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HATCHABILITY OF END-OF-LAY BROILER BREEDER EGGS AS INFLUENCED BY COOLING DURING INCUBATION

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by

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ABSTRACT

Over the last few decades, hatchability of chicken eggs has been a topic of concern for researchers. Most of this research is concerned with incubation and the optimal conditions for it. There are many factors affecting hatchability after the eggs are laid and during incubation. This research was conducted to determine the effects on hatchability of end-of-lay broiler breeder eggs as a result of cooling during incubation.

Three experiments were conducted to determine if the hatchability of eggs produced by end-of-lay broiler breeders could be improved by cooling during the incubation period. In the first experiment, 1,320 freshly laid eggs from two flocks of 51 week old Ross 708 broiler breeders were obtained from a commercial breeder farm. The eggs were placed in an egg cooler at 15.5°C at a relative humidity of 60% for three days. On the third day, all eggs were randomly distributed before being assigned to a treatment. These treatments were cooled to room temperature (approximately 24°C) either 0, 40, 80, or 120 minutes on days 18-20 of incubation. On day seven, eggs were candled, and the infertile and early-dead removed. These eggs were broken to confirm fertility. Eggs were transferred on the eighteenth day. Chicks and unhatched eggs were recorded and the resulting hatchability calculated. Data were analyzed by a randomized block design, with level in the incubator serving as the block. The experimental unit was a group of 30 eggs. None of the treatments improved fertile hatchability. All of the cooling treatments lengthened the incubation process.

The second experiment was conducted similar to the first, with the exception that eggs came from 60 week old Hubbard broiler breeders. In this experiment, the treatments were cooled either 0, 18, 24, or 30 hours at 22°C on the 16th day of incubation. Eggs were candled on the seventh day. At hatch, chicks and unhatched eggs were recorded and hatchability data calculated. Once again, fertile hatchability was not improved by cooling during the incubation process. The incubation period was lengthened for eggs that were cooled during the incubation period.

The third experiment also was conducted similar to the second, with the exception that eggs came from a flock of 59 week old Hubbard and a flock of 58 week old Ross 708 birds. In this experiment, the treatments were cooled either 0, 6, 12, or 18 hours at 22°C on the sixteenth day of incubation. Egss were candled on the seventh day. At hatch, chicks and unhatched eggs were recorded and hatchability data calculated. Yet again, fertile hatchability was not improved by cooling during the incubation process. The incubation period was lengthened for eggs cooled during the incubation period.

INTRODUCTION

The poultry industry has advanced from millions of backyard flocks to less than fifty vertically integrated and highly specialized agribusiness firms. In 1934, 11,405 facilities hatched all the chickens in the United States. Those hatcheries had an average incubator capacity of 24,244 eggs. In 2001, only 323 chicken hatcheries were located in our nation, but incubator capacity had reached 862 million eggs (National Agricultural Statistic Service, 2002). Throughout the latter half of the twentieth century and early twenty-first century, the demand for poultry in the U.S. has steadily increased. As poultry has gained market share of consumer meat expenditures, beef has lost market share and pork has remained relatively constant. Chosen for its low fat content and price, poultry is seen in a diverse set of food products that include wings, drumsticks, and tenders.

Commercial poultry production is one of the fastest growing capital-intensive animal industries in North America. The value of the United States broiler industry was estimated to be $5.68 billion in 1985. This estimate has exploded to $21.5 billion in 2007 (Crop Reporting Board, 1986; National Agricultural Statistics Service, 2008). U.S. exports of poultry during this same time increased substantially by 502% (Schaal and Cherian, 2007). In accordance with this trend, each year the number of broiler breeder eggs set has increased in domestic commercial hatcheries. The number of turkey eggs set has increased by 33% over the past twenty years. Over 11 billion eggs were set in commercial hatcheries in 2005, compared with 5.6 billion eggs in 1985, an increase of 98% (Schaal and Cherian, 2007).

The success of this industry is dependent on the ability to set and hatch fertilized eggs. The chicks must then grow quickly and produce sufficient meat. Successful companies are able to supply large orders of breeding stock that perform competitively in the global market. Therefore, the industry's primary concern is the ability to hatch each chick successfully in a commercial hatchery.

Broiler breeders are the most reproductively inefficient type of commercially raised chicken and are second only to turkeys. An increase, even if only as small as 1%, in hatchability of broiler breeder eggs would increase cost effectiveness tremendously. This degree of improvement would be highly desirable on both a national and international scale. The same incubation and egg handling techniques that were developed more than half a century ago are still used today, although today's broiler is considerably different from those of the 1950s.

The reproductive inefficiency of broiler breeders increases with age. In broiler breeders greater than 30 weeks of age, a decline is seen in the hatchability of their eggs by 15% (Leeson and Summers, 2000). Lower hatchability of eggs from post-peak broiler breeders is a result of many contributing factors, including larger egg size (Leeson and Summers, 2000), increased early and late embryo mortality (Haggar et al. 1986; Elibol and Brake, 2003), poorer shell quality (North and Bell, 1990), and other complications unique to large eggs from post-peak broiler breeders.

The larger than average post-peak broiler breeder eggs hatch larger than average chicks. Additionally, during the latter part of incubation, chicken embryos are subjected to stressors due to an increase in metabolic heat. With these larger eggs, it is believed that the metabolic heat generated is also greater than normal eggs, and may be to blame for decreased hatchability in post-peak broiler breeders. To test this hypothesis, eggs were cooled during late incubation (Experiment 1). In Experiments 2 and 3, the goal was to replicate and expand upon the results obtained by Sarpong and Reinhart (1985)where post-peak broiler egg mortality was lowered and hatchability improved by cooling on the sixteenth day of incubation. This presumptiously providing a longer resting phase for the revitalization of the embryo prior to the expenditure of energy needed for pipping and emerging from the shell (Sarpong and Reinhart, 1985). A second possibly is that Sarpong and Reinhart lowered chicken embryo stress, which resulted in the increased hatchability.

Although improvements have been obtained over the years in livability and in feed efficiency, very little improvement has been seen in hatchability. These results produced by Sarpong and Reinhart (1985) are very promising, and prompted this additional research, which will hopefully lead to adjusting recommended egg management and incubation procedures for end-of-lay broiler breeders. Finding the optimum handling and incubation techniques has the potential of a huge economic impact on the industry.

LITERATURE REVIEW

Hatchability is used in hatchery management and broiler breeder operations as an economic efficiency indicator and to monitor reproductive success. No major advances in hatchability have been achieved during the last twenty years, as hatchability rates have hovered between 79-82% (Schaal and Cherian, 2007). However, in this same time period, improvements have been made in growth rate, meat yield, and feed conversions, resulting in a decreased number of days between birth and market, increased bird growth, and increased carcass yield. This move towards higher yielding birds will continue as a result of market requirements, and as a result, greater effort will be required in breeder management to maintain reasonable fertility (Pollock, 1999). Current breeder management does not place enough emphasis on hatchability.

Numerous factors influence hatchability, but there are three main effects of concern: breeder flock, storage, and incubation. The breeder flock effect includes pre-oviposition variables that can directly affect the parent stock. The storage period exists from the time of oviposition until the commencement of incubation. Industry standards recommend a period of at most four days in length at the hatchery or farm. The incubation period lasts for exactly 21 days and has many variables that can be manipulated, such as relative humidity, temperature, turning angle, position of egg, and turning frequency. Though the process from time of egg formation to hatch is very complex and involves rapid differentiation of blastodermal cells, the poultry industry is currently researching ways of improving hatchability by manipulating controllable factors during incubation.

Breeder Flock Effect

Characteristics of the parent flock are important to understand because of their effect on the reproductive cycle. Genetic differences naturally exist between different lines of birds. In addition, the poultry industry selects for certain physiological traits based on genetics, but this selection can affect the embryo and egg development. For example, McNary *et al*., (1960) found advanced embryonic

development in the eggs from hen populations selected for low body weight. Furthermore, eggs from low body weight hens had increased hatchability and more advanced embryonic development at oviposition than hens selected for high body weight (Coleman and Siegel, 1966). Thus, it would appear a negative correlation exists between hen body weight and reproductive success, which may explain the static hatchability rates over the last twenty years as birds have become heavier (Creel and Maurice, 1998). As selection continues toward high yield and body weight, hatchability, and fertility could be severely devalued.

Breeder Age

Researchers that have studied the effect of broiler breeder age have found in various commercial broiler breeder operations with a multitude of management variables have found that numerous factors affect hatchability, but that hatchability is essentially a function of age (Creel and Maurice, 1998). At first, it was believed that hatchability declined at a linear rate, similar to the egg production cycle. However, it has been shown that shown that hatchability increases to an average peak of 87.3% at 33 weeks old, when it begins to decline. At 49 weeks of age, hatchability drops precipitously. Creel and Maurice (1998) used a segmented regression model to describe this trend.

The decline in fertility and hatchability due to age has both male and female counterparts. There are many female factors due to aging in females that influence hatchability. Examples include multiple or internal ovulations, impaired capacity of sperm tubules, faulty shell deposition, and retrograde transport (Creel and Maurice, 1998). Another age-related explanation for the decline in hatchability is an increase in the number of first-of-sequence eggs. These eggs are larger, have thinner shells, and are less viable (Creel and Maurice, 1998). Likewise, a correlation has been found between eggs from older laying hens and a rise in albumen pH, which must remain relatively stable (Brake *et al*., 1997). It has been noted that eggs from older flocks have the highest hatchability rates if set at the time

of lay, while eggs stored from younger flocks do not have decreased hatchability rates when stored for short periods of time (Reis *et al*., 1997).

The male effects on hatchability are alleviated with the commercial practice of spiking. Spiking involves adding young roosters in the hen house in the hopes of increasing fertility by overcoming the negative effects of older males. Younger males have higher volumes of ejaculate that contain greater concentrations of sperm than their older counterparts (Bramwell *et al.*, 1996). As males age, there is a reduction in the number of spermatozoa in the ejaculate and the volume of semen produced (Bramwell *et al*., 1996). Additionally, male competition, physical injuries, and decreased libido are contributing factors to fertility. However, females have a large effect because of their reproductive qualities.

Egg Quality

There are different aspects of egg formation and composition that can have an effect on hatchability. As the developing embryo relies on the diffusion of gas in order to survive, eggshell pores pay an important role. Eggshell quality has been quantified commonly by calculating egg specific gravity, which is the egg's density compared to water. The specific gravity of an egg is positively correlated to shell porosity. Roque and Soares (1994) observed that eggs with a larger specific gravity had a higher hatchability overall. Higher specific gravity eggs had lower mid- and late-embryonic mortality when compared to thin-shelled eggs. Thick-shelled eggs also are more resistant to impact, and thus less likely to break during transportation.

A certain level of weight loss must be reached during incubation in order for an egg to hatch. The initial egg weight, which is dictated by the female, will play a role in determining the full weight loss. Eggs have been found to vary in weight by time of lay. The weights of early laid eggs were significantly higher than middle laid and late laid eggs (Zakaria *et al*. 2005). Also of interest to researchers is the state of embryonic development at oviposition because of the subsequent influences on hatching

results. Mather and Laughlin (1979) reported that eggs from older birds hatched earlier and that embryonic development progressed more quickly.

Kirk et al. (1980) conducted a study to evaluate for peak performance of post-peak broiler breeders. It was observed that hatchability and fertility decreased with age. Sixty-week old post-peak broiler breeder fertility declined by 11% in this study, and hatchability declined 9% in eggs weighing more than 70 grams. This effect can be explained in part by a relationship that exists between breeder age and egg weight where younger breeders produce smaller eggs. It has been found that superior hatchability occurs at an average egg weight of 60 grams. Similar results were obtained by Reis *et al.*, (1997) who also examined weight loss due to evaporation between large eggs from post-peak broiler breeders and small eggs from young broiler breeders. The results indicated that eggs from post-peak broilers usually lost more weight in grams, but less in total percentage lost when compared to eggs from younger broiler breeders. This can be explained by the fact that larger eggs have less shell area per unit of interior egg weight than smaller eggs (Kirk *et al.*, 1980; North and Bell, 1990; Reis *et al.*, 1997; Roque and Soares, 1994). Secondly, yolk size increases more than the quantity of albumen as egg size increase.

Incubation Effect

From the moment of fertilization of the ovum, embryogenesis starts, and the environment begins playing an important role in embryo development. Half a century ago, the poultry industry determined what environmental conditions were the most conducive to hatchability of fertile eggs, and little has been changed since. The industry's current incubation practices were summarized by Wilson (1990). Eggs are set in an incubator after an optional storage period large end up. For the first nineteen days of incubation, constant temperature is provided and a relative humidity of 60% is maintained in the incubator. On days nineteen and twenty, relative humidity is increased and temperature is decreased. Temperature is the single most important environmental factor in determining or influencing hatchability.

Temperature

The temperature that will maximize both chick quality and hatchability is around 37.8°C (Yalcin and Siegel 2003). This should not vary more than 0.3°C (Wilson, 1990). The commonly held belief is that chick embryos need a fixed amount of heat for complete development, which explains the longer incubation period for eggs incubated at lower temperatures. Variation in incubation temperatures has been shown to affect functional processes, embryonic growth, and hatchability. Tolerance for this variation is smaller when the optimum temperature is exceeded, but can be offset by dramatic variations in temperature. Variation also varies within and between strains. As embryos age, heat must be decreased due to an increase in heat production and metabolic rate of the un-hatched chick. Subsequently, eggs are transferred to hatching baskets on the nineteenth day of incubation because the hatcher operates around 37.0°C. This is in accordance with the findings of Leksrisompong *et al*., (2007) which showed that high incubation temperatures at the end of incubation reduce body weight when compared to normal incubation temperatures.

Turning

Discovering the optimum turning of eggs during artificial incubation has been of historic interest to scholars. Olsen observed that hens moved their eggs during natural incubation approximately ninety-six times per day in 1949. Since this original report, many subsequent reports described similar results, and the turning of eggs is now an industry standard. Hatchability was maximized at a turning frequency of ninety-six times per day, but a more practical frequency is twenty-four times per day (Wilson, 1990). This artificial turning is believed to mimic the natural process of mother hens. Turning prevents the adhesion of the embryo or extra-embryonic membranes to the eggshell. Adhering may stunt normal development of the chorioallantoic membrane, which leads to fatality (Zakaria *et al*. 2005). These malformations due to adhering are more likely in end-of-lay broiler breeder eggs, and a higher rate of turning is beneficial as flock age increases.

The angle of turning also has been characterized as a critical aspect of incubational turning. When turning at angles between 20 and 75 degrees from vertical, the best results occurred at 45° (Funk and Biellier, 1944). Many companies use a reduced turning angle to increase incubator capacity, reduce costs, and alter airflow. The interaction among position, turning, and angle remains ambiguous. Elibol and Brake (2003) found that the occurrence of malpositioned embryos was increased by lowering the turning angle, but this effect was corrected for by an increase in turning frequency. These data lead one to believe that a lower turning angle could be used commercially, but only in conjunction with an increase in turning frequency.

Storage Effect

Eggs are not incubated immediately after laying because of the demand of large numbers of chicks within a constrained period. Fluctuations in the demand for eggs are dealt with by storing eggs and decreasing individual incubations. Eggs are stored for up to four days at the breeder farm while enough eggs are being collected for the next pick-up, and up to two days at the hatchery. During storage, the temperature, length of the storage period, gaseous environment, humidity, and orientation of the eggs all influence hatchability (Meijerhof 1992; Fasenko, 2007) It has long been recognized by the poultry industry that egg storage longer than seven days is detrimental to hatchability (Fasenko *et al*. 2001). After seven days of storage, the initiation of the development of the embryo can be delayed (Mather and Laughlin, 1977). However, this problem can be partially corrected by raising incubation temperature during the first days of incubation.

Unfortunately, egg storage also prolongs incubation time, retards embryonic development, and reduces chick weight (Ruiz and Lunam 2002). Additionally, egg storage depresses albumen quality, affects embryonic viability in all flock ages and results in less percentage of good quality day-old chicks (Lapao *et al.*, 1999; Tona *et al.*, 2004). Storage causes malformations of the embryo and morphological

changes in the blastoderm, which could negatively impact embryonic development, and subsequently hatchability.

Storage Position

The poultry industry long believed that keeping eggs in the small end down position would produce the highest rate of survival because it maintained the air space in the original position. However, it is now known that the developing embryo moves around greatly in this position. After storage, the embryo elevates towards the air space and eventually comes into contact with the shell membrane. In this position, the embryo is more likely to dehydrate and adhere to the membrane. A better alternative to storing eggs large-end up is storing eggs small-end up. This keeps the central yolk and blastoderm in the position for optimal hatchability (Mayes and Takeballi 1984). The blastoderm is maintained in the standard equatorial zone as opposed to moving to various areas on the yolk.

Storage Temperature

Once an egg is laid, it begins to cool to the temperature of its surroundings. As it cools, embryonic development slows down, and eventually comes to a complete halt. "Physiological zero" refers to the temperature at which the embryo begins to develop or the temperature at which embryonic development ceases, depending on whether the eggs are being warmed during incubation or cooled during storage or incubation. Depending upon which report you are reading, physiological zero varies between 28°C and 21°C (Edwards 1902; Funk and Biellier 1944; Mayes and Takeballi 1984). It is now thought there is an acceptable range of appropriate storage temperatures, depending on how long the eggs will be stored. Storage periods for longer than one week will have the highest hatchability if they are cooled to 10-15°C. For storage periods of four to seven days, a temperature of 16-17°C is ideal. Finally, storing eggs at 20-25°C is suggested when cooling for fewer than four days.

Humidity and air movement work along with temperature to impact weight loss through evaporation. Relative humidity (RH) is a term used to describe the amount of water vapor that exists in

a gaseous mixture of air and water vapor. It is measured at a given temperature and expressed as a percentage (Mayes and Takeballi 1984). High relative humidity during preincubation storage is advantageous to the producer in regards to hatchability and should be maintained near the condensation point (Proudfoot 1970). Mayes and Takeballi (1984) reiterated this when they concluded that water loss lowers the hatchability rate. The recommended relative humidity when storing and cooling eggs is 75% (North and Bell, 1990).

Storage Length

Ideally, eggs should be set in the incubator immediately after they are laid in order to optimize hatchability and reduce problems associated with storage. However, in commercial hatcheries storage is inevitable, and as a result hatcheries often aim to minimize storage losses by setting eggs after only three days. Studies have shown that a dramatic negative correlation exists between storage length and hatchability (Meijerhof 1992), especially when the storage period is longer than seven days (Heier and Jarp, 2001). For example, Mather and Laughlin (1979) reported that every day storage is increased means an increase in malformed embryos and an added hour to hatching time, most likely as a result of blastoderm shrinkage. Storage increases embryonic mortality (Kuurman *et al*. 2002) and negatively affects chick quality (Tona *et al*. 2003). Albumen pH increased with storage time, and albumen height significantly decreased (Lapao et al., 1999). In post-peak broiler hens, viability of eggs that were not stored was three to six percentage points higher than that of stored eggs (Reis *et al*. 1997). Hatchability of older flocks decreases at a higher rate than their younger counterparts when storage time is increased (Kirk *et al*., 1980). Long-term storage has a negative effect on hatchability, especially in older birds, but this can be alleviated by exposure to greater incubation temperatures early in incubation (Christensen *et al*. 2003).

Cooling During Incubation

The reproductive inefficiency of broiler breeders increases with age. In broiler breeders greater than 30 weeks of age (post-peak), a decline is seen in the hatchability of their eggs by 15% (Leeson and Summers, 2000). Lower hatchability of eggs from post-peak broiler breeders is a result of many contributing factors, including: larger egg size (Leeson and Summers, 2000), increased early and late embryo mortality (Haggar et al. 1986; Elibol and Brake, 2003), poorer shell quality (North and Bell, 1990), and other complications unique to large eggs from post-peak broiler breeders. Although improvements have been obtained over the years in livability and in feed efficiency, very little improvement has been seen in hatchability. However, Sarpong and Reinhart (1985) lowered mortality and raised hatchability of post-peak broiler eggs by cooling on the sixteenth day of incubation. This presumptiously providing a longer resting phase for the revitalization of the embryo prior to the expenditure of energy needed for pipping and emerging from the shell (Sarpong and Reinhart, 1985). A second possibly is that Sarpong and Reinhart lowered chicken embryo stress, which resulted in the increased hatchability. Previously, Tullett (1990) summarized results to indicate that during the latter part of incubation the chicken embryos are subjected to stressors due to an increase in metabolic heat.

However, some research with cooling during incubation found the exact opposite result. A series of experiments was conducted to determine the effects of cold stress during incubation on chick weight, egg weight loss, hatching time and embryonic mortality (Suarez *et al.*, 1996). Eggs were cooled for various lengths of time to various temperatures on day 8, 12, 14, 16, or 18 of incubation. Chick weights were lower at hatching in chicks from cooled eggs than those of chicks from eggs incubated at normal temperatures and chicks from cooled eggs were more susceptible to dehydration. Cooling during incubation lengthened the incubation period and significantly increased embryonic mortality.

A third conclusion drawn by researchers investigating cooling during incubation is that cooling has no significant effect on any of the variables studied when compared with controls (Zakaria and Al-

Anezi 1996). These results are in accordance with those previously reported by Lancaster and Jones (1988). Lancaster and Jones (1988) cooled eggs from thirteen to eighteen days of incubation for eight to seventy-two hours at 18.3 to 26.7°C. They also concluded that cooling broiler hatching eggs does not have significant effects on hatchability.

Research on cooling during incubation has shown conflicting results and should be further examined to determine the effect of cooling during incubation on hatchability of end-of-lay broiler breeder eggs. It is believed that the metabolic heat generated in post-peak broiler breeder eggs is greater than normal eggs, and may be to blame for decreased hatchability in post-peak broiler breeders. To test this hypothesis, eggs were cooled during late incubation in Experiment 1, when metabolic heat is greatest. In Experiments 2 and 3, the goal was to replicate and expand upon the results obtained by Sarpong and Reinhart (1985) where post-peak broiler egg mortality was lowered and hatchability improved by cooling on the sixteenth day of incubation. This presumptiously providing a longer resting phase for the revitalization of the embryo and lowered chicken embryo stress, which resulted in the increased hatchability.

MATERIALS & METHODS

In Experiment 1 a total of 1,320 eggs laid within a two hour time frame from a flock of 61 week old Ross 708 birds were transported to LSU. When they arrived, they were randomized and assigned to one of four treatments before being cooled at 15.5°C at a relative humidity of 60% for three days. Upon completing the storage period, eggs were randomly distributed before being assigned to a treatment. Eggs were then placed in a Natureform incubator in a randomized block design for eighteen days. During incubation, eggs were candled and the infertile and early fertile dead embryos were removed on day 7. The removed eggs were broken to confirm infertility or embryonic mortality.

At eighteen days of incubation, the treatment groups were transferred to a Natureform hatcher and placed in hatching baskets by treatment. Cooling of the eggs took place over the next three days (days 18, 19, and 20) in hatching baskets for 40, 80, and 120 minutes daily. Eggs were cooled to room temperature (approximately 24°C) . After 504 hours of incubation, chicks were removed and counted. Variables measured were percent true fertility, percent fertile hatchability, percent early dead (1-7 days), percent mid-dead (8-14 days), percent late dead (15-21 days), percent pips, and percent total hatchability.

In Experiment 2, 1,320 fresh broiler breeder eggs from 60-week old Hubbard broiler breeders were transported to LSU where they were placed in an egg cooler at 15.5°C at a relative humidity of 60% for three days. On the third day, all eggs were randomly distributed before being assigned to a treatment. These treatments were subjected to 0, 18, 24, and 30 hours of cooling at 22°C on day sixteen of incubation. A randomized block design was used in the setter for eighteen days. On day seven of incubation, eggs were candled and the infertile and fertile early dead embryos were removed. These eggs were broken to confirm fertility. On day sixteen, the three treatment groups that were to be cooled were removed from the incubator and placed in egg cartons. Upon completion of cooling, eggs were put back into the incubator. At eighteen days of incubation, the treatment groups were

transferred to a Natureform hatcher and placed in hatching baskets by treatment. After 504 hours of incubation, chicks were removed and counted. All unhatched eggs were removed and pips recorded. Variables measured were the same as Experiment 1.

In Experiment 3, 960 eggs from a flock of 59 week old Hubbard and a flock of 58 week old Ross 708 birds were transported to LSU, randomized and assigned to one of four treatments before being cooled at 15.5°C at a relative humidity of 60% for up to 72 hours. Treatments were set at six-hour intervals; the 6-, 12-, and 18-hour groups at 6, 12, and 18 hours earlier than the control, which was set after exactly 72 hours of storage. During incubation, eggs were candled and the infertile and early fertile dead embryos were removed on day 7. The removed eggs were broken to confirm infertility or embryonic mortality. On day sixteen of incubation, the treatments were subjected to 6, 12, and 18 hours of cooling at 22°C. The control group was not cooled on day sixteen. Eggs were transferred to a Natureform hatcher after cooling and placed in hatching baskets that were each randomized into four blocks per level. After 504 hours of incubation, chicks were removed and counted. Variables measured were the same as in previous experiments.

Statistical Analysis

Hatchability data were analyzed by the analysis of variance procedures appropriate for a randomized block design, using the GLM procedure of SAS (SAS, 1996). A flat of 30 eggs was the experimental unit for all hatchability trials. Arcsine of the square root of all dependent variables was used to convert all means prior to analysis. When significant effects were found, means were separated by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

True fertility was not a response variable in this particular experimental research as fertilization of the eggs occurred in the hens at the breeder farms. However, true fertility represents an important ratio that indicates the productivity of the breeder flock as it is necessary in calculating embryonic mortality and fertile hatchability. True fertility for these three experiments ranged from 81-94%, which according to McDaniel et al. (1981) is normal for broiler breeders of these ages.

In experiment 1, no significant effects were observed as a result of the cooling treatments. Fertile and total hatchability were not significantly increased nor decreased by cooling treatments (Figure 1, Table 1). Early-dead and mid-dead embryonic mortality could not be related to cooling since it would have occurred prior to cooling treatments (Figure 2, Table 2). Percent late dead, pips and total mortality were not affected by cooling treatments (Figure 2, Table 2). It can be reasoned that the cooling times were not long enough to do any significant harm or good in regards to hatchability. Percent early-dead was higher than that observed by Elibol and Brake (2003). However, the eggs were transported for four hours from the commercial farm to our research farm which may have contributed to increased early-dead mortality.

In the second experiment, fertile hatchability means ($p = 0.001$) and total hatchability means ($p = 0.009$) were significantly affected by cooling (Figure 3, Table 3). Early dead embryonic mortality means are given in Figure 4 and Table 4. It was not possible for cooling treatments to affect these variables since cooling treatments were applied on day 16 of incubation. Percent late dead ($p = 0.009$), pips ($p = 0.03$) and total mortality ($p = 0.05$) were significantly affected by cooling treatments (Figure 4, Table 4). Cooling eggs for up to 24 hrs beginning on day 16 of incubation did not significantly affect late dead, pips and total mortality. However when eggs were cooled for 30 hrs these variables were significantly decreased. Fertile hatchability, total hatchability, percent early-dead, percent mid-dead, and percent late-dead are consistent with observations made by Elibol and Brake (2003).

In experiment 3, no significant effects were observed as a result of the cooling treatments. Fertile and total hatchability were not significantly increased nor decreased by cooling treatments (Figure 5, Table 5). Early dead and mid dead embryonic mortality could not be related to cooling since it would have occurred prior to cooling treatments (Figure 6, Table 6). Percent late dead, pips and total mortality were not affected by cooling treatments (Figure 6, Table 6). It can be reasoned that the cooling times were not long enough to do any significant harm or good in regards to hatchability.

Figure 1: The effect of cooling on days 18-20 of incubation on fertility, fertile hatchability, and total hatchability of eggs from 51-week old Ross 708 broiler breeders (Experiment 1).

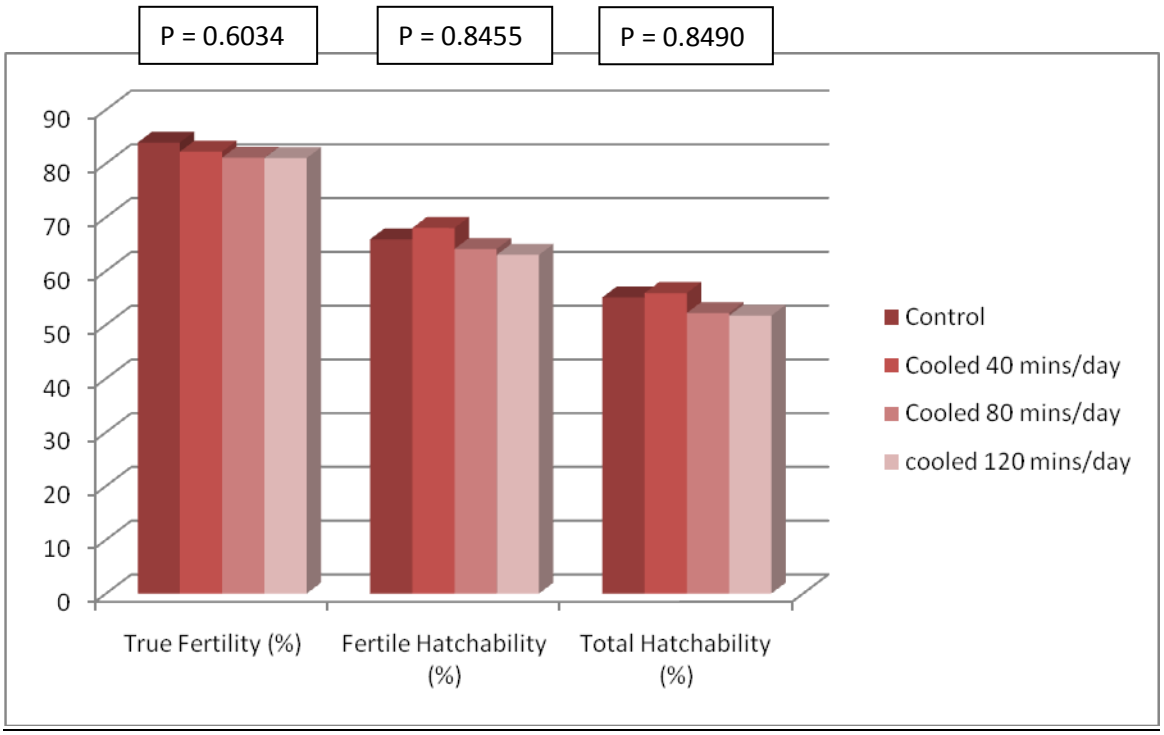

Table 1: The effect of cooling on days 18-20 of incubation on fertility, fertile hatchability, and total hatchability of eggs from 51-week old Ross 708 broiler breeders (Experiment 1).

Cooling Time (Minutes/Day)	True Fertility	Fertile Hatchability	Total Hatchability
	----------(%)----------		
0	83.93±2.0	65.92±3.7	55.15±3.1
40	82.25±1.6	68.08±5.0	55.99±4.1
80	81.15±1.6	64.19±4.8	52.29±4.1
120	81.05±1.9	63.08±4.7	51.72±4.6
P>F	**0.60**	**0.85**	**0.85**

Values are means ± SEM

Figure 2: The effect of cooling on days 18-20 of incubation on embryonic mortality in eggs from 51-week old Ross 708 broiler breeders (Experiment 1).

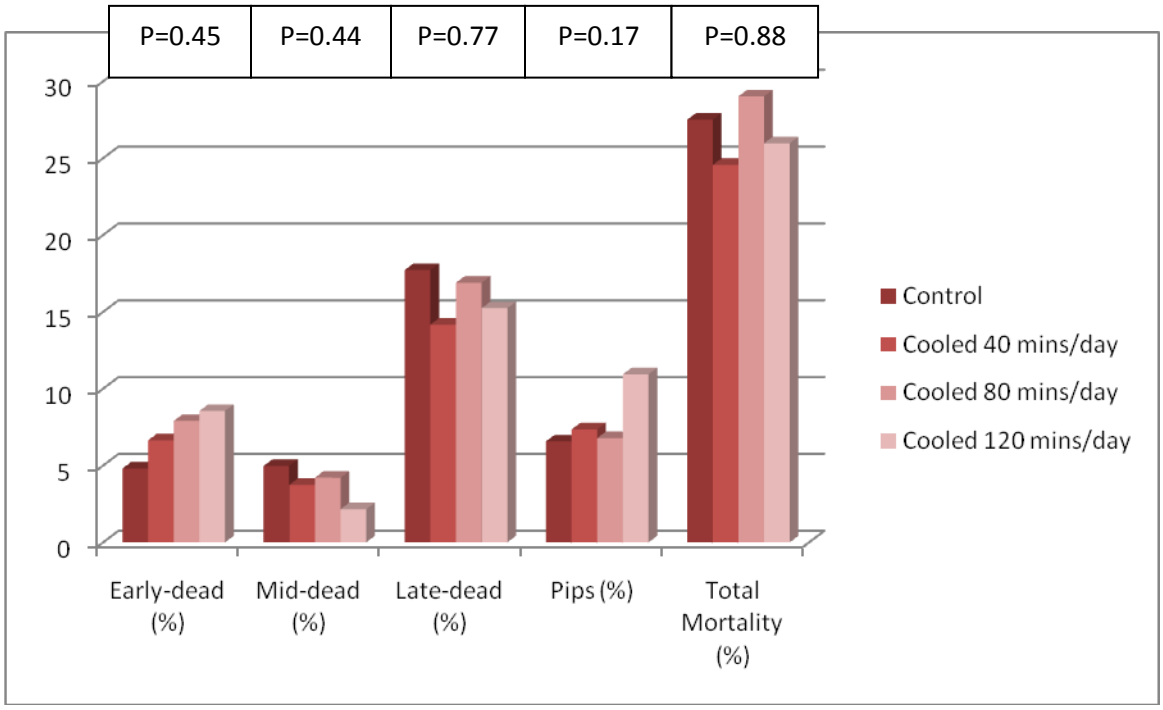

Table 2: The effect of cooling on days 18-20 of incubation on embryonic mortality in eggs from 51-week old Ross 708 broiler breeders (Experiment 1).

Cooling Time (Minutes Per Day)	Early-dead	Mid-dead	Late-dead	Pips	Total Mortality
	----------(%)----------				
0	4.80±1.3	4.97±1.3	17.72±2.9	6.57±1.6	27.50±3.4
40	6.64±1.4	3.74±1.6	14.17±2.3	7.35±2.3	24.56±3.0
80	7.90±1.4	4.21±1.5	16.90±3.3	6.78±1.1	29.02±4.8
120	8.55±1.9	2.16±1.0	15.24±3.5	10.94±1.2	25.97±4.6
P>F	0.4514	0.4456	0.7751	0.1746	0.8838

Values are means ± SEM

Figure 3: The effect of cooling to 22°C on day 16 of incubation on fertility, fertile hatchability, and total hatchability of eggs from 60-week old Hubbard broiler breeders (Experiment 2).

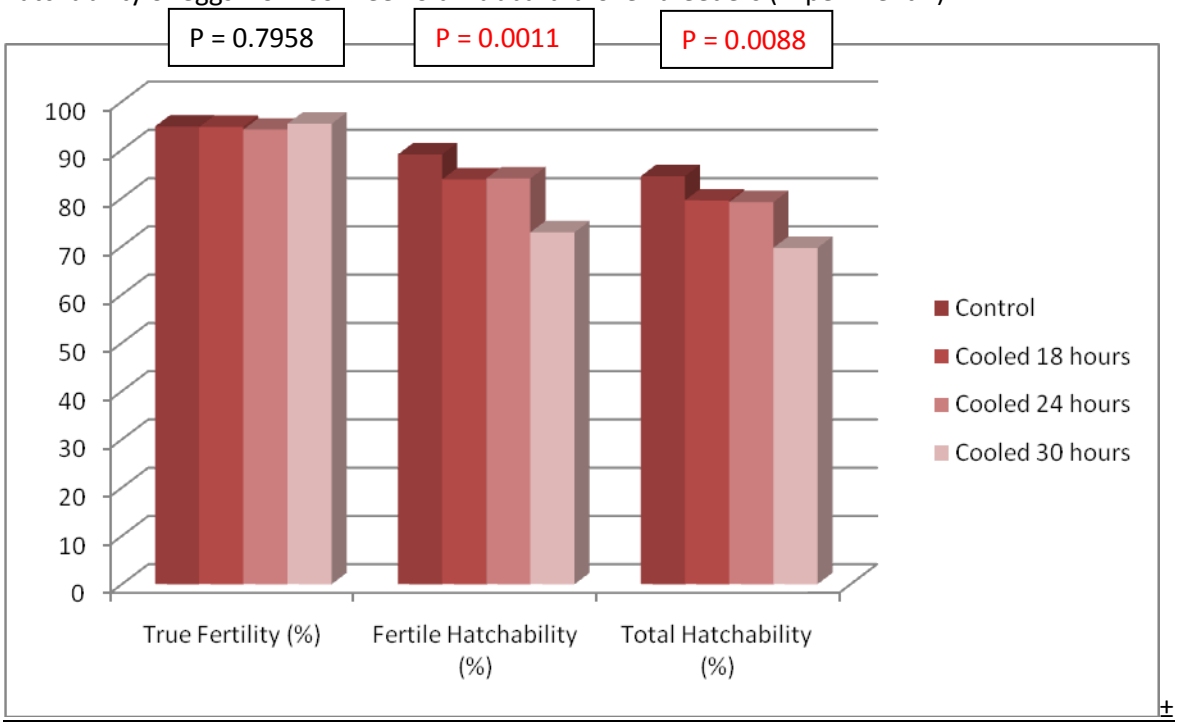

Table 3: The effect of cooling to 22˚C on day 16 of incubation on fertility, fertile hatchability, and total hatchability of eggs from 60-week old Hubbard broiler breeders (Experiment 2).

Cooling Time (Hours)	True Fertility	Total Hatchability	Fertile Hatchability
	----------(%)----------		
0	94.84±2.7[a]	84.54±6.7[a]	89.05±5.2[a]
18	94.78±3.8[a]	79.52±6.9[a]	83.87±5.9[a]
24	94.20±3.6[a]	79.16±7.9[a]	84.08±8.4[a]
30	95.41±4.3[a]	69.69±13.5[b]	72.91±13.5[b]
P>F	**0.79**	**0.001**	**0.009**

a,b Means with different letters in the same column are significantly different (P<0.05).

Figure 4: The effect of cooling to 22°C on day 16 of incubation on embryonic mortality in eggs from 60-week old Hubbard broiler breeders (Experiment 2).

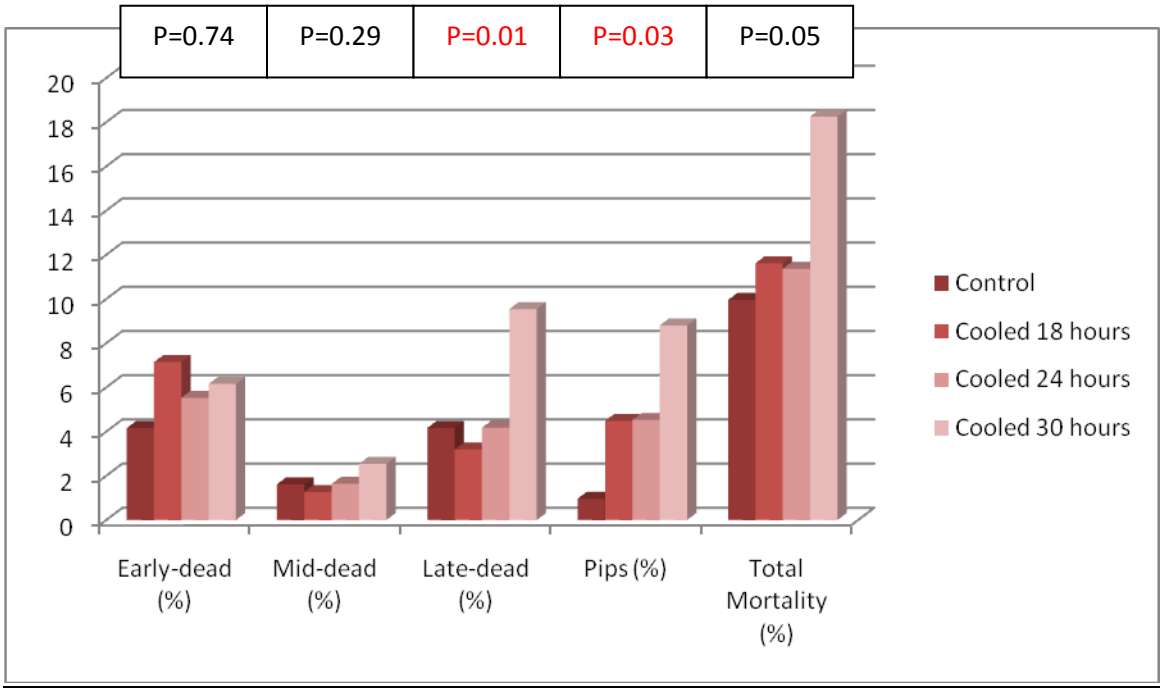

Table 4: The effect of cooling to 22°C on day 16 of incubation on embryonic mortality in eggs from 60-week old Hubbard broiler breeders (Experiment 2).

Cooling Time (Hours)	Early-dead	Mid-dead	Late-dead	Pips	Total Mortality
	----------(%)----------				
0	4.16±1.4^{a}	1.62±2.9^{a}	4.17±3.8^{a}	0.97±1.6^{a}	9.96±3.9^{a}
18	7.15±4.5^{a}	1.27±3.2^{a}	3.19±3.24^{ab}	4.49±3.8^{b}	11.62±6.3^{a}
24	5.53±4.6^{a}	1.64±2.4^{a}	4.18±4.1^{ab}	4.53±3.58^{b}	11.37±6.8^{a}
30	6.16±4.4^{a}	2.54±1.6^{a}	9.55±6.9^{b}	8.81±11.2^{ab}	18.26±7.8^{a}
P>F	0.7443	0.2950	0.0087	0.0308	0.0574

a,b Means with different letters in the same column are significantly different (P<0.05).

Figure 5: The effect of cooling to 22°C on day 16 of incubation on fertility, fertile hatchability, and total hatchability of eggs from 59-week old Hubbard and Ross 708 broiler breeders (Experiment 3).

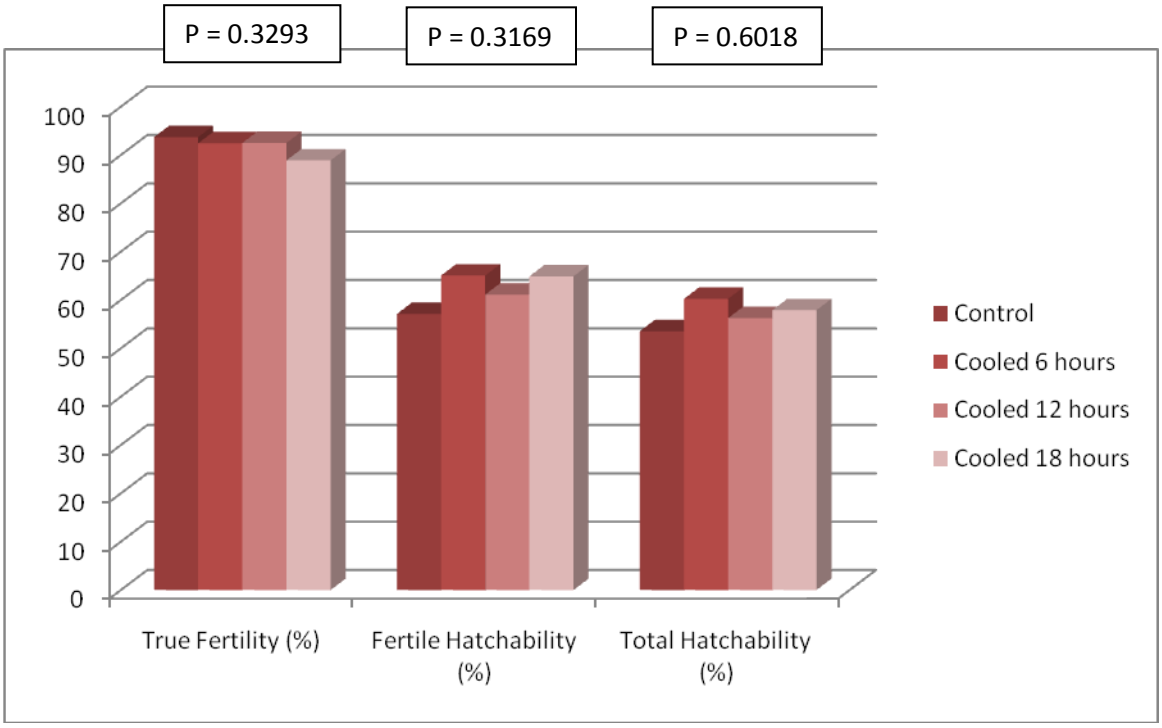

Table 5: The effect of cooling to 22°C on day 16 of incubation on fertility, fertile hatchability, and total hatchability of eggs from 59-week old Hubbard and Ross 708 broiler breeders (Experiment 3).

Cooling Time (Hours)	True Fertility	Fertile Hatchability	Total Hatchability
	----------(%)----------		
0	93.69±5.2	57.12±9.5	53.54±9.8
6	92.44±6.4	65.06±8.6	60.24±9.8
12	92.50±5.2	61.13±12.0	56.25±9.5
18	88.89±6.0	64.90±9.3	57.89±10.7
P>F	**0.32**	**0.31**	**0.60**

Values are means ± SEM

Figure 6: The effect of cooling to 22°C on day 16 of incubation on embryonic mortality in eggs from 59-week old Hubbard and Ross 708 broiler breeders (Experiment 3).

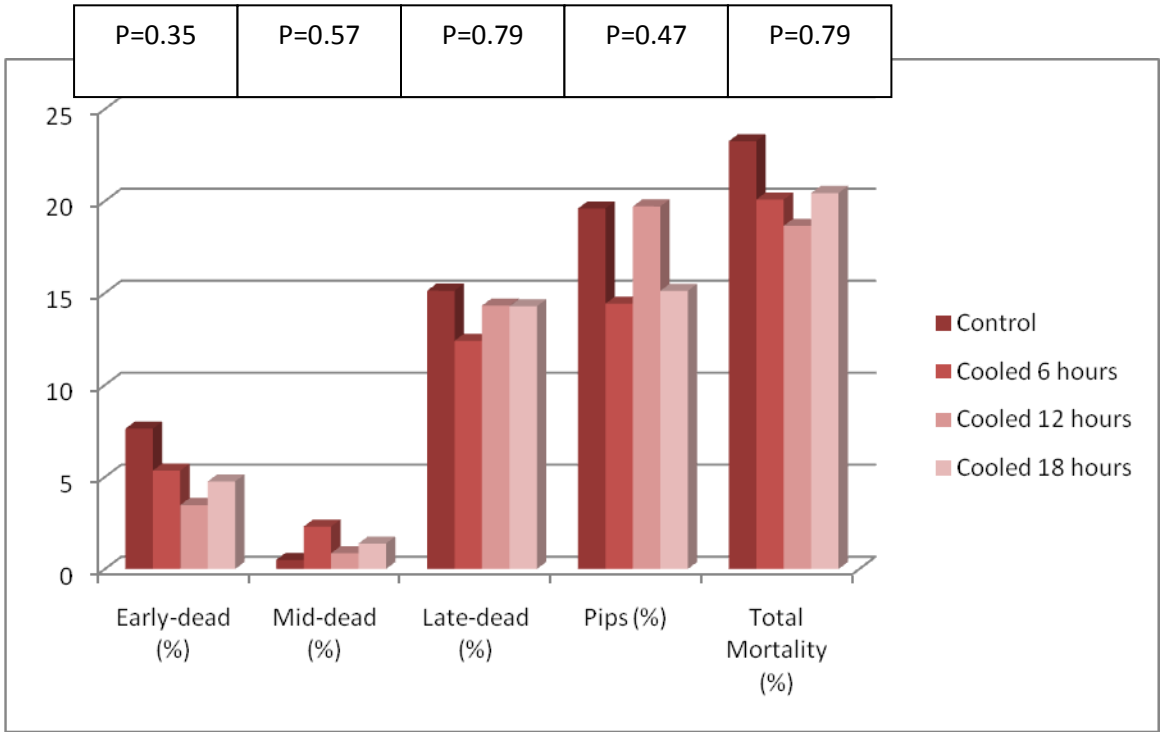

Table 6: The effect of cooling to 22°C on day 16 of incubation on embryonic mortality in eggs from 59-week old Hubbard and Ross 708 broiler breeders (Experiment 3).

Cooling Time (Hours)	Early-dead	Mid-dead	Late-dead	Pips	Total Mortality
	----------(%)----------				
0	7.63±6.3	0.50±1.4	15.13±8.4	19.60±10.4	23.26±10.1
6	5.35±4.3	2.31±4.0	12.41±9.6	14.42±5.0	20.08±9.5
12	3.48±4.1	0.86±1.5	14.32±3.2	19.71±9.1	18.66±7.0
18	4.76±3.3	1.39±1.9	14.28±6.1	15.12±11.1	20.44±8.1
P>F	0.3584	0.5711	0.7940	0.4740	0.7941

Values are means ± SEM

SUMMARY

This study was conducted to determine the effects of cooling for 0,6, 12, 18, 24, or 30 hours during the sixteenth day of incubation on percent fertile hatchability, percentage total hatchability, embryonic mortality, and pips of eggs from post-peak Ross 708 and Hubbard Classic broiler breeders. Additionally, the effect of cooling eggs during incubation for 40, 80 or 120 minutes on the eighteenth, nineteenth and twentieth days of incubation was investigated in regards to the same dependent variables. The results are as follows:

1) Percent fertile hatchability, total hatchability, early dead, late dead, pips, and total embryonic mortality were not affected by cooling for up to 120 minutes daily on days eighteen through twenty of incubation.

2) Percent fertile hatchability, total hatchability, early dead, mid dead, and total embryonic mortality were not affected by cooling on day sixteen of incubation for periods up to thirty hours. Decreased fertile hatchability and total hatchability was observed by cooling for 30 hours when compared to eggs cooled for 0, 18, and 24 hours during incubation. Increased amounts of late-dead embryos were observed upon cooling for 30 hours when compared to the control eggs. Finally, increased amounts of pips were observed by cooling for 18, 24, or 32 hours.

3) The incubation period was lengthened for eggs that were cooled.

CONCLUSIONS

It appears that cooling post-peak broiler breeder eggs during incubation is not beneficial in improving hatchability or decreasing mortality. Pausing embryonic development for up to thirty hours, as was done in the second experiment, decreased fertile hatchability and total hatchability while increasing late-dead embryos and pips. No significant results were observed in the first and third experiments.

Current management practices call for incubating eggs continuously from setting through day eighteen, when the eggs are transferred to a hatcher. However, it is a known fact that hens allow their eggs to experience periods of cooling and warming during incubation. Attempts to mimic this process do not result in improved hatchability rates when compared to chicks hatched using the current industry standards. But, the data from this study indicate that eggs can be cooled for up to eighteen hours without negatively affecting hatchability.

There is no obvious benefit from cooling eggs during incubation. The results of this study do not agree with results reported by Sarpong and Reinhart (1985) who suggested cooling on the sixteenth day of incubation increases fertile hatchability. More research is needed to investigate the effect of cooling for shorter periods of time at a higher frequency during incubation.

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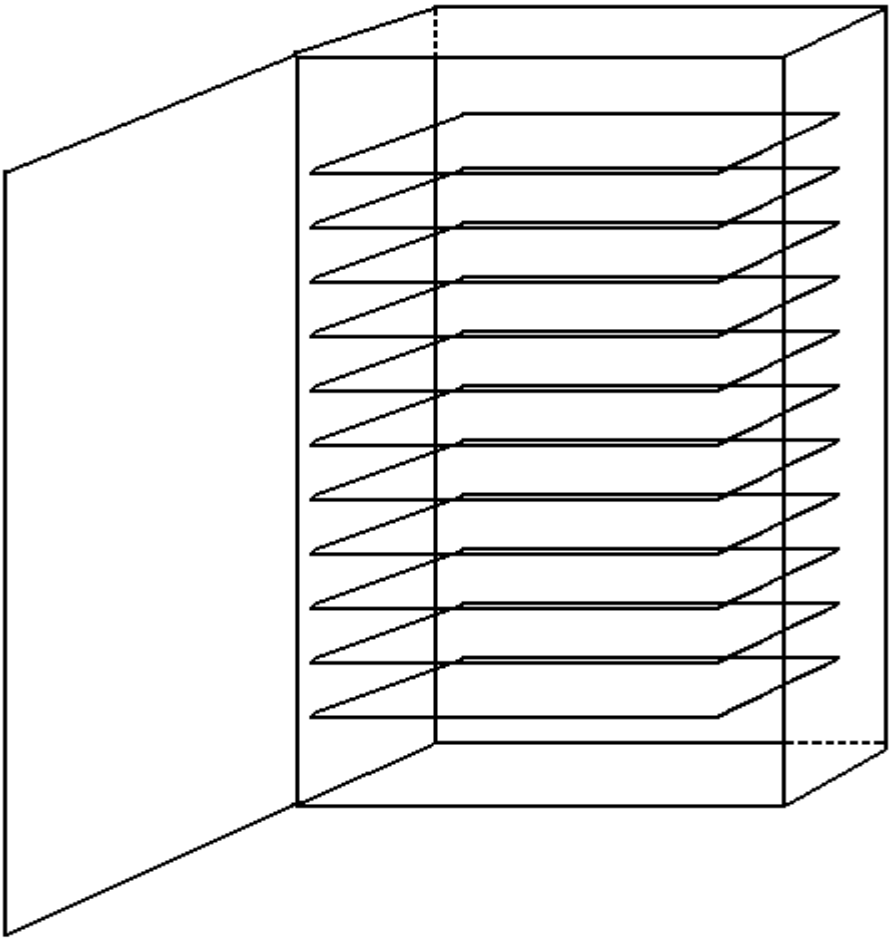
APPENDIX: SUPPLEMENT DATA



1	2	3
4	5	6

Figure A. Natureform setter #2000 showing eleven levels, and position of experimental eggs within a level (Wiggins, 2008).

VITA

Jim Rabalais was born in Lafayette, Louisiana on August 21, 1988. He is the oldest son of Myron and Emily Rabalais, who reside in Katy, Texas. He has one younger brother, Reid (18), who attends Seven Lakes High School and a younger sister, Anna (11), who is in fifth grade. His father, Myron Rabalais, is a District Sales Manager for DuPont Land Management, where he covers the states of Texas and Louisiana. His mother teaches business courses at Seven Lakes High School. Jim lived in Lafayette, Louisiana until the age of fourteen before moving with his family to Katy, Texas. He attended Cinco Ranch High School where he was an active member of the FFA, student council and the football team. While involved in FFA he served as vice-president of the chapter, showed market hogs at local livestock shows and competed in Land & Range Judging, Personal Skills in Agriculture, and Job Interview. In 2006, Jim graduated from high school in the top 10% of his graduating class and is currently in his fourth year at Louisiana State University. He is currently pursuing both a B.S. in Biological Sciences and an Honors B.S. in Animal, Dairy, and Poultry Sciences. During Jim's undergraduate years, he has been an active member and officer of Pi Kappa Phi fraternity, a student government senator, a member of the LSU poultry judging team, and president of Alpha Zeta, the honorary agricultural fraternity. Additionally, Jim was recognized as the College of Agriculture's Outstanding Freshman and Sophomore, and also as one of LSU's Top 10 Freshmen. Jim will be graduating in May, 2010 and will enroll at LSU's School of Medicine in Shreveport in July, 2010. This thesis, under the direction of Dr. Dennis Ingram, serves as the final requirement of his Honors College curriculum.