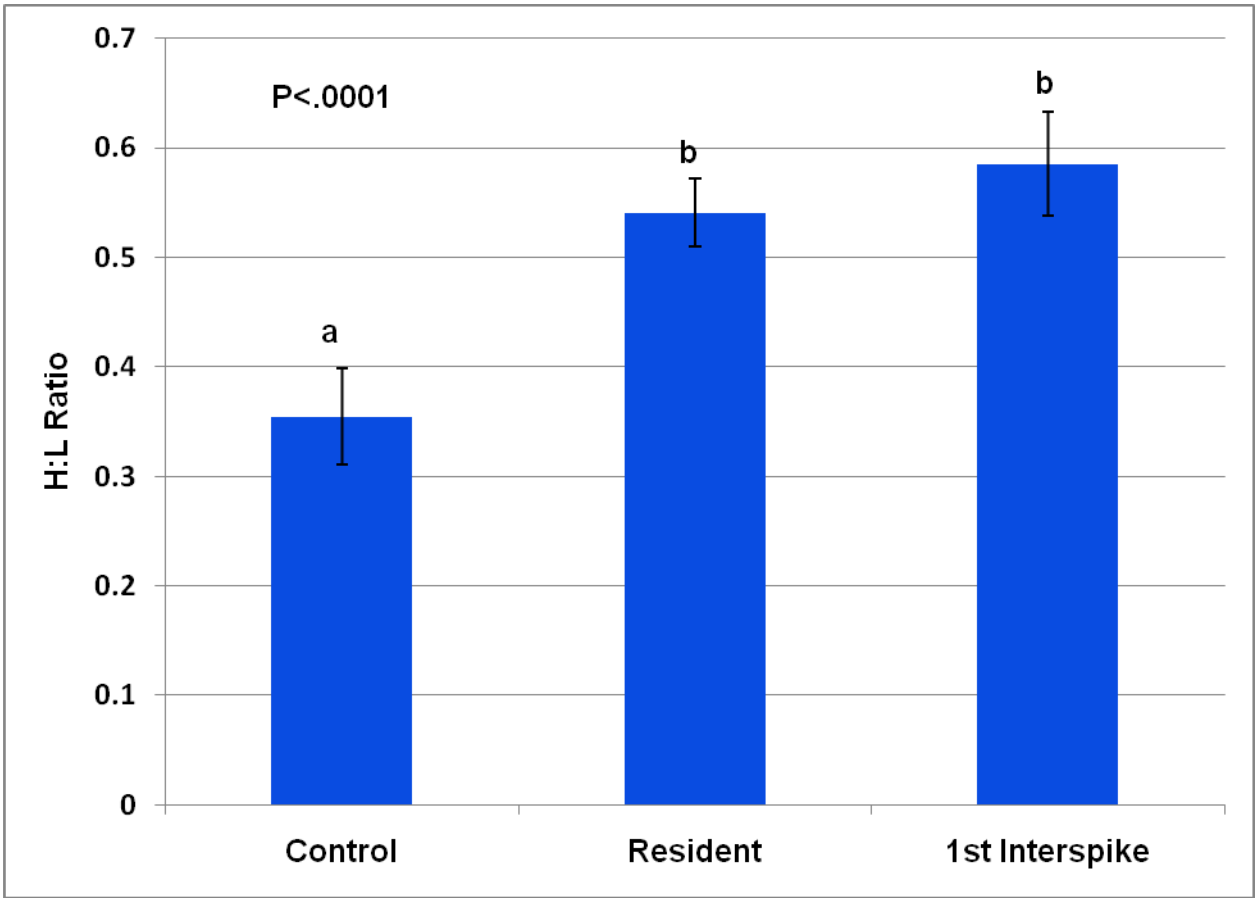

Figure 18. H:L ratios among control, resident, and 1st interspike groups at 42 weeks of age.

Values are expressed as mean ± SE. Means with different letters differ (P<.05).

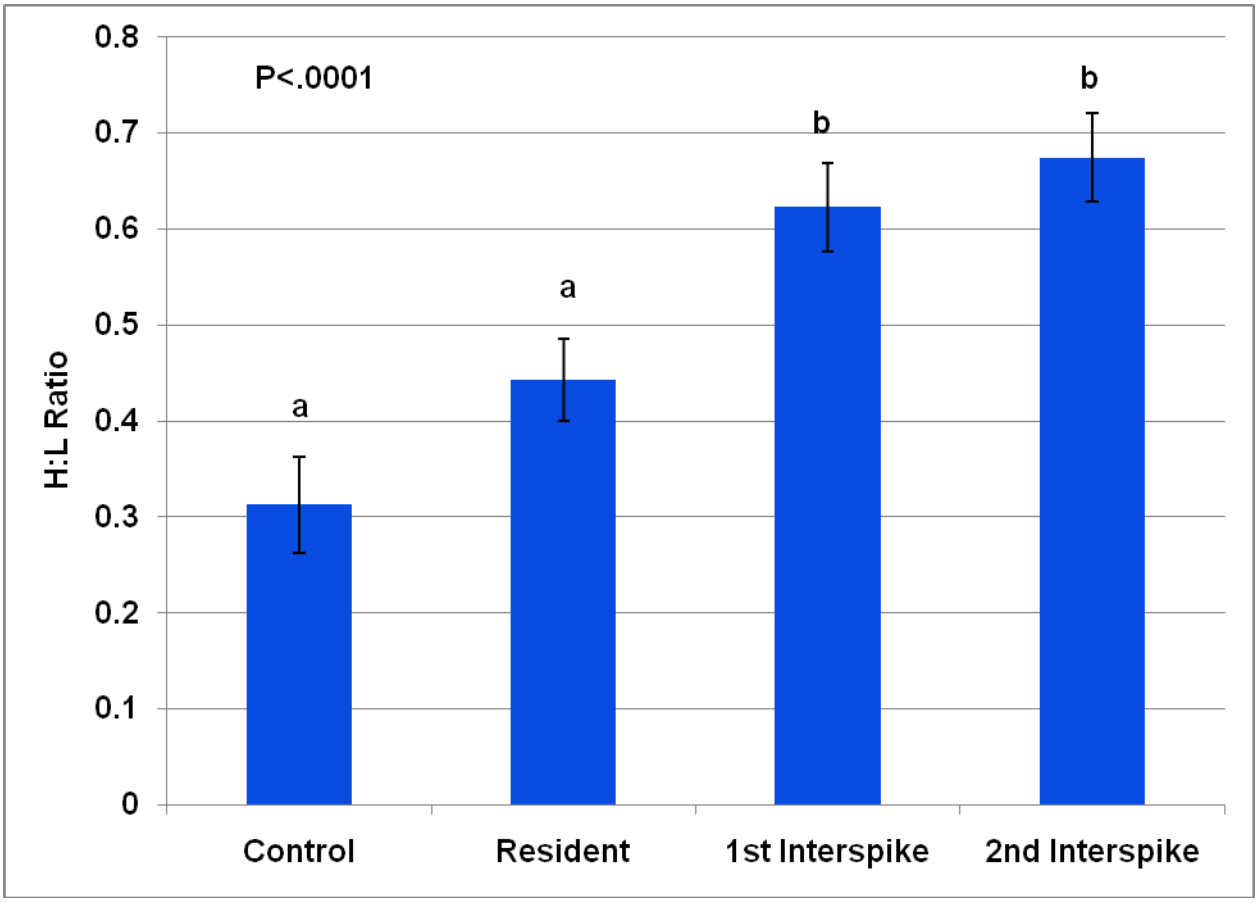

Figure 19. H:L ratios among control, resident, 1st interspike, and 2nd interspike groups at 52 weeks of age.

Values are expressed as mean ± SE. Means with different letters differ (P<.05).

Discussion

The male management practice of double interspiking shows great promise as a tool to increase fertility levels in an aging broiler breeder flock. With the addition of benefits such as decreased breeder cost and improved biosecurity, the practice of double interspiking could allow for greater advantages to the broiler breeder industry that the common industry practice of spiking does not. This research followed this male management practice through one mature life cycle of Ross 708 broiler breeder chickens housed in heat stress conditions.

Our study concludes that regardless of treatment, fertility will decline with age. This is consistent with the work of Sexton et al. (1989), Hocking and Bernard (2000), and McGary et al. (2002), who showed that fertility declines with age through 48, 57, and 50 weeks of production respectively. As a result of double interspiking, fertility percentages of the interspiked flocks were significantly elevated for four weeks in comparison to the control flock. Interspiked flocks also showed a trend for increased fertility levels for the following six weeks. Therefore, the interspiked flocks showed a trend for increased fertility levels for a total of ten weeks before birds were interspiked again. The results from the second interspike of the flocks did not show similar changes to fertility levels as the first interspike. After the second interspike, fertility levels were fairly comparable to the control flock and were not significantly different. Casanovas (2002) and Wilson (2002) both agreed that as a flock ages and approaches 40 WOA, fertility levels begin to decline due to a problem affecting the roosters. In this research, the first interspike was scheduled at 42 WOA in order to counteract this fertility decline.

For the second interspike, we followed Casanovas' study and performed the second transfer around 50 weeks of age since that is when that study showed mating activity beginning to decline (Casanovas, 2002). Unlike that study which concluded that the effects of interspiking on fertility lasted for only six weeks, our study conducted in high temperature environments only showed a significant increase for four weeks and a trend of elevated fertility levels for a total of ten weeks following the first interspike.

In contrast to previous studies (Fiser and Chambers, 1981; Creel et al., 1998; Hocking and Bernard, 2000; Barna et al., 2007), hatchability in our study did not decrease towards the end of the life cycle. At peak production recorded at 32 WOA, we calculated high hatchability values at 98.3%. At the end of the production cycle, hatchability levels were not different. There was a slight decline of approximately eight percent in hatchability at 44 WOA, but this decline was recovered eight weeks later at 52 WOA which corresponds to the second interspike. Kirk et al. (1980) reported a decline in hatchability at 44 WOA, which remained low and did not return to baseline. The application of double interspiking to our flocks did not seem to affect hatchability levels either since treatment was not significant. This was consistent with the work of Hocking and Bernard (2000) who stated that aging roosters are not the cause of declining hatchability levels.

Dependent on the results of the first interspiking, a second interspiking might be more effective if completed earlier. Individual recognition is of utmost importance when studying double interspiking since individuals must be able to recognize if a new individual is a former flock mate or a stranger. It has been shown that birds do not lose

full recognition of another individual until after two weeks of separation (Guhl and Ortman, 1953). This would mean that after only two weeks, individuals can be transferred again since the three birds that were initially transferred would have forgotten all members of their previous flock. Therefore, further research is needed to determine the optimal age(s) to perform the interspikings in order to understand the full potential and benefits of double interspiking on fertility levels. When unfamiliar birds are introduced into a pen, birds will perform exploratory-type behavior which usually causes an initial reaction from the other flock mates (Guhl and Ortman, 1953). A bird that is introduced into a strange pen will not attack unfamiliar birds unless it is attacked first (Guhl and Ortman, 1953). This is a result of the individual not recognizing any members of the flock and only responding to any type of attack. Therefore, as long as individual recognition is gone, multiple interspikings could be performed throughout the life of the flock in order to cause disruption to the social hierarchy.

All behaviors were initially analyzed individually in order to find more explanations for the expression of certain behaviors during different stages of the male management practice of double interspiking. However, after an analysis of two hour recordings, there were very few counts of behavioral frequencies that were expressed regardless of treatment. Therefore, behaviors were grouped into the type of social interaction it explained. For instance, fighting, pecking, and retreating represented male-to-male behaviors, attempted mating and completed mating represented male-to-female behaviors, and preening, wing flapping, and feather ruffling were represented as individual type behaviors. Most studies classified these behaviors under these types of categories. However, some behaviors could be classified under two different types of

interactions. Kruijt (1964) reported that aggressive behavior would sometimes follow wing flapping while Jones and Mench (1991) found a correlation between wing flapping and dominance status. Some other researchers classified wing flapping and feather ruffling as male-to-female behaviors due to their possible use as courtship displays, but it was mentioned that these behaviors could also be classified as an individual's expression of a bird's presence and territory (Kruijt, 1964; Jones and Mench, 1991; McGary et al., 2003). Regardless of these few studies, both wing flapping and feather ruffling were counted as individual type behaviors because they were behaviors that did not seem influenced by any other individual. Rather, it was a behavior that was performed as an announcement by the individual of their presence within the flock. Another behavior, pecking, was reported to be motivated by curiosity or hunger and was described as a more exploratory type behavior (Komai and Guhl, 1960). However, many studies showed the importance of pecking in aggressive behaviors that were used in order to establish and maintain the pecking order.

Our findings for the male-to-male and male-to-female interactions as well as individual type behaviors were expected. The frequency of male-to-male interactions increased during the weeks that the birds were interspiked. This increase in aggressive behavior among roosters following double interspiking is likely a result of the disruption of the established pecking order in the flock. Guhl and Ortman (1953) explained that when strangers are introduced into a new pen, they perform exploratory behavior which alerts resident birds that this is a stranger and a new individual to the pen. Aggressive acts are performed by the residents of the pen towards the strangers, in order to establish the new pecking order. Guhl (1968) noted that aggressive behavior should be

fairly tense until domination and subordination statuses are determined within the flock mates of the pen. This increase in fights and aggressive behavior seems to be a result of the high ranking roosters attempting to maintain their dominant status within the group. Other individuals are able to decide whether or not to fight with the stranger by witnessing the settlement of a conflict by a stranger and a known (dominant or subordinate) flock mate (Hogue et al., 1996). The expectation for this study was that double interspiking would cause a disruption of the pecking order, and the increased expression of aggressive behavior between the roosters confirmed this disruption. Our findings regarding age on male-to-female interactions were consistent with results presented by Craig and Bhagwat (1974), Duncan et al. (1990), Hocking and Bernard (2000), and McGary et al. (2003). As with these studies, all expressions of male-to-female type behavior in our study declined with age of the roosters. Regardless of the treatment, there was a trend for a decrease in sexual behavior with age. This decline is attributed to the decline in mating interest of the roosters over time. The practice of double interspiking was not able to completely resolve the loss of mating interest in roosters. Some researchers have not been able to find a relationship between fertility and sexual behavior (Duncan et al., 1990), while others have concluded that mating frequency does not necessarily correlate with the number of offspring that the rooster parented (Bilcik et al., 2005; Bilcik and Estevez., 2005). Therefore, fertility decline might not be a result of the infrequent matings or infrequent cloacal contacts (McGary et al., 2003). However, although we were not able to correlate male-to-female sexual interactions with fertility levels in the flock or find a relationship, both parameters declined throughout the life of the flock. Expression of individual behaviors did not

change in frequency throughout the entire experiment. This was not unexpected since maintenance type behaviors are likely to be expressed by all individuals regardless of external stressors. These results are consistent with a study by Duncan and Wood-Gush (1972) in which they concluded that preens could be modified in the presence of frustration or a stressor, but the frequency of the preening expression did not differ.

In this study, we found a trend for a correlation between comb area and testosterone concentration. This is consistent with Allee et al. (1939) and Verhulst et al. (1999) since both saw a correlation between plasma testosterone concentrations and comb size. Sexual behavior was also slightly correlated with comb area and showed a possible relationship with wattle area. This was not unexpected since secondary sexual characteristics are used by hens to select their mate. McGary et al. (2003) and Bilcik et al. (2005) both found positive correlations between reproductive type behaviors and a large comb. In contrast, Wood-Gush and Osborne (1956) could not find a correlation between the comb size and the expression of sexual behaviors, and Wolanski et al. (2004) and Bilcik et al. (2005) both agreed that secondary sexual characteristics did not play a large role in reproductive success either. Lastly, it was concluded that the calculated area for the comb and wattle would not differ by date. This is not surprising since this characteristic is a tool used by other individuals within the flock for identification purposes. Larger combs are usually related to high ranking individuals, and any size changes would affect the identification and possible social ranking of the individual. Similar to Allee et al. (1939) and Bilcik et al. (2005) who saw a relationship between comb size and aggressive behavior, our study found a trend for a correlation

between wattle area and aggressive behavior. Aggressive behavior is important in order to maintain a pecking order.

Consistent with the work of Sexton et al. (1989), Weil et al. (1999), and Hocking and Bernard (2000), in the present study, plasma testosterone concentrations declined as roosters aged. Regardless of treatment, testosterone concentrations dropped throughout the life of the flock. At the end of the productive life, measurements were as low as 0.11 ng/mL at 62 WOA in comparison to 1.9 ng/mL at 32 WOA. These concentrations were very low in comparison to the concentration range reported by Culbert et al. (1977). In that study, researchers saw an increase in testosterone concentrations with the production of sperm and testes maturation of cockerels raised under thermoneutral conditions. Their analysis of testosterone values showed sustained testosterone values from 35 WOA to 44 WOA whereas in our study, conducted under heat stress, those values declined over this period. As expected, sexual behavior was positively correlated with testosterone levels of the roosters. Brougher et al. (2005) found a correlation between sexual behavior and testosterone levels as well. However, Chappell et al. (1997) did not find any relationship between testosterone and sexual behavior. Regardless, our study showed a decline in testosterone values which likely caused a decline in the mating interests of the roosters. If this is the case, a decline in mating interest would translate into the decline in sexual activity.

As expected, H:L ratios increased during both weeks that the birds were double interspiked. The ratios declined during the weeks following interspikings and remained

low for all other time points in the experiment. The change in H:L ratios was likely influenced by the heterophil counts instead of the lymphocyte counts. Heterophil counts were elevated during both 42 and 52 weeks of age whereas lymphocyte counts were fairly variable throughout the experimental time period. This increase in heterophils is due to heterophilia which was also shown in studies by Siegel (1980), Davis et al. (2000), and Shini et al. (2008). A study by Post et al. (2003) proposed that heterophil counts could be used as an indicator of stress levels. Our study is consistent with these results since heterophil counts were elevated during both weeks that birds were interspiked. Using the less variable parameter of H:L ratios, this experiment showed that birds that were housed in the control rooms did show lower levels of H:L ratios. This was expected since only those birds in the interspiked rooms should have been stressed resulting in an increase in H:L ratios. It would be expected that all birds housed in the interspiked rooms would show higher levels of CS than the birds in the control room. However, this was not the case. It was surprising that double interspiking did not increase plasma CS concentrations during both interspiking weeks. Corticosterone concentrations were shown to be elevated the week of the first interspiking, and this measurement returned to baseline levels when blood was sampled two weeks later. However, CS concentration did not increase during the week of the second interspiking. Corticosterone levels were high towards the end of the life of the flock though. From the first interspiking at 42 WOA, as was expected, the groups of birds housed in the interspiking rooms showed trends for higher levels of CS expression in comparison to the control birds.

Regarding the hormone CS, concentrations could have been affected by the sequence that blood was sampled resulting in a change in stress response. Davis et al. (2000) performed an experiment on single comb white Leghorn hens and found a difference in the CS levels of hens dependent on the sequence at which blood was sampled. They reported that the hens that were sampled towards the end of the bleeds showed higher levels of CS concentrations than those hens sampled at the beginning of the sequence. Therefore, this could have occurred in our roosters as well. For each bleed, all roosters from each pen were collected and placed into a holding enclosure. Then, individuals were randomly selected and blood was sampled. A rise in plasma CS concentrations could have been a result of the time it took to obtain the blood sample, the amount of time the bird waited for blood sampling or the order in which the birds were sampled. Corticosterone concentrations increase fairly rapidly therefore handling of the birds probably affected the measured CS concentration as well. Upon capturing the birds to draw a blood sample, the length of time the bird is captured and restrained will affect the plasma CS levels. The shortest amount of time that CS takes to rise was reported by Beuving and Vonder (1978) which stated that CS values had shown a significant increase after 45 seconds of immobilization. When birds are handled for a longer period of time, CS concentration will rise rapidly for five minutes and then will rise gradually (Beuving and Vonder, 1978). Romero and Reed (2005) explain that CS needs to be recorded within two to three minutes, because this time frame will represent baseline concentrations without the effects of handling. Due to this variable nature of CS, it is hard to fully depend on the results of CS alone.

It is known that the concentration of CS could have been affected by some other interaction with another hormone in the body which was not analyzed during this study. Hormones such as thyroid hormones (Decuypere and Kuhn, 1988) have been found to affect CS concentrations. In this study, we did not find a relationship between the two hormones of interest, testosterone and corticosterone. However, it would be interesting to study the effects of CS or the hormones involved in the HPA axis (i.e. CRF and ACTH) on the hypothalamic-pituitary-gonadal (HPG) axis. In the HPG axis, the hypothalamus secretes gonadotropin-releasing hormone (GnRH) which stimulates the anterior pituitary gland to release luteinizing hormone (LH). LH stimulates the Leydig cells in the testes to produce testosterone. It could be possible that any hormones in the stress axis could negatively affect the production of the hormones in the HPG axis.

There is a large negative genetic correlation between meat yielding capabilities and reproduction, so it is very important to try to maintain a good optimal body weight for both roosters and hens in order to also curb some of the fertility problems (McDaniel et al., 1981; Hocking, 1990; Dunnington et al., 1990; Casanovas, 2002; McGary et al., 2003; Bilcik et al., 2005). If roosters have a large amount of breast fleshing or fat pad deposition, they will have more difficulty in mounting a hen correctly and will not be able to successful inseminate semen into then hen's oviduct. Therefore, it is very important to have good management practices in place in order to avoid obesity problems in chickens. Precautions like sex separate feeders, weighing birds weekly to check their growth progress, calculating feed according to the production manual, and adjusting feed amounts to keep birds on their target weights were taken. Special care was taken to avoid sudden weight loss since Casanovas (2002) concluded that such rapid weight

loss could impair both mating activity and production of sperm. However, in our housing set-up, it appears that the large weight of the chickens and the fact that they were reared on slatted floors, resulted in feet and leg problems. In other research conducted at the university, the use of slatted pen floors was not detrimental and did not cause a lowered feet score for a lighter type of bird. However, we saw with the increase in weight of the broiler breeders that there was a negative effect on the foot pads of our birds. This is consistent with the reports that state a higher incidence of both obesity and leg problems in broiler breeders in production (Barbato, 1999).

A problem that possibly complicated the effects of double interspiking on our flock was the limited number of roosters in the three pens. As is normal to industry standards, prior to the practice of spiking, some older roosters are culled if their health is poor or they are not in good physical condition. This is because when young roosters are introduced into a flock, there will only be an increase in fertility levels if existing roosters are in good physical condition and are capable of mating successfully (Wilson, 1999). Due to our small sampling size, we were not able to cull any roosters that were both unusually large and inactive or those that needed to be culled due to feet and leg problems. This could have lowered the frequency of sexual behavior or aggressive behavior displayed and could have had an impact on the fertility levels of the spiked flocks. Without the optimal health of each individual rooster, it would be impossible for the roosters housed in those chicken pens to respond to their highest capability and successfully mate with the hens. This poor physical condition could also reduce mating efficiency and therefore result in a decline in mating interest or libido of the rooster.

Although infertility issues have been associated with a problem with the roosters, there has been some research done regarding the possible contribution of the hen to the infertility issue. Instead of fertility decline being a problem solely with the roosters, some researchers have contended that the age of the hen is a possible cause for fertility decline as well. In a study by Bramwell et al. (1996), older hens experienced lower fertility levels than younger hens. When artificially inseminated with an older rooster's sperm, young hens showed higher levels of fertility and sperm penetration in comparison to the older hens. In that study, the older roosters actually showed higher levels of sperm penetration and fertility levels in comparison to the younger males. Therefore, age did not seem to affect the fertilizing potential of the male. A study on native Thai chickens also concluded that roosters from nine months to two years will show high fertilizing ability and researchers blamed hens for the fertility decline (Chotesangasa, 2001). It was even reported that the mating ratios had no affect on the fertility of the flock as ratios ranged from 1:7 to 1:16 and did not differ in fertility levels. This would indicate that the roosters are capable of fertilizing more hens and that lower fertility percentages could be attributed to the hen.

Finally, this study of double interspiking combined with a study of spiking needs to be replicated under both thermoneutral and heat stress conditions. This study was performed under heat stress conditions due to the fact that the decline in fertility is exacerbated in the presence of high temperatures. Although broiler breeder producers in temperate climates suffer from economic losses due to fertility declining with age, producers in hot or tropical climates experience a greater economic loss due to detrimental effects on health as well as impaired reproduction. In this experiment,

double interspiking was applied to see if this practice was capable of increasing fertility levels when all members of a flock were under a type of environmental stress. Double interspiking did allow for some improvements although the flocks were exposed to chronic heat as a stressor. It would be interesting to investigate the other male management practice of spiking under these same conditions in order to compare the results of the two practices under elevated environment stress. An experiment using the male management practice of spiking in heat stressed environments could explain the impact of spiking in industry today since this practice is the one most commonly used. It is also important to investigate the effects of double interspiking and spiking in a thermoneutral environment. In comparison to the results that we found in this study, if double interspiking was practiced in a thermoneutral flock, it might be possible that greater effects might be observed due to the fact that the birds are not experiencing an extra environmental stressor.

Literature Cited

Allee, W., N. Collias, and C. Lutherman. 1939. Modification of the social order in flocks of hens by the injection of testosterone propionate. Physiological Zoology 12:412-440.

Al-Murrani, W., A. Kassab, H. Al-Sam, and A. Al-Athari. 1997. Heterophil/lymphocyte ratio as a selection criterion for heat resistance in domestic fowls. British Poultry Science 38:159-163.

Altan, O., A. Altan, M. Cabuk, and H. Bayraktar. 2000. Effects of heat stress on some blood parameters in broilers. Turkish Journal of Veterinary and Animal Sciences 24:145-148.

Barbato, G. 1999. Genetic relationships between selection for growth and reproductive effectiveness. Poultry Science 78:444-452.

Barna, J., B. Végi, E. Varadi, Z. Szoke, and K. Liptói. 2007. Analysis of Fertility in Broiler Breeder Flocks - Male Side Approaches. Proc. Proceedings of the 5th Vietnamese-Hungarian International Conference on "Animal Production and Aquaculture for Sustainable Farming", Can Tho, Vietnam.

Bartlett, J., and M. Smith. 2003. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poultry Science 82:1580-1588.

Bedanova, I., E. Voslarova, V. Vecerek, V. Pistekova, and P. Chloupek. 2007. Haematological profile of broiler chickens under acute stress due to shackling. Acta Vet. Brno 76:129-135.

Beuving, G., and G. Vonder. 1978. Effect of stressing factors on corticosterone levels in the plasma of laying hens. General and Comparative Endocrinology 35:153-159.

Bilcik, B., and I. Estevez. 2005. Impact of male-male competition and morphological traits on mating strategies and reproductive success in broiler breeders. Applied Animal Behaviour Science 92:307-323.

Bilcik, B., I. Estevez, and E. Russek-Cohen. 2005. Reproductive success of broiler breeders in natural mating systems: the effect of male-male competition, sperm quality, and morphological characteristics. Poultry Science 84:1453-1462.

Bramwell, R., H. Marks, and B. Howarth. 1995. Quantitative determination of spermatozoa penetration of the perivitelline layer of the hen's ovum as assessed on oviposited eggs. Poultry Science 74:1875-1883.

Bramwell, R., C. McDaniel, J. Wilson, and B. Howarth. 1996. Age effect of male and female broiler breeders on sperm penetration of the perivitelline layer overlying the germinal disc. Poultry Science 75:755-762.

Braw-Tal, R., S. Yossefi, S. Pen, D. Shinder, and A. Bar. 2004. Hormonal changes

associated with ageing and induced moulting of domestic hens. British Poultry Science 45:815-822.

Brougher, S., I. Estevez, and M. Ottinger. 2005. Can testosterone and corticosterone predict the rate of display of male sexual behaviour, development of secondary sexual characters and fertility potential in primary broiler breeders? British Poultry Science 46:621-625.

Bshary, R., and J. Lamprecht. 1994. Reduction of aggression among domestic hens (Gallus domesticus) in the presence of a dominant third party. Behaviour 128:311-324.

Campo, J., and S. Davila. 2002a. Estimation of heritability for heterophil: lymphocyte ratio in chickens by restricted maximum likelihood. Effects of age, sex, and crossing. Poultry Science 81:1448-1453.

Campo, J., and S. Davila. 2002b. Influence of mating ratio and group size on indicators of fearfulness and stress of hens and cocks. Poultry Science 81:1099-1103.

Casanovas, P. 2002. Management Techniques to Improve Male Mating Activity and Compensate for the Age-Related Decline in Broiler Breeder Fertility: Intra-Spiking. The Poultry Informed Professional.

Chappell, M., M. Zuk, T. Johnsen, and T. Kwan. 1997. Mate choice and aerobic capacity in red junglefowl. Behaviour 134:511-529.

Cheng, H., G. Dillworth, P. Singleton, Y. Chen, and W. Muirt. 2001. Effects of group selection for productivity and longevity on blood concentrations of serotonin, catecholamines, and corticosterone of laying hens. Poultry Science 80:1278-1285.

Chotesangasa, R. 2001. Effects of mating ratio, cock number in the flock and breeder age on fertility in Thai native chicken flock. Kasetsart Journal (Natural Sciences)(Thailand); Witthayasan Kasetsart (Sakha Witthayasat).

Craig, J., and A. Bhagwat. 1974. Agonistic and mating behavior of adult chickens modified by social and physical environments. Applied Animal Ethology 1:57-65.

Creel, L., D. Maurice, W. Bridges, and L. Grimes. 1998. A model to describe and predict post-peak changes in broiler hatchability. The Journal of Applied Poultry Research 7:85-89.

Culbert, J., P. Sharp, and J. Wells. 1977. Concentrations of androstenedione, testosterone and LH in the blood before and after the onset of spermatogenesis in the cockerel. Reproduction 51:153-154.

Davis, G., K. Anderson, and A. Carroll. 2000. The effects of long-term caging and molt of Single Comb White Leghorn hens on heterophil to lymphocyte ratios, corticosterone

and thyroid hormones. Poultry Science 79:514-518.

Davis, G., K. Anderson, and D. Jones. 2004. The effects of different beak trimming techniques on plasma corticosterone and performance criteria in Single Comb White Leghorn hens. Poultry Science 83:1624-1628.

De Jong, I., A. van Voorst, J. Erkens, D. Ehlhardt, and H. Blokhuis. 2001. Determination of the circadian rhythm in plasma corticosterone and catecholamine concentrations in growing broiler breeders using intravenous cannulation. Physiology & behavior 74:299-304.

De Jong, I., M. Wolthuis-Fillerup, and R. Van Emous. 2009. Development of sexual behaviour in commercially-housed broiler breeders after mixing. British Poultry Science 50:151-160.

Decuypere, E., and E. Kuhn. 1988. Thyroid hormone physiology in galliformes: Age and strain related changes in physiological control. Integrative and Comparative Biology 28:401-415.

Duncan, I. 1998. Behavior and behavioral needs. Poultry Science 77:1766-1772.

Duncan, I., P. Hocking, and E. Seawright. 1990. Sexual behaviour and fertility in broiler breeder domestic fowl. Applied Animal Behaviour Science 26:201-213.

Duncan, I., and D. Wood-Gush. 1972. An analysis of displacement preening in the domestic fowl. Animal Behaviour 20:68-71.

Dunnington, E., P. Siegel, and N. Anthony. 1990. Reproductive fitness in selected lines of chickens and their crosses. Journal of Heredity 81:217-219.

Fiser, P., and J. Chambers. 1981. Determination of male fertility in thirteen commercial lines of broiler parents. Poultry Science 60:2316-2321.

Gross, W., and G. Colmano. 1971. Effect of infectious agents on chickens selected for plasma corticosterone response to social stress. Poultry Science 42:896–905.

Gross, W., and H. Siegel. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Diseases 27:972-979.

Gross, W., and P. Siegel. 1973. Effect of social stress and steroids on antibody production. Avian Diseases 17:807-815.

Gross, W., and P. Siegel. 1981. Long-term exposure of chickens to three levels of social stress. Avian Diseases 25:312-325.

Guémené, D., M. Debut, M. Couty, M. Garreau-Mills, Y. Jego, N. Sellier, C. Berri, and

E. Le Bihan-Duval. 2006. Pre-slaughter stress responses and adrenal responsiveness in broilers of fast and slow growth rate genotypes. Proc. Proc. XII European Poultry Conference, Verona.

Guhl, A. 1949. Heterosexual dominance and mating behavior in chickens. Behaviour 2:106-120.

Guhl, A. 1950. Social dominance and receptivity in the domestic fowl. Physiological Zoology 23:361-366.

Guhl, A. 1968. Social behavior of the domestic fowl. Transactions of the Kansas Academy of Science (1903):379-384.

Guhl, A., and L. Ortman. 1953. Visual patterns in the recognition of individuals among chickens. Condor 55:287-298.

Guhl, A., and D. Warren. 1946. Number of offspring sired by cockerels related to social dominance in chickens. Poultry Science 25:460-472.

Hamilton, W., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites? Science 218:384-387.

Hocking, P. 1990. The relationships between dietary crude protein, body weight, and fertility in naturally mated broiler breeder males. British Poultry Science 31:743-757.

Hocking, P., and R. Bernard. 1997. Effects of dietary crude protein content and food intake on the production of semen in two lines of broiler breeder males. British Poultry Science 38:199-202.

Hocking, P., and R. Bernard. 2000. Effects of the age of male and female broiler breeders on sexual behaviour, fertility and hatchability of eggs. British Poultry Science 41:370-376.

Hocking, P., and S. Duff. 1989. Musculo-skeletal lesions in adult male broiler breeder fowls and their relationships with body weight and fertility at 60 weeks of age. British Poultry Science 30:777-784.

Hogue, M., J. Beaugrand, and P. Lagu 1996. Coherent use of information by hens observing their former dominant defeating or being defeated by a stranger. Behavioural Processes 38:241-252.

Jones, M., and J. Mench. 1991. Behavioral correlates of male mating success in a multisire flock as determined by DNA fingerprinting. Poultry Science 70:1493-1498.

Keeling, L., and I. Duncan. 1991. Social spacing in domestic fowl under seminatural conditions: the effect of behavioural activity and activity transitions. Applied Animal

Behaviour Science 32:205-217.

Kirk, S., G. Emmans, R. McDonald, and D. Arnot. 1980. Factors affecting the hatchability of eggs from broiler breeders. British Poultry Science 21:37-53.

Komai, T., and A. Guhl. 1960. Tameness and its relation to aggressiveness and productivity of the domestic chicken. Poult. Sci 39:817–823.

Korte, S., G. Beuving, W. Ruesink, and H. Blokhuis. 1997. Plasma catecholamine and corticosterone levels during manual restraint in chicks from a high and low feather pecking line of laying hens. Physiology & behavior 62:437-441.

Kruijt, J. 1964. Ontogeny of social behaviour in Burmese red junglefowl (Gallus gallus spadiceus) Bonnaterre. Behaviour. Supplement.

Lake, P., and D. Wood-Gush. 1956. Diurnal rhythms in semen yields and mating behaviour in the domestic cock. Nature 178:853.

Maxwell, M. 1993. Avian blood leucocyte responses to stress. World's Poultry Science Journal 49:34-43.

McDaniel, C., R. Bramwell, and B. Howarth Jr. 1996. The male contribution to broiler breeder heat-induced infertility as determined by sperm-egg penetration and sperm storage within the hen's oviduct. Poultry Science 75:1546-1554.

McDaniel, C., R. Bramwell, J. Wilson, and B. Howarth Jr. 1995. Fertility of male and female broiler breeders following exposure to elevated ambient temperatures. Poultry Science 74:1029-1038.

McDaniel, G., J. Brake, and M. Eckman. 1981. Factors affecting broiler breeder performance. 4. The interrelationship of some reproductive traits. Poultry Science 60:1792-1797.

McFarlane, J., and S. Curtis. 1989. Multiple concurrent stressors in chicks. 3. Effects on plasma corticosterone and the heterophil: lymphocyte ratio. Poultry Science 68:522-527.

McGary, S., I. Estevez, M. Bakst, and D. Pollock. 2002. Phenotypic traits as reliable indicators of fertility in male broiler breeders. Poultry Science 81:102-111.

McGary, S., I. Estevez, and E. Russek-Cohen. 2003. Reproductive and aggressive behavior in male broiler breeders with varying fertility levels. Applied Animal Behaviour Science 82:29-44.

Millman, S., and I. Duncan. 2000. Effect of male-to-male aggressiveness and feed-restriction during rearing on sexual behaviour and aggressiveness towards females by

male domestic fowl. Applied Animal Behaviour Science 70:63-82.

Millman, S., I. Duncan, and T. Widowski. 2000. Male broiler breeder fowl display high levels of aggression toward females. Poultry Science 79:1233-1241.

Moyle, J., D. Yoho, R. Harper, and R. Bramwell. 2010. Mating behavior in commercial broiler breeders: Female effects. The Journal of Applied Poultry Research 19:24-29.

Mumma, J., J. Thaxton, Y. Vizzier-Thaxton, and W. Dodson. 2006. Physiological stress in laying hens. Poultry Science 85:761-769.

Novikov, B. 1979. Hypothalamic Mechanisms of Reproductive Function in Poultry. World's Poultry Science Journal 35:214-226.

Pizzari, T., and T. Birkhead. 2001. For whom does the hen cackle? The function of postoviposition cackling. Animal Behaviour 61:601-607.

Post, J., J. Rebel, and A. ter Huurne. 2003. Automated blood cell count: a sensitive and reliable method to study corticosterone-related stress in broilers. Poultry Science 82:591-595.

Puvadolpirod, S., and J. Thaxton. 2000. Model of physiological stress in chickens 3. Temporal patterns of response. Poultry Science 79:377-382.

Queiroz, S., and V. Cromberg. 2006. Aggressive behavior in the genus Gallus. Revista Brasileira de Ciência Avícola 8:1-14.

Ramsay, A. 1951. Familial recognition in domestic birds. The Auk 68:1-16.

Reis, L., L. Gama, and M. Soares. 1997. Effects of short storage conditions and broiler breeder age on hatchability, hatching time, and chick weights. Poultry Science 76:1459-1466.

Romero, L., and J. Reed. 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology 140:73-79.

Rushen, J. 1983. The development of sexual relationships in the domestic chicken. Applied Animal Ethology 11:55-66.

Sands, J., and M. Smith. 1999. Broilers in heat stress conditions: effects of dietary manganese proteinate or chromium picolinate supplementation. The Journal of Applied Poultry Research 8:280-287.

Selye, H. 1976. Forty years of stress research: principal remaining problems and misconceptions. Canadian Medical Association Journal 115:53-56.

Sexton, K., J. Renden, D. Marple, and R. Kempainen. 1989. Effects of dietary energy on semen production, fertility, plasma testosterone, and carcass composition of broiler-breeder males in cages. Poultry Science 68:1688-1694.

Shini, S., P. Kaiser, A. Shini, and W. Bryden. 2008. Differential alterations in ultrastructural morphology of chicken heterophils and lymphocytes induced by corticosterone and lipopolysaccharide. Veterinary immunology and immunopathology 122:83-93.

Siegel, H. 1980. Physiological stress in birds. Bioscience 30:529-534.

Siegel, H. 1995. Stress, strains and resistance. British Poultry Science 36:3-22.

Siegel, P. 1963. Selection for body weight at eight weeks of age. 2. Correlated responses of feathering, body weights and reproductive characteristics. Poultry Science 42:896–905.

Singh, H. 1999. Optimizing delivery of genetic merit in subtropical climates through advanced reproductive technologies. Poultry Science 78:453-458.

Turkyilmaz, M. 2008. The Effect of Stocking Density on Stress Reaction in Broiler Chickens during Summer. Turkish Journal of Veterinary and Animal Sciences 32:31-36.

Vahdatpour, T., K. Adi, Y. Nezhad, N. Sis, S. Riyazi, and S. Vahdatpour. 2009. Effects of Corticosterone Intake as Stress-Alternative Hormone on Broiler Chickens: Performance and Blood Parameters. Asian Journal of Animal and Veterinary Advances 4:16-21.

Vakili, R., and F. Akbarogli 2006. Effect of feed restriction method during rearing on growth and blood indices of stress in broiler breeder. Proc. XII European Poultry Conference, Verona, Italy.

Végi, B., Z. Szoke, and J. Barna. 2006. Monitoring of the effects of various spiking techniques in broiler breeder flocks by counting of IPVL holes of the eggs. Proc. Proc. XII European Poultry Conference, Verona, Italy.

Verhulst, S., S. Dieleman, and H. Parmentier. 1999. A tradeoff between immunocompetence and sexual ornamentation in domestic fowl. Proceedings of the National Academy of Sciences 96:4478-4481.

Weil, S., I. Rozenboim, A. Degen, A. Dawson, M. Friedländer, and A. Rosenstrauch. 1999. Fertility decline in aging roosters is related to increased testicular and plasma levels of estradiol. General and Comparative Endocrinology 115:23-28.

Wilson, H., N. Piesco, E. Miller, and W. Nesbeth. 1979. Prediction of the fertility

potential of broiler breeder males. World's Poultry Science Journal 35:95-118.

Wilson, J. 2002. Hatchery/Breeder Tip... Understanding the Factors that Influence Broiler Breeder Flock Fertility. The University of Georgia Cooperative Extension Service

Wilson, J. L. 1999. Hatchery/Breeder Tip... Implementing a Spiking Program. The University of Georgia Cooperative Extension Service

Wolanski, N., R. Renema, F. Robinson, and J. Wilson. 2004. End-of-season carcass and reproductive traits in original and replacement male broiler breeders. The Journal of Applied Poultry Research 13:451-460.

Wood-Gush, D. 1958. The effect of experience on the mating behaviour of the domestic cock. Animal Behaviour 6:68-71.

Wood-Gush, D. 1960. A study of sex drive of two strains of cockerels through three generations. Animal Behaviour 8:43-53.

Wood-Gush, D., and R. Osborne. 1956. A study of differences in the sex drive of cockerels. The British Journal of Animal Behaviour 4:102-110.

Ylander, D., and J. Craig. 1980. Inhibition of agonistic acts between domestic hens by a dominant third party. Applied Animal Ethology 6:63-69.

Zulkifli, I., P. Liew, D. Israf, A. Omar, and M. Hair-Bejo. 2003. Effects of early age feed restriction and heat conditioning on heterophil/lymphocyte ratios, heat shock protein 70 expression and body temperature of heat-stressed broiler chickens. Journal of thermal biology 28:217-222.

Zulkifli, I., and P. Siegel. 1995. Is there a positive side to stress? World's Poultry Science Journal 51:63-76.

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