INCUBATION TEMPERATURE EFFECTS ON BROILER CHICKEN EMBRYOS

by

KATIE ELAINE COLLINS

(Under the Direction of Jeanna L. Wilson)

ABSTRACT

Extreme variations in incubation temperature affect the broiler embryo and, ultimately, post hatch broiler performance. Previous studies have implied that incubation temperature may impact chick quality and sex of the chick. The control of both parameters would be economically beneficial to the poultry industry. This work evaluated embryonic differences between a modern high yielding broiler and a 1950s meat-type chicken strain when incubated at a 1950s incubation temperature (37.7°C) and investigated incubation temperature effects on modern broiler sex ratios. These data suggest that the incubation temperature of 50 years ago is too high for the modern broiler. Wide temperature variations (0.8°C) away from standard temperature were not found to influence the sex ratio of broiler chicks at hatch.

INDEX WORDS: broiler, incubation temperature, sex, Athens Canadian Random Bred

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DEDICATION

This Thesis is dedicated in honor of my father, Dr. Gearl Martin “Marty” Collins, Jr.

and to Abigail, my first chicken.

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CHAPTER 1

INTRODUCTION

A proper incubation temperature is essential in order to hatch high quality broiler chicks. Previous studies have investigated various effects of high and low incubation temperatures on the broiler embryo and post hatch broiler performance. Both variations in incubation temperature can alter embryo metabolism, hinder the number of chicks that hatch, and decrease hatchling body weight and broiler weights at market age (Romanoff et al., 1938; Lourens et al., 2005; Joseph et al., 2006; Hulet et al., 2007; Molenaar et al., 2010; Willemsen et al., 2010, 2011).

Today's commercial broiler can achieve four times the body weight in half the time as compared to the commercial broiler of 50 years ago (Havenstein et al., 1994, 2003). Since incubation time has not changed, the percentage of a broiler's life spent as an embryo inside the incubator has increased over the years (Hulet, 2007). Changes in the broiler embryo due to this post hatch growth selection are uncertain. Some researchers believe modern embryos have a higher heat production than in the past (Hulet, 2007), although broiler embryo comparisons with a 1978 female broiler parent strain found no difference in metabolism or eggshell temperatures (O'Dea et al., 2004). This thesis compares how the broiler embryo of today differs from a 1950s broiler strain when incubated at a 1950s incubation temperature. This comparison can aid in assessing the proper incubation temperature for the modern broiler.

Another uncertainty concerning incubation temperature is the ability to skew chicken sex ratios at hatch by altering the incubation temperature. The ability to control broiler sex ratios

could be economically beneficial to the poultry industry. Incubation temperature was reported to skew Rhode Island Red chicken sex ratios in a published U.S. patent (Ferguson, 1996), but no other known research has successfully reproduced this effect in chickens. Sex skews due to high and low incubation temperatures were found in Australian brush turkeys via sex-biased embryo mortality (Göth and Booth, 2005; Göth, 2007; Eiby et al., 2008). Female biased embryo mortality has been found in certain chicken breeds at a standard incubation temperature (Li et al., 2008; Wu et al., 2012). This thesis investigates if commercial broiler sex ratios can be altered due to incubation temperature.

CHAPTER 2

INCUBATION TEMPERATURE EFFECTS ON BROILER CHICKEN EMBRYOS

LITERATURE REVIEW

Artificial Incubation for Poultry

A chicken of any breed or variety requires approximately three weeks to develop and hatch. Hatch time can be delayed on the order of hours to days due to high elevation, strain (such as White Leghorns selected for slow hatch time), longer storage time prior to incubation, and temperature and humidity (Funk, 1934; Landauer, 1948; Bohren et al., 1977). The proper incubation parameters for a successful hatch have long been investigated. In general, incubating embryonated eggs requires four main factors: temperature, turning, humidity and ventilation (Romanoff, 1929). An appropriate temperature (>21°C, see next section) is necessary to reinitiate cell divisions of the blastodisc (Edwards, 1902). Eggs should be turned frequently and gently, at least 3 and no more than 96 times every 24 hours optimally at a 45° angle from either side of the vertical, particularly during the first two weeks of incubation (Olsen and Byerly, 1936; Funk and Forward, 1960), with the setting Q1 hen observed to rotate her eggs as much as 65 times a day (Olsen, 1930). Turning functions to prevent the embryo from adhering to the shell membranes prior to amnion closure which causes abnormal development and mortality (New, 1957), and promotes the full development of the chorioallantoic membrane, which is responsible for embryo respiration before internal pipping (Tullett and Deeming, 1987). Turning is essential to prevent the chorioallantoic membrane from adhering to the inner shell membrane

too early (prior to day 11 of incubation) and to prevent the allantois adhering to the yolk sac since merely tilting the eggs will not prevent these occurrences (Buhr, 1989). Humidity is an important factor that controls water vapor loss from the egg (Landauer, 1948). Water loss is measured by the weight of the egg before incubating and at 18 days of incubation; a loss of 11-12% for a multistage incubator is considered ideal in order to achieve high hatchability and chick quality (Cobb-Vantress, 2008; Aviagen, 2012b). The majority of nondomestic avian species have a moisture loss of 15% (Rahn et al., 1980). Domestic poultry have <15% moisture loss due to their larger egg size (Landauer, 1948; Reinhart and Moran, 1979). Humidity is also related to temperature as percent humidity increases, temperature should be decreased (Townsley, 1931). Lowering humidity at a constant temperature increases incubation time and decreases chick weight (Townsley, 1931). Adequate ventilation during incubation is required to maintain a uniform temperature, supply oxygen and remove carbon dioxide which is increasingly needed as embryo development progresses (Atwood and Weakley, 1915; French, 1997). As ventilation maintains incubation temperature, oxygen and carbon dioxide are usually kept at an acceptable level.

The artificial incubation of poultry species has a long history with the earliest evidence from 246-207 B.C. (Landauer, 1948). The technology started in China and Egypt using various methods to incubate duck and chicken eggs, including the use of fire below the eggs, using fermented animal manure, heated crop husks and even human body heat in the Philippines (de la Gironière, 1854; Landauer, 1948). Cornelius Drebbel (1575-1633) was the first to invent incubators with temperature controlled by an alcohol-mercury thermostat (Tierie, 1932; Landauer, 1948). Bonnemain invented the first bimetallic thermostat made of iron and brass in 1828 and hatched poultry commercially using his invention (Landauer, 1948). Incubators still

needed improvements in the 20th century (Romanoff, 1929). The origin and eventual increase in incubator technology emerged due to an increased need of food for a growing human population (Landauer, 1948).

Early incubators were still-air, and in the absence of electrical power, relied on convection currents to provide ventilation, having holes for air to enter through the bottom and air to exit out the top of the incubator (Landauer, 1948). In these machines, a "temperature gradient" exists with temperatures "declining from upper to lower surface of the eggs" by as much as 0.6-1.1°C (Landauer, 1948). Commercial incubators are forced-draft incubators which use a fan to provide a more uniform temperature for all eggs in the incubator by both bringing in fresh air and circulating the air within the incubator. The ideal air temperature for a still-air incubator at the top of the eggs is from 38.9-39.4°C and for forced-draft incubators from 37.2-37.8°C (Landauer, 1948).

The history of modern "mammoth incubators" has a shorter timespan on the order of 50-100 years (Chickmaster, 2012). The most frequently seen commercial incubators used in Georgia hatcheries are from companies founded within this last century. NatureForm was founded in 1982 to update the Robbins Incubator Company's commercial incubators of the 1900s (Jefferson County Historical Commission, 1999; NatureForm, 2012). The Jamesway Incubator Company started as the James Manufacturing Company in 1890 (Jamesway, 2010). Chickmaster was founded in 1948 (Chickmaster, 2012). Each of these companies have made their own advances to more efficiently produce high quality and mass quantities of poultry for the same reason as the ancient Egyptians and Chinese: our growing human population.

The studies of this thesis focus on the effects of incubation temperature on the developing commercial broiler embryo. Previous studies have investigated how varied incubation temperatures affect the developing embryo and chick.

Incubation Temperature

Importance and Range of Poultry Incubation Temperatures

Temperature is regarded as the most significant factor for successful incubation (Romanoff, 1960); without the appropriate temperature, embryonic development will not occur, and modest changes in temperature can significantly affect the chick that emerges. Development, though very slow, can occur at temperatures just over 20°C (Edwards, 1902). Division of cells occurs in the 41.7°C body of the hen as the egg transcends the reproductive tract so the freshly laid (stage X) egg contains a blastodisc consisting of as many as 60,000 cells (Eyal-Giladi and Kochav, 1975; Simkiss, 1991). Birds are known to incubate their eggs between 30-40°C (Webb, 1987). Higher temperatures tend to advance early embryonic development while lower temperatures retard the progression of development; however, elevated temperatures have a less dramatic impact on developmental speed than lower temperatures (Barott, 1937; Romanoff, 1960). Generally, cooler temperatures are said to affect embryos and the resulting hatch to a lesser degree than higher temperatures (French 1997; Willemsen et al. 2010). There are temperature limits that will kill a developing embryo. To kill an embryo within the egg with a low temperature requires internal egg temperatures of ice crystal formation at -1.7°C to -1.11°C for 70-95 minutes (Moreng and Shaffner, 1951). Younger embryos were more resistant to these cold temperatures and required longer exposure time to cause death. The high internal egg temperatures required to cause embryonic death range from 41.1°C to 48.3°C. In this high

temperature range, the higher temperatures were required to cause death in older embryos (Moreng and Shaffner, 1951). The most ideal air temperature for forced-draft incubators has been regarded as 37.5°C (Barott, 1937; Deuchar, 1952; Romanoff, 1960). These known temperature ranges for poultry incubation are important for the selection of the incubation temperatures in the studies of this thesis. These temperature ranges are particularly important for the selection of incubation temperatures to test for potential broiler sex ratio manipulation due to incubation temperature.

Methods of Measuring Incubation Temperature

Incubation temperatures can be measured using several different methods: air temperature, eggshell temperature, and internal egg temperatures. The air temperature of the incubator is the measurement of the air surrounding the eggs. Eggshell temperatures are the temperature of the shell of the egg measured at the equator of the egg using an infrared thermometer (Aviagen, 2012a). Internal egg temperatures are measured in the interior center of the egg contents using a thermometer inserted in the middle location of the egg contents (Moreng and Shaffner, 1951). These temperature measurements relate to each other. Higher air temperatures will cause higher eggshell and internal temperatures. As an embryo develops, its metabolic heat production increases, so initially, eggs are cooler than the air temperature, but halfway through incubation, the eggs become warmer than the air temperature (French, 1997). Accordingly, during early incubation, internal temperatures are lower than the air and eggshell temperatures (French, 1997). During later incubation, the internal temperature becomes higher than the eggshell temperature, and the eggshell temperature becomes higher than the air temperature (French, 1997). Heat exchange for the embryo depends on the incubator's temperature and the heat produced by the embryo (French, 1997). Another recently developed

method to measure incubation temperature is the use of temperature transponders placed in the air cell of 10.5-14 day incubated embryonated eggs (Pulikanti et al., 2011a,b; Pulikanti et al., 2012). The researchers have reported 75-90% survival of the embryos following implanting transponders. This air cell temperature records a higher temperature than eggshell temperature and allows for a less destructive method than internal egg measurements (Peebles et al., 2012).

Commercial hatcheries measure air, eggshell, and internal temperatures. Air cell temperatures are not yet widely used due to the price of the transponders. The Jamesway Pilot measures eggshell temperatures of four eggs in three different locations in the incubator and the incubator regulates incubation temperature based on the eggshell temperature (Jamesway, 2013). Hatchery managers also check eggshell temperatures typically using an infrared ear thermometer on select eggs (Tullett, 2009). Commercial machine sensors measure air temperatures and these can be recorded using temperature data loggers such as Tinytags (Tullett, 2009). Internal temperatures have been measured by hatchery managers, yet this method is not as favored compared with eggshell temperatures since internal temperatures destroy the egg.

Many previous studies have investigated the various effects of altered incubation temperatures on the chick embryo and on hatching success. Understanding the various ways to measure incubation temperatures are important to distinguish how these previous studies manipulated the incubation temperature.

Incubation Temperature Effects on Embryonic Growth

Incubation temperatures affect the growth rate of the embryo. Romanoff et al. (1938) found that higher temperatures (40.5°C) during the first week of incubation would increase embryo weights 240% on day 3 of incubation compared to standard (37.5°C) embryo weights, and low incubation temperatures (33.5°C) decreased embryo weight to 10% of the standard

weight on day 3 of incubation. High temperature after the middle of incubation decreases embryo weight (Romanoff et al., 1938). A high eggshell temperature (constant 38.9°C and from days 7-19 of incubation) was found to decrease yolk-free body weights and reduced the efficiency of protein utilization in broiler chicks (Lourens et al., 2007; Molenaar et al., 2010). High late incubation temperatures (18-21 days at 39.5°C) also decreased yolk-free chick weight at hatch (Joseph et al., 2006). Low early incubation temperatures (0-10 days at 36.6°C) increased broiler chick weight at hatch but only due to a larger yolk sac weight, not from an increase in yolk-free chick weight (Joseph et al., 2006). Layer chicks weighed less than control chicks when cooled during incubation (18 or 24°C at various days and hours continuously for 12, 24, 36, 48, or 72 hours on days 8, 12, 14, 16, or 18 or intermittently cooled for 6 hours every 48 hours or for 12 hours every 96 hours) (Suarez et al., 1996). Christensen et al. (2001) found that IGF-I, an anabolic growth factor, was increased in a turkey strain that maintained their body weight in a high temperature (37.2°C) during the last three days of incubation, while another turkey strain that did not increase IGF-I levels had 10% decrease in body weight due to the temperature treatment. Bone development was also affected by incubation temperature in turkeys by changing the air temperature from 37.5°C to 36°C, 37°C, 38°C, or 39°C during late incubation (days 24-28) (Oviedo-Rondón et al., 2008). Femur weight was not affected by the temperature, but the tibia weighed less at 39°C than 36°C while the metatarsus increased in weight between 36°C and 39°C. The femur and tibia decreased in length between the 36°C treatment and the 39°C treatment. Molenaar et al. (2011) found 3.0g heavier yolk-free chick weight in standard temperature chicks at hatch (37.8°C eggshell temperature) than high incubation temperature chicks (38.9°C days 7-21 of incubation). Both low and high incubation

temperatures appear to hinder the yolk-free hatch weight of chicks when compared with standard incubation temperatures.

Incubation temperature changes have been found to alter heart and lung growth. High incubation temperatures (37, 38, and 39°C) on the last four days of turkey incubation (days 25-28 of incubation) were found to decrease heart size and cardiac glycogen and increase cardiac lactate (Christensen et al., 2004). Molenaar et al. (2011) found broiler eggs incubated at a high eggshell temperature of 38.9°C from days 7-21 days of incubation had 0.1g lighter hearts at hatch compared to an eggshell temperature of 37.8°C. Similar results were found at high eggshell temperatures of 39.5°C after day 14 of incubation and at an eggshell temperature of 38.9°C during days 9-19 of incubation (Leksrisompong et al., 2007; Lourens et al., 2007). Yalçin and Siegel (2003) exposed broiler embryos to one of the following incubation temperature treatments: cooled to 36.9°C for 6 hours each day during 0-8 days of incubation, heated to 39.6°C for 6 hours each day from 0-8 days of incubation, cooled to 21.0°C on day 14 for 24 hours, cooled to 36.9°C for 6 hours each day from 10-18 days of incubation, heated to 39.6°C for 6 hours from 10-18 days of incubation, or a control maintained at 37.8°C. Heart weights on day 18 of incubation (measured as a percentage of body weight) were heaviest for the high treatment on days 0-8 and the control had the lowest heart weights. Heart measurements on day 10 of incubation and at hatch did not reveal any incubation treatment effects. Thus, heart weight seems to be negatively affected by longer high temperature exposures during late incubation periods. Relative lung weights (measured as a percentage of body weight) did not differ between the air temperature treatments on day 18 of incubation, but at hatch, the highest lung weights were found in the control (constant 37.8°C) and the lowest were in the two treatments exposed to high (39.6°C) incubation air temperatures. The lung weights of the low

(36.9°C and 21°C) incubation treatments were intermediate. The authors concluded that higher rather than lower temperatures have a greater negative effect on the lungs. Barri et al. (2011) found that high air temperature during late broiler incubation (39.6°C from days 13-21 of incubation) had similar duodenum villi height and crypt depth to a control (37.4°C) until day 10 post hatch when the controls had longer villi and deeper crypt depth showing a delay in the incubation treatment's effect on the intestine. This effect on the intestine could be related to the fact that embryos that experience high temperatures late in development have larger yolk sacs (Willemsen et al., 2010) and have white colored down instead of yellow (Leksrisompong et al., 2007). Larger yolk sacs indicate less yolk utilization by the embryo and the pigment is not deposited into the white down feathers of commercial broilers.

Temperature has great effects on the metabolic reactions of the embryo. Turkey embryos kept in high (39°C) air temperatures in the last four days of incubation had higher plasma glucose levels than those incubated at 36, 37 or 38°C (Christensen et al., 2004). The turkeys incubated at 36° and 38°C had lower plasma glucose levels than the 37°C embryos. Increasing the temperature to 37.2°C the last three days of incubation also caused higher plasma glucose levels compared to 36.8°C in turkey embryos during external pipping (Christensen et al., 2001). Broilers also had high glucose levels in high temperature (40.6°C eggshell temperature days 16-18.5 of incubation) treated chicks which is thought to be due to liver gluconeogenesis conversion of lactate, formed from anaerobic respiration, to glucose (Willemsen et al., 2010). High incubation eggshell temperature (40.6°C) late in broiler incubation (between days 16-18.5) decreased plasma thyroid hormone and liver glycogen while a low incubation temperature (34.6°C) had no effect in chicks at hatch (Willemsen et al., 2010); however, when these eggshell temperatures were applied for only 4 hours during late incubation (days 16, 17, and 18), the

embryos altered their metabolism during the temperature exposure (Willemsen et al., 2011). Embryos in the high temperature treatment had lower partial pressure of oxygen measured in the air cell of the egg, a higher partial pressure of carbon dioxide in the air cell, lowered blood pH, and greater amount of lactic acid which may result from an increased anaerobic metabolism (Willemsen et al., 2011). Embryos from the lower temperature had higher yolk weights, higher plasma triglyceride levels, and higher glycogen in the liver which may indicate a slowed metabolism of lipids and carbohydrates (Willemsen et al., 2011). These embryos recovered by the time they hatched (Willemsen et al., 2011). Thus, high temperatures during late incubation appears to decrease yolk utilization, increase plasma glucose levels, decrease glycogen stores, and decrease thyroid hormone levels.

Incubation Temperature Effects on Hatch Time

Because of the effect of temperature on developmental speed, the duration of incubation is also affected by incubation temperature. Increasingly higher temperatures (range of air temperatures tested are 35°C-39°C constant temperatures at 60% relative humidity and 0.1mile/minute ventilation and 21% oxygen and <0.5% carbon dioxide) have been found to decrease the time required to incubate poultry up to a temperature of 39.5°C (Barrott, 1937; Romanoff, 1936). Air temperatures above 39.5°C increase the time required for incubation (Romanoff, 1936; Willemsen et al., 2010). Lower temperatures (air temperatures tested include: 18°C and 24°C for up to 72 hours on days 8, 12, 14, 16, or 18 of incubation or for 6 hours every 48 hours or 12 hours every 96 hours then incubated at 37.5°, 34.6°C at 30°C wet bulb for 4 hours on days 16, 17, and 18 of incubation lowered from 37.6°C) have been seen to increase the time required for incubation with longer cooling times causing longer incubation times (Suarez et al., 1996; Willemsen et al.,2010). Romanoff et al. (1938) found that high (40.5°C) or low (33.5°C)

incubation temperatures during the first week of incubation have the greatest effect shortening or increasing incubation time than the second and third weeks of chicken incubation.

Incubation Temperature Effects on Hatchability

The number of chicks that hatch can be greatly affected by the incubation temperature. The number of chicks that hatch are recorded in two different ways: hatch of eggs set and hatch of fertile eggs. Hatch of eggs set, or hatchability, is the number of chicks that hatch from the total number of eggs incubated. Hatch of fertile is the number of chicks hatched from the number of fertile eggs that were incubated. Hatchability is important for producing mass quantity and quality of chicks as better hatch of fertile eggs has been found to produce the largest chick relative to the egg weight (Romanoff, 1936). High temperature (38.5-40.5°C) causes more mortality and decreased hatchability when applied during the last week of incubation than low temperatures (33.5-36.5°C) (Romanoff et al., 1938). Low temperatures (33.5-36.5°C) during the first week of incubation cause more mortality and decreased hatchability than high temperatures (38.5-40.5°C) (Romanoff et al., 1938). Continuous exposure of high (40.5°C) and low (33.5°C) temperatures killed all White Leghorn embryos by day 14 of incubation (Romanoff et al., 1938). Turkeys exposed to high air temperatures (38°C or 38.5°C) during one week of incubation or throughout the majority of incubation (0-25 days) had significantly decreased hatch of fertile eggs when compared to eggs incubated at 37.5°C (French, 2000). The lowered hatches had increased late dead (3rd and 4th week of incubation) mortalities. Eggshell temperatures above 39°C from days 16-18.5 of incubation in broilers lowered percent hatch of set by 10.2-15.9% compared to a low (34.6°C) and control (37.6°C) eggshell temperatures with higher embryo mortality from 18.5-21 days of incubation in the higher temperature treatment (Willemsen et al., 2010). Low temperatures (eggshell temperature of 36.6°C) during early incubation (days 0-10 of

incubation) reduced percent hatch of eggs set by 4% while high late incubation temperatures (eggshell temperature of 39.5°C from days 18-21 of incubation) increased percent hatch of eggs set by 2% compared to a control eggshell temperature of 37.8°C (Joseph et al., 2006). However, the early low temperature had 6% less saleable chicks at hatch with 1.5% more external pips than the control which could indicate that the cool chicks did not have sufficient incubation time before the chicks were removed from the incubator, and the high late incubation treatment did not differ in percent saleable chicks at hatch compared to the control (Joseph et al., 2006). Yahav et al. (2004) found 5% greater hatchability in eggs incubated at 39.5°C and 65% relative humidity for 3 hours on days 16-18 of incubation compared to control eggs incubated at 37.8°C and 56% relative humidity. Romanoff et al. (1938) found that the highest hatch of eggs set occurred at constant 37.5°C air temperature when at 60% relative humidity, but found that lowering air temperatures to as low as 33.5°C after day 16 of incubation did not negatively affect hatch and helped keep air temperatures below 37.5°C (Romanoff, 1936). Lourens et al. (2005) investigated maintaining a set eggshell temperature and reported that the highest hatchability from this study was from a constant eggshell temperature of 37.8°C, and that these chicks were able to maintain a higher body temperature after hatching than alternate incubation temperature treatments. Hatchability may be changed due to the temperature increase or decrease, the duration of the temperature change, and the day(s) of incubation that the temperature change occurs.

Incubation Temperature Effects on Post Hatch Growth

High and low incubation temperatures also do not appear to aid broilers in future growth. Low (37.5°C) and high (39.7°C) eggshell temperature (in the hatcher on days 16-21 of incubation) treated broilers weighed less than standard temperature treatment (38.6°C eggshell

temperature) broilers at 35 days post hatch (Hulet et al., 2007). The cool treatment weighed significantly more (59g) than the hot treatment broilers at 35 days post hatch (Hulet et al., 2007). Molenaar et al. (2011) found that standard incubation broilers (37.8°C eggshell temperature) maintained a heavier weight throughout a 42 day post hatch growout than a high incubation treatment (38.9°C eggshell temperature from 7-21 days of incubation).

Despite hindered growth, high incubation temperatures have been found to improve breast meat yield. Brief (3 hours per day) late elevated incubation temperatures (from 37.8°C and 56% relative humidity to 39.5°C and 65% relative humidity on days 16-18 of incubation) were found to increase breast meat yield in females by 1% compared to the high incubation temperature during days 8-10 of incubation or from 8-10 and 16-18 days of incubation, but these treatments were not significantly different from a control of constant 37.8°C and 56% relative humidity (Collin et al., 2007). Molenaar et al. (2011) also found high eggshell temperature (38.9°C compared to 37.8°C) from 7-21 days of incubation increased breast meat yield by 1%.

Incubation temperatures may have implications in ascites incidence in post hatch broilers. Exposing broiler embryos to cold (15°C) air temperatures for 30 or 60 minutes 2 or 3 times during late incubation in the hatcher (18 and 19 days of incubation) increased body weights and reduced the occurrence of ascites (Shinder, 2009). A higher incubation temperature (38.9°C eggshell temperature from days 7-21 of incubation compared to 37.8°C eggshell temperature) caused a higher (3.8%) incidence of ascities in broilers (Molenaar et al., 2011).

Summary of Known Incubation Temperature Effects on Broiler Embryos

These previous studies show that incubation temperature can have great effects on chick quality and hatch performance. How the embryo and hatchling are affected depends on the temperature, which day of incubation, and the amount of time during incubation that the

temperature is altered. Generally, high temperatures decrease incubation time up to a point, decrease yolk-free body weight at hatch, decrease hatchability, decrease heart and lung weights, cause anaerobic metabolism effects, and hinder post hatch growth. Cool incubation temperatures generally have opposite effects but can also have similar effects to high temperatures depending on the temperature and the duration. These known effects are important to understand the constraints on temperature fluctuations that could be applicable for potential commercial poultry sex ratio manipulation and to understand how 1950s incubation temperature could affect modern broiler embryos.

Strain Differences in Embryonic Development

Chickens come in all shapes and sizes from the 12 pound Brahma to the 16 ounce Serama (American Poultry Association, 2010; American Bantam Association, 2011). Different breeds and strains have been found to tolerate heat better than others (Hutt, 1938; Lee et al., 1945; Wilson et al., 1966; Wilson et al., 1975), and this observation has been extended to speculate that there are differences in the embryo's heat tolerance between breeds and strains as well (Ande and Wilson, 1981). Within bantam breeds, embryo weights have been found to be significantly different between breeds (Hardin, 1972). The heat produced by an embryo has been found to follow a set pattern, increasing heat production until 80% of incubation, then increasing heat production again when the chick internally pips into the air cell (Janke et al., 2004; Tzschentke, 2008). The exact amount differs between both species and breeds of poultry (Janke et al., 2004; Tzschentke, 2008). Thus, to accommodate the needs of modern embryos, an increasing number of studies are examining how incubation conditions affect different breeds and strains of

commercial poultry such as examining metabolism, heat production, incubation time, hatchability, mortality, etc.

Recent authors believe the modern commercial broiler's heat production has increased over the years and is detrimental to the performance of the bird when the incubator is not adjusted for this change in heat production (Hulet, 2007; Meijerhof, 2011). The possible overheating effects on the embryo include, as previously discussed (see temperature section), an earlier hatch, influences on muscle development, and higher incidences of ascites (Meijerhof, 2011).

Metabolic comparisons of two strains of broilers, Cobb and Ross, have revealed differences between the embryos. Meijerhof (2011) states that Ross 308 embryos lose more moisture than Cobb 500, indicating a slower gas exchange for Cobb embryos. Cobb 500 embryos have a higher heat output than Ross 308 (0.35-0.5°C higher eggshell temperature), and Cobb 500 hatch 8-12 hours earlier than Ross 308 (Meijerhof, 2011). Tona et al. (2010) found similar results with Cobb embryos exhibiting faster growth in the first and third week of incubation, a quicker hatch time, higher heat production prior to hatch and continued elevated heat production during the chick's first days as compared with Ross broilers. Even within Cobb and Ross strains, embryos exhibit different heat production: Cobb 700 produce more heat than Cobb 500, and Ross 708 and Ross 508 produce more heat than Ross 308 (Janke et al., 2004; Meijerhof, 2011). Druyan (2010) found metabolic differences between Ross 308 and Cobb 500 embryos. Ross 308 had higher oxygen use (on days 16-19 of incubation), higher T3 levels (days 14, 16, and 21), and a higher heart rate than Cobb 500 embryos.

Greater differences have been found between broiler and layer embryos. Druyan (2010) found layers, compared with Cobb and Ross broilers, hatched a day later than the broilers with

lighter embryo weights. The layer embryos consumed less oxygen and had lower plasma triiodothyronine concentration, hematocrit, and hemoglobin in their plasma (Druyan, 2010). Druyan (2010) also found a faster embryo heart rate late in incubation in the layer embryos. The layer and broiler embryos had a similar relative yolk weight. The one day delay in hatch may have allowed the layer embryos more time to consume the same amount of yolk as the broilers (Druyan, 2010). Janke et al. (2004) also showed that layers hatch one day later than broilers and consume less oxygen. Broiler embryos also produced more heat than layers (Janke et al., 2004). Ohta et al. (2004) also found faster development and yolk utilization in broiler than layer embryos, and concluded that broiler eggs have inadequate amounts of protein that may actually be limiting the broiler embryo's already fast growth.

These strain comparison studies all agree that incubation conditions should be tailored to the needs of the specific strain, and that the heat production and metabolic activities of each embryo strain should be monitored periodically so that the requirements of the particular embryo will be known in order to produce high quality commercial birds.

A meat type chicken control strain from the 1950s (See Appendix C), the Athens Canadian Random Bred, can be a useful comparison strain not only for the growing broiler, but also for the growing broiler embryo. Incubation conditions to optimize hatchability and chick quality are likely different for modern broilers with today's broiler embryo's higher heat production and altered metabolism (Hulet, 2007). Despite the changes in broilers already evidenced by ACRB comparison studies (Havenstein et al., 1994a,b; Qureshi and Havenstein, 1994; Cheema et al., 2003; Havenstein et al., 2003a,b), the time required for the development of the embryo has remained the same, increasing the percentage of a bird's life spent in its shell from 20-25% to 30-40% over the past 20 years as market age has decreased from 12 weeks to 6

weeks (Havenstein et al., 1994a; Hulet, 2007). Today's hatcheries are also different from the past with greater egg incubation capacity, and yet hatchability values have changed very little over the past 20 years (Schaal and Cherian, 2007). Schaal and Cherian (2007) calculated that even an average 1% increase in hatch of eggs set (for broilers and turkeys) would equal over $25 million in reducing production losses, not counting the profits from the larger numbers of birds placed and reared. As previously discussed, research on the appropriate incubation conditions for the modern embryo have been occurring, though, no known research has compared the ACRB embryo to the modern broiler embryo. The closest study is a 2004 study (O'Dea et al.) comparing of modern broiler strains to a 1978 female broiler parent strain. The unselected 1978 female strain had the lightest eggs and the shortest length chicks measured from the tip of the beak to the end of the third toe on the right leg. The modern strains had 0.65% less moisture loss than the unselected strain (11.15% vs. 11.8%). Otherwise, no differences were seen between the strains including hatchability, metabolism, conductance, eggshell temperatures, intestine, heart and liver weights, and glycogen content in the liver.

Sex Differences in Embryonic Development

Besides breed and strain differences in embryonic development, there are also sex differences. Sexual dimorphism is actually present within the hatchery. Some disagreement exists about chick weight differences between the sexes at hatch. Reasons for the lack of similar results include different breeds and strains, different pull times and incubator models affecting moisture loss/drying of the chick, and differences in measurement such as if the yolk should be removed from the chick before weighing and if the weight should be represented as a percentage of the initial egg weight (Burke, 1994). Of the papers that found sex weight differences in

chicks, the male chick was found to outweigh the female at hatch (Romanoff, 1933; Romanoff, 1948; Godfrey and Jaap, 1952; Khan et al., 1975). Zawalsky (1962) found heavier male chicks, but determined that the males actually weighed the same as female chicks due to differences in hatch times between the sexes. The males hatched an average 1.2 hours later than the females and this was deemed to have affected their weight (Zawalsky, 1962). Hays (1941) found that female Rhode Island Red chickens hatch before males. Williams et al. (1951) also found that New Hampshire females hatched sooner than males (57.4% of early hatching chicks were female). Romanoff (1933) found that male chicks had longer intestines and heavier hearts and gizzards than females, while females had longer cecum and heavier livers. Pulse rate and red blood cell count was found to be higher for males than for females (Romanoff, 1948), although differences between the sexes embryo heart rate has been found to overlap and not exhibit any difference (Glahn et al., 1987). As expected, sex differences exist in the embryo. In both turkeys and ACRBs, male embryos outweigh females at middle-late ages (days 12-20 in the turkey and days 8-15 in the chicken) (Burke and Sharp, 1989; Burke, 1994). Muscle differences were also found between male and female broiler embryos with males having greater amounts of myofibers that were smaller of size than in females (Henry and Burke, 1998). We can identify the sexes of embryos via histology and progressively by necropsy evaluation of the gonads as early as 7 days of incubation (Romanoff, 1960). Sex can be determined by necropsy with the naked eye at hatch: male chicks have two testes and females have a left ovary (Romanoff, 1933).

Chick sexing can also be ascertained molecularly to determine the genotypic sex. The chicken is known to possess Z and W sex chromosomes as a female and ZZ as a male (Hance, 1926). Polymerase Chain Reaction (PCR) procedures have been successful in confirming sex of chicks and embryos using either tissue or blood. Two examples are sexing birds by amplifying

genes on the W and Z chromosome (CHD1W and CHD1Z) and sexing birds with a fragment of the W chromosome and a control portion of the 18S ribosomal gene (Fridolfsson and Ellegren, 1999; Haunshi et al., 2008). These methods could even be used on an unincubated egg to determine the sex, but this technique is considered inaccurate due to contamination from both parents on the vitelline membrane (Arnold et al., 2003).

Sex Determination and Differentiation

Sex differentiation in chickens is considered to be genetically controlled. As the heterogametic sex, the hen determines the sex of the offspring. Even though sex in birds is genetically determined, the exact mechanism is still unknown (Bloom, 1974; Carré et al., 2011). Current ideas about sex determination in birds include a dosage dependent gene on the Z chromosome and a dominant gene on the W chromosome (Smith et al., 2007). A dose-dependent gene on the Z chromosome would mean that male birds would have two copies of the gene whereas a female bird would only have one (Smith et al., 2007). After reviewing possible sex determining genes in birds, Smith et al. (2007) concluded that the most likely gene for the dose dependent idea is DMRT1 (doublesex and mab3 related transcription factor 1), and HINTW (histidine triad nucleotide binding protein on the W chromosome) is currently considered the most likely gene to be a dominant female sex determining gene on the W chromosome. Other known genes, factors, and hormones contributing to the formation of the gonad include DAX1 (dosage-sensitive sex reversal-adrenal hypoplasia critical region, on X chromosome 1), SF1 (steroidogenic factor 1), and P450aromatase in the female's ovary and DMRT1, SOX9 (sry-related HMG box gene 9), and anti-müllerian hormone in the male's testes (Yamamoto et al., 2003). While the brain's interaction with the gonad has been thought to occur after the gonad

had differentiated between the male and female pathway, sex specific genes have been found to be expressed before the gonad differentiates (Bruggeman et al., 2002; Lee et al., 2009).

Generally, sex is thought to be determined prior to the differentiation of the embryo's gonad, which begins at approximately day 6.5 of incubation for both sexes (Romanoff, 1960; Smith et al., 2007). Prior to differentiation, the chick's gonad is considered bipotential or exactly the same for both sexes and able to differentiate into either sex (Romanoff, 1960; Halverson and Dvorak, 1993; Smith et al., 2007). Each sex also has both Wolffian and Mullerian ducts (Kar, 1947; Bellairs and Osmond, 2005). The Wolffian duct initially is used for excretion, but later becomes the vas deferens of the male and degenerates in the female (Kar, 1947; Bellairs and Osmond, 2005). The Mullerian duct becomes the oviduct of the female and degenerates in the male and on the right side of the female (Kar, 1947; Bellairs and Osmond, 2005). The gonad begins development from the intermediate mesoderm (Nakamura et al., 2007). Primordial germ cells, which form the germ cells, migrate around the vascular system and arrive at the gonad area at stage 15 (Romanoff, 1960; Nakamura et al., 2007). From stages 15-17, the majority of primordial germ cells arrive on the left side in both males and females, although females were found to have greater numbers of primordial germ cells than males (Nakamura et al., 2007). After the formation of the bipotential gonad and the arrival of the primordial germ cells, the gonad begins specializing into either an ovary or testes under the influence of some of the before mentioned genes. Zhao et al. (2010) examined gynandromorph chickens that were half male and half female and created chimaera embryos with male and female cells in the gonad and concluded that each individual cell is able to determine and maintain its own sex despite the surrounding environment. Barske and Capel's (2010) review of the paper questioned this conclusion since extensive past research has found that chicken sex can be reversed with

hormones. An early example of this type of work was dipping fertile black sex-linked eggs into an estrogen solution producing genetic males that possessed a left ovary which later became testes with a few female organs still present in some birds after rearing for 6 months (van Tienhoven, 1957).

Bird Incubation Temperature Sex Ratio Investigations

Sex ratio manipulation in domestic fowl has long been of interest to man. Commercial poultry would benefit financially from control over their sex ratios. While roosters are needed in layer breeding, they are unnecessary for infertile table egg production (Halverson and Dvorak, 1993). Commercial broiler and turkey industry would prefer to hatch predominantly male chicks due to performance differences between the sexes (Halverson and Dvorak, 1993). According to the Cobb Vantress 2012 Cobb 500 Broiler Performance and Nutrition Supplement, each week during growout, males continually outgrow females in daily gain and weight for age, and consume more feed yet have a similar feed conversion to females. Similar results were also found in Ross broilers (May and Lott, 2001). Primary breeders of commercial poultry would benefit from decreased amounts of by-product chicks. Depending on the cross, either males or females could be preferred to have the majority hatch. For hatching breeders that will lay eggs for broiler production, more females would be desired to hatch than males since predominantly more females are needed in the laying house.

As mentioned, a chicken's sex can be phenotypically changed with hormone treatments, and hormones injected into the hen can alter sex ratios genetically (Pinson et al., 2011). Alternative techniques are worth examining since hormone treatments would be expensive, labor

intensive, and the public perception is already negative toward any possible hormone administration (Dale and Davis, 2001).

Reptiles and birds share common evolutionary lineage dating back to Archaeopteryx (Xu et al., 2011). Reptiles have genetic sex determination (GSD) as well as temperature-dependent sex determination (TSD) (Deeming and Ferguson, 1991). In TSD, the incubation temperature will determine the resulting sex of the offspring (Deeming and Ferguson, 1991). In reptiles, three types of TSD are known: a higher incubation temperature produces more females and a lower temperature produces more males (known in turtles), the opposite where a higher temperature produces more males and the lower temperatures produce more females (seen in crocodilians and lizards), and more females hatch when exposed to the extremes (high and low temperatures) and more males hatch when incubated at middle temperatures (seen in other crocodilians, turtles, and one species of lizard) (Deeming and Ferguson, 1991; Shoemaker and Crews, 2009). The temperature must be applied during a "temperature sensitive period" where temperature-sensing molecules direct molecular differentiation of the bipotential gonad before morphological differentiation of the gonad to become either male or female (Shoemaker and Crews, 2009). TSD is considered more common in reptiles than other vertebrates who exhibit TSD including fish and amphibians (Georges et al., 2010). TSD may be the only sex determining mechanism in some reptile species, while others have both genetic and temperature sex determination, and others (such as snakes) have only genetic sex determination (Sarre et al., 2004; Showmaker and Crews, 2009). Even though TSD does not exist in snakes, sex-biased embryo mortality does whereby more females die at higher temperatures and more males die at lower temperatures; snakes have heterogametic females or homomorphic sex chromosomes (Burger and Zappalorti, 1988; Janzen and Paukstis, 1991). Sex chromosomes in reptiles are seen

both as either heterogametic males or heterogametic females with all crocodilians having homomorphic sex chromosomes as well as all crocodilians exhibiting TSD (Deeming and Ferguson, 1991; Janzen and Pauksis, 1991). Homomorphic sex chromosomes are considered "a necessary precondition for TSD" (Janzen and Paukstis, 1991). In a lizard with both TSD and GSD, the temperature can override the genetic sex determination (Radder et al., 2008). Since TSD and GSD do exist together and related organisms can have either TSD or GSD, these mechanisms are believed to have evolved back and forth multiple times, and that TSD may have even been a cause for dinosaur extinction (Miller et al., 2004; Sarre et al., 2004). Sarre et al. (2004) thinks that TSD and GSD should not be viewed separately since TSD and GSD have similar molecular mechanisms.

As previously described, although the exact mechanism is not known, birds have genetic sex determination (Deeming and Ferguson, 1991). The primary sex ratio of chickens is very close to 50% males and 50% females (Hays, 1945). In birds, the ability to skew the offspring sex ratio with incubation temperature is uncertain. Several studies have investigated this possible mechanism due to its potential implications and probable ease of application by simply changing the incubation temperature. TSD is uncertain in birds since they have heteromorphic sex chromosomes instead of homomorphic, which again, is thought to be required for TSD, and birds have a narrow range of acceptable incubation temperatures compared with reptiles (Webb, 1987; Deeming and Ferguson, 1991; Janzen and Paukstis, 1991).

Deeming and Ferguson (1991) and Ferguson (1996) cited a Russian article (Shubina, Zhmurin and Vedeneeva, 1972) claiming that chicken eggs incubated at 37.5°C, and then, starting after 72 hours of incubation, incubated at 22°C for 7-8 hours hatched 150 males for every 100 females. Ferguson's patent (1996) explored this phenomenon and claimed that by

pulsing eggs with a high or low incubation temperature, the embryo's sex could be reversed while keeping mortality values low. He used Rhode Island Red chicken eggs pulsed for 24-36 hours at 22°C to produce more males (1.6 males for each female) and for 18-96 hours at 36°C to produce more females. He changed the incubation temperature during a sex-sensitive time which he found for the chicken is on day 3 of incubation at Hamburger-Hamilton stage 18/19. Altering the temperature on and after day 5 increases the mortality. He also tested if breeder flock age affected this sex ratio skew due to temperature. He tested this using Isabrown auto-sexing strain and changed the temperature on day 3 of incubation to 22°C for 24 hours or 36°C for 48 hours. Eggs from older breeders produced more of a sex skew than younger flocks (range of ages was from 30-52 weeks old). He also claimed to have produced sex reversed birds whose genotype differed from their phenotype where if bred to a normal sexed bird could theoretically produce WW offspring. No other sources claimed to have been able to reproduce this work.

Studies with the Australian brush-turkey (Göth and Booth, 2005; Göth, 2007) examined sex ratios of offspring from eggs incubated at different temperatures. In both artificial incubation (at 31°C, 36°C and 34°C) and then in the natural soil/leaf mounds maintained by the male (overall range in temperatures was 27-37.3°C) more males hatched at lower temperatures and more females hatched at higher temperatures with equal amounts of both sexes at an intermediate temperature. Investigating the cause of these sex ratio differences, Eiby et al. (2008) repeated the artificial incubation experiment and sexed all chicks and dead embryos: finding that the reason for the sex skew was that lower temperature incubation treatment had higher female embryo death and the higher incubation temperature had greater male embryo death with the middle temperature having similar amount of mortality for both sexes. This was concluded as the first finding of temperature-dependent sex-biased embryo mortality in a bird.

Ratite birds are a bit different from other bird species having virtually homomorphic sex chromosomes (Bloom, 1974). Ratites are also considered "more closely related to reptiles in a number of characteristics than other birds" (Deeming and Ferguson, 1991). One common characteristic is that the ureter opens into the coprodeum of the cloaca in both ratites and crocodiles (Oliveira et al., 2004). Chicken ureters open into the urodeum (Oliveira et al., 2004).

In a Japanese quail experiment (Yılmaz et al., 2011), eggs from different age parents (P) (ages 8-10, 16-18, and 22-24 weeks) were incubated at 5 different temperatures (ranging 36.7 to 38.7°C) and separately reared and bred. The F1 eggs were incubated at a standard temperature and sex ratios for the resulting offspring (F2) were examined. At 6 weeks of age, 23.6% and 26.66% more males were present from the lower temperature (36.7°C and 37.2°C) incubated P; 10.26% and 14.28% more females more females were present from the higher temperature (37.7°C-38.7°C) incubated P, but only from the oldest age P birds (age 22-24 weeks). This study concluded that incubation temperature of a parent generation may alter the sex of the next generation and that grandparent age may also play a role in offspring sex for birds. Jull (1932), however, concluded from breedings of both White Leghorns and Rhode Island Red chickens that sex ratio skews is not an inherited trait. Hays (1954) also did not find inheritance of sex ratios in Rhode Island Reds.

When Li et al. (2008) incubated several different breeds of chickens at a standard incubation temperature (37.5°C), a bias for greater female mortality was seen during the first week of incubation. Wu et al. (2012) found greater female embryonic mortality throughout incubation in similar chicken breeds as Li et al. (2008). The University of Massachusetts examined multiple possible influences on sex ratio in Rhode Island Red chicken from 1946-1952. These experiments also found that female embryos were more likely to die than males

throughout the incubation period (Hays, 1949; Hays and Spear, 1950). Thus as hatchability decreased, the amount of male offspring increased (Hays, 1949; Hays and Spear, 1950, 1952; Hays, 1951, 1952). The female chicken embryo is thought to predominantly perish since it is the heterogametic sex, and other animals are known to experience greater mortality in whichever sex is heterogametic (Hays, 1949; Hays and Spear, 1950, 1952; Hays, 1951, 1952). Of hatched chicks, more females died during the 8 week growout (Hays, 1951). Modern high yielding Ross broilers, however, have higher (1.75%, 2.85%, and 3.18%) male mortality compared to females when raised to 32, 38, and 48 days post hatch (Schwean-Lardner et al., 2013).

Literature Review Summary

These past studies show that manipulation of incubation temperature can cause detrimental or beneficial effects for the broiler embryo and post hatch broiler growth. Various strains of poultry react differently to incubation temperature manipulation, and determining differences in the modern broiler to a control strain could clarify what incubation temperature is most ideal for today's modern high yielding broiler. The ability for incubation temperature to affect the sex of broilers is uncertain and could have great financial impact on the poultry industry; however, potential broiler sex manipulation by incubation temperature could cause the detrimental effects reviewed here.

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CHAPTER 3

HATCHABILITY AND EMBRYONIC DEVELOPMENT OF ATHENS CANADIAN RANDOM BRED BROILERS AND THE MODERN HIGH YIELDING BROILER[1]

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ABSTRACT

The 2012 Athens Canadian Random bred (ACRB), a 1950s meat-type chicken control strain, was compared to the 2012 Cobb 500 high yielding broiler during incubation and hatch. Two trials were completed using a Cobb 500 breeder flock of similar age to the ACRB breeding population. Eggs were incubated and at 37.7°C and 53% relative humidity and 37.5°C and 53% relative humidity in the hatcher. Three eggs per strain were sampled on eight days during incubation and measured for eggshell temperature, embryo stage of development, embryo weight and length, and heart and intestine weight. A hatch pattern was recorded every 6 h starting at 468 h of incubation. Chicks were removed from the hatcher at 498 h of incubation, all unhatched eggs were evaluated for age of embryo mortality, and chicks and yolk sacs were weighed. The Cobb 500 hatched 6 h earlier than the ACRB in trial 1 and 12 h earlier in trial 2. The ACRB had a larger percentage of early dead embryos (0-7 d of incubation), compared to the Cobb 500 while the Cobb experienced greater late dead mortality (15-21 d of incubation). No difference in eggshell temperature was found between the two strains. The modern broiler had significantly larger egg size, yolk-free chick weight and greater percentage yolk sac at hatch. The ACRB eggs had a greater egg moisture loss during incubation. The Cobb 500 also had greater embryo lengths and heavier weights, but as a percentage of egg weight, embryo weights between the two strains did not differ. The heart and intestine weights were also greater in the modern broiler, but as a percentage of the embryo weight, these weights did not differ between the strains. While body size has increased for the modern broiler embryo, proportionally, the embryo is very similar to the meat-type embryo of 50 years ago.

Key words: embryo, broiler, Athens Canadian Random Bred, heart, intestine

INTRODUCTION

Genetic selection over the past 50 years has produced a broiler that can reach a body weight that is four times heavier than the broiler of 50 years ago in half the time (Havenstein et al., 1994, 2003). These studies compared modern broilers to the Athens Canadian Random Bred (ACRB), a 1950s meat-type chicken control strain maintained at the University of Georgia. The ACRB is a subpopulation of the Ottawa Meat Control strain developed in 1955 from commercial broiler strains raised at the time (Hess, 1962; Merritt and Gowe, 1962; Merritt, 1968).

Although studies (Havenstein et al., 1994, 2003) have documented changes in the broiler during growout, only one known study has documented differences in the ACRB embryo compared to a modern broiler (Christensen et al., 1995). This study examined ACRB and Arbor Acres (AA) embryos from 17 d of incubation to hatch. This comparison found that AA embryos were an average 13g heavier than ACRB embryos during these days of incubation. The percentage liver weight was greater for the ACRB from 19 d of incubation to hatch. The percentage heart was greater for the ACRB on days 17-19, but percentage heart was greater for AA on day 20 and at hatch. There was no difference in conductance constants adjusted for egg weight between the ACRB and AA. These embryos also exhibited differences in metabolism with higher heart glycogen in the ACRB, increased liver glycogen on day 19 of incubation in the ACRB, increased blood glucose on day 19 of incubation for the AA, and higher T3 concentration for the AA on day 20 at external pipping. These chicks had similar hatch time and hatchability values were 89% for the AA and 93% for the ACRB.

In a similar study, O'Dea et al. (2004) compared eggs, hatchability, and chicks at hatch of modern broiler strains (a commercial broiler strain selected for high breast meat yield and a commercial broiler strain selected for whole bird market) to a 1978 unselected female broiler

parent strain. The unselected 1978 female strain produced lighter eggs and shorter and lighter chicks at hatch. No other differences were seen between the strains in eggshell temperatures, conductance, hatchability, percentage heart or liver weights or glycogen concentrations in these organs, intestine weights at hatch, yolk sac weights at hatch, and embryonic mortality.

Broiler embryonic comparisons would be insightful with increasing concerns over modern broiler incubation management and performance. Authors have stated that the modern commercial broiler embryo's heat production has increased over the years and failure to compensate for this increase in heat production during incubation has been detrimental to broiler growout performance (Hulet, 2007). A calculated 1% increase in hatchability (for broilers and turkeys) would reduce production losses by more than $25 million, not counting the profits from the larger numbers of birds placed and reared (Schaal and Cherian, 2007).

In the current study, we compared the hatchability of the 2012 ACRB to the 2012 Cobb 500 broiler embryo. Understanding how embryonic development may have changed could help direct hatchery management adjustments needed to produce the optimum number and quality broiler chicks. This study also compared small sample sizes of earlier embryonic development (<17 d) between the ACRB and the modern broiler.

MATERIALS AND METHODS

This study was conducted in two trials. Eggs were collected from the ACRB breeder flock at the University of Georgia that was artificially inseminated using pooled semen. In trial 1, the ACRB breeders were 41 wk old, so eggs from a commercial 41 wk old Cobb 500 breeder flock were obtained for comparison. We collected eggs from the same breeder flocks for trial 2 when the breeders were 48 wk old. In both trials, due to the low number of ACRB breeders, eggs

incubated from the ACRB flock ranged in age from 1-7 d. Cobb 500 eggs obtained from a commercial flock were all laid on the same day, obtained from a commercial hatchery the day after they were laid, and stored for 1-2 d prior to incubation at the University of Georgia in an 18.4˚C and 70% relative humidity cooler so the eggs were set 2-3 d after being laid. In trial 1, five trays of 70 eggs each in a 90 egg capacity tray (350 eggs) were incubated per strain and seven trays (490 eggs) per strain were incubated in trial 2. Initial egg weights were taken for a sample of 30 eggs (individually) per strain. In trial 1, 29 additional eggs per strain and 28 additional eggs per strain in trial 2 were set for eggshell temperatures and embryo measurements.

All eggs were stored in the 18.4˚C and 70% relative humidity cooler until placed in a Natureform NMC2000 incubator running at 37.7°C and 53% relative humidity in the evening at 22:00. We began timing incubation once the incubator came back up to temperature after the eggs were placed in the machine which occurred at approximately 00:00 midnight in both trials. ACRB eggs were located higher in the egg mass with Cobb 500 eggs beneath the ACRB eggs in both trials with the initial intention of detecting air temperature differences between the strains. The same incubator and hatcher were used in both trials with automatic turning of the eggs 12 times every 24 hours in the incubator. The 30 eggs per strain that were weighed individually were located in the middle of each strain's egg mass in the middle of the tray. The additional eggs set for embryo breakouts and eggshell temperatures were equally distributed in all trays within their strain to limit any location effects within the strain. The incubator (temperature and humidity) was calibrated prior to incubation in both trials. Air temperatures in the incubator were recorded every 15 minutes using HOBO temperature probes (HOBO U30 Station, Onset Computer Corporation, Bourne, MA) located throughout the top, middle and bottom locations of each incubator. The top probe rested with the setter's temperature probe, the middle remained in

the middle of the ACRB egg mass and the bottom probe was located in the middle of the Cobb 500 egg mass.

Prior to incubation, a sample of eggs (five per strain in trial 1 and nine per strain in trial 2) were opened and the upper surface of the blastoderm was examined under a microscope and staged according to Eyal-Giladi and Kochav (1976) staging. The blastoderm was visualized by injecting fast green dye in PBS under the germ. These stages were determined using the appearance of the area pellucida, area opaca, Koller's sickle, and the hypoblast. Infertile and eggs where the yolk was broken to the extent the blastoderm was lost were not staged. On 8 sample days (0.5, 3.5, 5.5, 7.5, 10.5, 12.5, 14.5 and 17.5 d of incubation), three of the additional sample eggs per strain were removed from the incubator at the same time; all remaining extra eggs were removed on day 17.5. Eggshell temperatures on these sample eggs were taken immediately after opening the incubator door with an infrared thermometer (Exergen Temporal Artery Thermometer Home Model 2000C, Exergen Corporation, Watertown, M.A.) along the bottom half of the egg (thermometer sensitivity was 0.1°C). The eggs were weighed, and the embryos were removed from the eggs, separated from the extra embryonic membranes, and drained of excess liquid using a tissue placed next to the embryo. The embryos were weighed and staged according to Hamburger and Hamilton's (1951) staging. The 0.5 d embryos were examined under a microscope using the same dye technique used for gastrulation. Day 0.5 stages were assigned by examining the extent the primitive streak extended across the area pellucida, the shape of the area pellucida, the appearance of Hensen's node and the appearance of the head process. Day 3.5 stages were based on eye pigmentation and the appearance of the limb buds such as symmetry and apparent length and width comparison. Day 5.5 stages examined the formation of elbow and knee joints in the limb buds and the appearance and the comparative

lengths of the three toes in the leg bud. Day 7.5 stages were based upon numbers of the scleral papillae. Day 10.5 and 12.5 stages examined the extent the eyelids covered the cornea. Day 12.5 stages also examined the length of the third toe. Day 14.5 and 17.5 stages were assigned based on the length of the third toe and the beak. The embryos were euthanized by decapitation and the heart and intestines were excised and weighed starting on day 7.5 for the heart and day 12.5 for the intestines. Intestine weights included from the duodenum (with pancreas) to the end of the large intestine at the cloaca.

Embryo lengths were also measured for all ages using digital calipers. Embryo measurements included body length, wing, leg, beak and third toe with and without the toenail. Embryo measurements were recorded as follows.

Embryo Length

Since embryos have a natural curled position, a natural diameter length was taken on sample days where an embryo was visible with the naked eye (days 3.5-17.5): measured from the outermost portion of the curled tail to the outermost part of the curved neck (See Figure 3.1). Embryos were additionally stretched out of their natural position and measured from the tip of the toe to the tip of the beak starting on sample day 7.5 in trial 2 and day 10.5 in trial 1. This stretched measurement technique is based on Hill's (2001) chick length measurement by stretching the chick and measuring its length from the tip of the beak to the end of the third toe on the right foot. This length measurement has also been taken on chicken embryos starting as early as 7 days of incubation in order to determine embryonic growth (Lourens et al., 2005). This specific measurement in younger embryos (<7 days of incubation) is not as appropriate since embryos have a natural curled position.

Wing and Leg Lengths

Wing measurements included the entire wing bud until the wing joint of the embryo developed whereupon the middle of the joint to the tip of the wing bud was measured and then the radius/ulna (wing flat) was measured. The leg measurements followed the same protocol as the wing: measuring the limb bud until the joint developed and then measuring only the metatarsus (shank). The third toe measurements were taken starting on day 7.5. The third toe nail could be distinguished starting on day 12.5 so measurements with and without the third toe nail were taken on these days. Toenail lengths were calculated by subtracting the third toe without the nail from the third toe with the nail measurements.

Beak Length

The beak was measured entirely from the tip to the meeting with the head of the embryo until the beak could be measured as in Hamburger and Hamilton's (1951) measuring of the beak "from anterior angle of nostril to tip of bill" beginning on sample day 10.5.

The eggs were transferred to plastic hatching baskets in a hatcher on day 18.5 of incubation at 36.9°C and 53% relative humidity. Relative humidity was set at 53% in the incubators and the hatchers since 10% higher and lower relative humidity during incubation have been found to lower hatchability, and 43% and 63% relative humidity in the hatcher caused no significant difference in hatchability compared to 53% relative humidity (Bruzual et al., 2000a,b). Two trays (140 eggs) were placed in one 76.2cm width, 71.1cm length, and 11.4cm deep hatching basket with a divider placed in the middle of the hatching basket between the trays. A divider was also placed behind the eggs to allow chicks that hatched to be counted and then moved behind the divider to eliminate counting all chicks at each time period. Eggs were

transferred in the same orientation as in the incubator with Cobb 500 eggs lower in the hatcher than ACRB eggs. Final egg weights were recorded at transfer. Air temperature in the hatcher was recorded every 20 minutes by two Chickmaster temperature eggloggers per strain placed in the hatching tray with the eggs.

The total number of chicks hatched was recorded every 6 h starting at 468 h until the hatch was pulled at 498 h of incubation. The end of hatch was determined once all chicks were hatched and dry and no additional eggs were progressing toward external pip. After the hatch was pulled, chicks from one tray per strain were euthanized by carbon dioxide asphyxiation, individually weighed and the yolk sac removed and weighed. The yolks were dried at 80°C and reweighed after 72 h. A residue breakout was completed on all remaining unhatched eggs as described by Wilson (2010). The eggs were assigned categories of infertile, early dead, middle dead, late dead, live pip, dead pip, dead chick, cull chick, set crack, transfer crack, and contaminated. Eggs were considered infertile when a fertilized blastoderm was not visible. Early (0-7 d), middle (8-14 d), and late (15-21 d) dead embryos were assigned to eggs that had a fully intact eggshell. Pips were considered live if the chick was still alive and breathing at the time the egg was opened or dead pips if the chick was dead. Set cracks were designated when a crack was present in the eggshell and the internal contents of the egg had dried down to a low region of the egg. Transfer cracks were designated for eggs with a crack in the eggshell with a white hardened shell membrane that the chick was unable to penetrate. Cracked eggs were not assigned a stage (early, middle, late) of embryonic death. Contaminated eggs were designated as eggs that had a distinctive black, blue, or red color inside with a noticeable odor and/or eggs that popped when the eggshell was broken. Cull chicks were chicks that hatched but were strikingly unable to survive, such as protruding internal organs (yolk sac, intestines).

Statistical Analysis

We used the general linear model procedure of SAS (Version 9.2, 2012) to determine any strain and trial effects and day of incubation when applicable using each tray (7 or 9) as the experiment unit. Any P value less than 0.05 were regarded as significant. We compared differences between the strains, trials, and day of incubation using Fisher's Least Significant Difference.

RESULTS

Cobb 500 eggs were an average 14.5 g heavier than ACRB eggs at setting (Table 3.1). We saw significantly heavier egg weight in trial 2 than in trial 1 when comparing the 30 eggs per strain that were weighed at set. Cobb 500 embryo weight was significantly heavier on days 12.5, 14.5, and 17.5 ($P<0.0001$; Figure 3.2). When the embryo weight was taken as a percentage of its initial egg weight, though, the percentages were not different between the strains ($P=0.9620$; Figure 3.3). Embryo weights and percentage embryo weight did not differ between trials ($P=0.7756$ and $P=0.2829$, respectively). The significant increase in egg weight between trials with no increase in embryo weight did not decrease the percentage embryo weight of egg weight for both strains because the embryos' initial egg weights did not significantly differ by trial ($P=0.0691$, n=93 eggs excluding infertile and early dead embryonated eggs). These eggs had an average 56.8g egg in trial 1 and an average 58.7g egg in trial 2. This result is due to a low sample size of embryonated eggs for embryo measurements. Based on strain averages and the embryo weight standard deviation, a large sample size of 404 embryos per strain would be necessary to detect a significant difference. Additionally, a larger sample size of 342 eggs per trial would be needed to confirm the trial differences in initial egg weight. Eggshell

temperatures were not significantly different between the strains (p=0.4314, Figure 3.4), but trial 2 had a significantly higher eggshell temperature than trial 1 (p<.0001). Percent moisture loss was significantly greater (2.2%) in ACRB eggs (11%) than Cobb 500 eggs (8.8%; Table 3.1). Moisture loss did not differ between trials. Moisture loss accounting for surface area, moisture loss divided by the initial egg wetight$^{2/3}$, remained higher for ACRB eggs (Table 3.1).

Cobb 500 embryos had significantly heavier hearts on days 14.5 and 17.5 (0.0322g heavier on day 14.5 and 0.0597g heavier on day 17.5; P=0.0008, Table 3.2). When the heart weight was taken as a percentage of embryo weight, the heart weight did not differ between the strains (P=0.4446, Table 3.3). The average percentage heart over the measured five sample days was 0.8%. The weight and percentage heart were very high on day 7.5 for trial 1 and on day 12.5 for trial 2 compared to the other measurements. These are most likely due to sampling error when trimming the heart.

The intestine weight was significantly heavier on day 17.5 for Cobb embryos (by an average 0.1968g; P=0.0021, Table 3.4); however, the intestine weight as a percentage of embryo weight was not significantly different between the strains at any time point (P=0.2013, Table 3.5). On average, intestine weights made up 1.6% of embryo weight. There were trial effects for the intestine weight (P=0.0495) and percentage intestine (P=0.0003). The percentage intestine was 0.5% greater in trial 1 on day 12.5 and in trial 2 was 0.2% greater on day 17.5. There was no difference in percentage intestine weight between trials on day 14.5.

The Cobb 500 embryos had significantly greater length for all parts measured (Tables 3.6, 3.7, 3.8, 3.9, 3.10, 3.11, and 3.12) with the exception of the third toe nail (Table 3.13), which did not differ in length between strains or trials (p=0.0600 and p=0.6907 respectively). When the

part lengths were standardized for length to mass relationship by dividing the part length by the initial egg weight$^{1/3}$, no parts differed between the strains (data not shown).

Of the eggs sampled for preincubation staging, we were able to stage five blastoderms per strain in trial 1 and for trial 2, we were able to stage five Cobb blastoderms and four ACRB blastoderms. There were no significant differences in preincubation stage between the strains (P=0.0988) or trials (P=0.2953) although a sample size of 25 blastoderms per strain would be needed to confirm this finding. The overall average Eyal-Giladi and Kochav stage was XII and the range was IX-XIV. The Hamburger-Hamilton staging of embryos from 12 h to 17.5 d (Table 3.14) had significant interactions of trial and day of incubation (p=0.0003), trial and strain (p=0.0106), and day of incubation and strain (p=0.0113). Over both trials, comparing the strains on each sample day, ACRB embryos overall were 0.5 stages higher on day 7.5 and Cobb embryos were 0.7 stages higher than ACRB embryos on day 17.5. Trial 2 had more advanced stages on day 3.5 (1 stage higher), day 7.5 (0.5 stage higher), and day 10.5 (0.7 stage higher). Between the trials, trial 2 Cobb embryos were a significantly higher stage than in trial 1 by 0.5 stage and were 0.4 stage higher than the ACRB in trial 1 and 2.

In the recorded hatch pattern, the Cobb chicks hatched 6 h earlier in trial 1 and 12 h earlier in trial 2 than the ACRB (Figure 3.5). No difference was found in the percentage of infertile eggs or hatchability between the strains (Table 3.15). The ACRB had a greater percentage of early (days 0-7) dead embryos (Table 3.15). In trial 1, the Cobb 500 experienced very high late (days 15-21) dead mortality (6.6%). No difference was found between the strains for percentage of middle (days 8-14) dead embryos (0.1% ACRB and 0.25% Cobb 500, Table 3.15). The Cobb 500 also had greater percentages of dead pips (P=0.0468; 0.3% vs. 0.9%) and dead chicks (P=0.0244; 0% ACRB vs. 0.5% Cobb chick mortality, Table 3.15).

Chicks from one randomly selected tray per strain were weighed so, in trial 1, 57 ACRB chicks and 56 Cobb chicks were weighed, and in trial 2, 62 ACRB chicks and 56 Cobb chicks were weighed. Yolk-free chick weights were an average 7.2g heavier for the Cobb than the ACRB (Table 3.1). Yolk-free chick weights were significantly heavier in trial 2 than in trial 1 by 1.5g. The percentage of dry yolk sac weight of total chick weight was significantly higher (1%) for the Cobb (Table 3.1). The percentage of moisture in the yolk was 1.3% higher in Cobb yolks and was different between the trials with 4.3% greater percent yolk moisture in trial 1 (Table 3.1).

Air temperatures recorded by the HOBO data loggers in different locations of the setter were significant for the interaction between trial, location and day of incubation ($p<.0001$; Figure 3.6). Air temperatures were highest in the bottom of the incubator where the Cobb eggs were located, the middle probe had the next highest air temperatures near the ACRB eggs, and the top probe, located with the incubator's temperature probe, had the lowest air temperatures closest to the set temperature of 37.7°C in the incubator. As an overall average of days 1-17, the middle location was 0.46°C warmer than the top probe, and the bottom probe was 0.31°C warmer than the middle probe. Trial 1 top air temperatures were higher than trial 2's top temperatures on each day of incubation. The middle location had a higher temperature in trial 2 during early incubation on days 1-5 after which no difference between trials was found. The bottom location had a higher temperature during trial 2 on days 1-5, 7, and 13-15. The bottom temperatures increased quickly from day 1 to 2 in both trials. In the hatcher on days 19, 20 and 21 of incubation, Chickmaster egglogger temperatures did not differ by strain ($p=0.7460$; Figure 3.7), and the interaction of trial and day was significant ($p=0.0010$). Temperatures between the trials did not differ by day of incubation in the hatcher until pull time.

DISCUSSION

The most noticeable difference between the broiler embryo of today compared to 50 years ago is size. The 23% larger Cobb 500 eggs resulted in larger embryos and 20% larger chicks. Embryos from the two strains started at similar weights, but the modern Cobb embryos became heavier by day 12.5 with longer body and appendage lengths. Cobb embryos had larger hearts by day 14.5 and larger intestine weights by day 17.5. The chicken embryo begins to orally intake albumin on day 11 of incubation when the seroamniotic connection ruptures (Baintner and Fehér, 1974; Moran, 2007), and lipid transfer from the yolk to the embryo accelerates after 12 days of incubation (Speake et al., 1998). These two metabolic changes in nutrient intake enabled the Cobb embryos to outweigh the ACRB in later incubation periods (starting on day 12.5). Proportional to egg size, though, the modern broiler embryo is similar to the ACRB. The trial differences of heavier eggs and chicks for both strains in trial 2 are likely due to increases in hen weight as the breeder flock ages (Peebles and Brake, 1987; Suarez et al., 1997; Vieira and Moran, 1998).

The trial differences of 0.5% greater intestinal percentage in trial 1 on day 12.5 and in trial 2 0.2% greater on day 17.5 with no trial difference between the trials on day 14.5 is unknown but may be due to unintentional slight trimming differences between trials. No difference was found for intestine weight between the strains until day 17.5 of incubation when the Cobb 500 intestine was heavier, but as a percentage of embryo weight, the intestine did not differ between strains. Since Cobb embryo weight became heavier than the ACRB by day 12.5 of incubation but the intestine weight did not differ between the strains until day 17.5 of incubation, this finding lends evidence of the intestine as a late maturing organ. Uni et al. (2003) examined the percentage of the small intestine of Ross embryos on days 17, 19, 20 of incubation

and in chicks at hatch. The percentage small intestine started at 1.4% on day 17 and increased to 3.4% at hatch. Comparatively, in this study, both the small and large intestines were weighed together and percentage determined on days 12.5, 14.5, and 17.5 of incubation. Our results including the small and large intestine are 0.8% heavier on day 17.5 compared to Uni et al. (2003)'s day 17 results. Our results show that over the 5 days of incubation from day 12.5 to day 17.5, the intestines increase by 1.2% of the embryo's body weight. Uni et al. (2003) found a 2% increase in the small intestine over the next four days of incubation from day 17 to hatch. Future work can investigate if ACRB and Cobb embryo intestines continue similar growth in the hatcher.

Christensen et al. (1995) compared ACRB and Arbor Acres broiler embryo heart weights from day 17 of incubation to hatch. This study's 2012 Cobb 500 relative heart weights on day 17.5 of incubation are 0.2% greater than the Arbor Acre values reported by Christensen et al. (1995). This study's 2012 ACRB relative heart weights on day 17.5 of incubation were only 0.04% greater than those of the ACRB embryos from Christensen et al. (1995). This difference in percentage heart weight could indicate an increase in heart weight between the Cobb high-yielding broiler embryo and the Arbor Acres broiler embryo with little change in the ACRB embryo's percentage heart. Christensen et al. (1995) found a heavier relative heart weight in the ACRB on days 17-18 of incubation, but found that during external pipping and at hatch the Arbor Acres broiler had larger relative heart weights. Our results from days 7.5-17.5 of incubation found no difference in percentage heart weight between the ACRB and the Cobb 500. Future studies can investigate if ACRB and Cobb 500 relative heart weights differ while in the hatcher. Great care should be taken when trimming early embryonic hearts, as demonstrated by this study's 7.5 day trial 1 and 12.5 d trial 2 large values, to obtain repeatable values.

Both strains began incubation at the same stage of development (Eyal-Giladi and Kochav stage XII). The Hamburger and Hamilton staging differences of <1 stage at all embryo ages do not represent drastic differences in developmental pace. The more advanced Cobb stage on the last sample day, 17.5, indicates a trend toward faster development prior to transfer to the hatcher. The more advanced stage of the Cobb in trial 2 compared to the trial 1 Cobb and both trials of the ACRB (average 0.4 stage more advanced) also supports the even earlier hatch that occurred in trial 2. Since the Cobb chicks hatched faster than the ACRB in both trials, future study could investigate if greater stage differences between the strains occurs in the hatcher.

The ACRB has been regenerated at the University of Georgia using the incubation temperatures of this study (37.7°C in the incubator and 36.9°C in the hatcher). Older reports such as Barott (1937) found higher hatchability near 37.8°C with 60% relative humidity with ventilation of 12cm/min. However, this incubation temperature is considered higher than standard incubation temperature. Romanoff et al. (1938) found that the highest hatch of eggs set occurred at constant 37.5°C air temperature when at 60% relative humidity, but found that lowering air temperatures to as low as 33.5°C after day 16 of incubation did not negatively affect hatch and helped keep air temperatures below 37.5°C (Romanoff, 1936). Lourens et al. (2005) reported that the highest hatchability was found at a constant eggshell temperature of 37.8°C. The average eggshell temperatures for both strains were higher than 37.8°C on days 12.5, 14.5 and 17.5 of incubation at an average 38.3°C. Eggshell temperatures are unknown for this study in the hatcher. When incubating Cobb 500 eggs, commercial hatcheries set the incubator temperature 0.1°C lower than the manufacturer setting in the incubator and 0.6°C lower in the hatcher (Scott Martin, Cobb-Vantress Inc., personal communication).

Due to the location effects within the incubator, the Cobb eggs were exposed to a 0.31°C higher temperature than the ACRB eggs in both trials. Future studies must intersperse eggs to eliminate localized temperature effects within the incubator. Even with the higher air temperature, no difference in eggshell temperature was found between the strains. A significant difference in eggshell temperature was found between the trials with a 0.4°C higher eggshell temperature in trial 2. Trial 1's average eggshell temperature was 37.7°C and trial 2's average eggshell temperature was 38.1°C. The average room temperature where the incubator was located was not different between trials (24.9°C and 39.2% relative humidity in trial 1 and 25.2°C and 43.2% relative humidity in trial 2). The reason for the difference in eggshell temperature between trials is uncertain.

The Cobb 500's earlier hatch in both trials and higher amounts of late mortality (dead chicks and dead pips and trial 1's high late embryonic mortality) could be due to overheating in these early hatching chicks which remained in the hatcher longer than the ACRB chicks and were characterized as late mortality (Barott, 1937; Cobb-Vantress, 2008). The ACRB had a more consistent hatch time between trials as compared to Cobb that hatched even earlier in trial 2. The reported pull time for the commercial Cobb 500 breeder flock at 41 weeks of age used in this study was 509.5 hours when incubated in a multi-stage incubator at a commercial hatchery. Druyan (2010) incubated Cobb 500 eggs at 37.8°C and 56% relative humidity for 18 d and at 37.2°C and 60% relative humidity in the hatcher. Druyan found the first chicks hatch at 478 h of incubation and finished at 501 h of incubation. These results indicate that the temperatures of this study decreased incubation time in the Cobb 500. Another potential factor could be if the Cobb eggs were exposed to warmer environmental temperatures during transport which was not controlled by the researchers.

Another indicator of overheating embryos is a larger yolk at hatch (Willemsen et al., 2010). The Cobb chicks hatched with larger dry yolk weight as a percentage of body weight as compared to the ACRB. This study did not evaluate initial yolk weights prior to setting. Christensen et al. (1995) found that ACRB eggs had 1.2% greater yolk prior to setting than Arbor Acres eggs. Buhr et al. (2006) compared the incidence of unabsorbed yolk sacs in modern commercially raised broilers to the ACRB at 8 wk of age post hatch and found double the occurrence of unabsorbed yolk sacs in commercial broilers. Our difference in yolk may explain the higher incidence of unabsorbed yolk sacs in modern market age broilers, but weighing initial yolk weights would need to clarify any difference in yolk utilization between the strains. Further study can determine if modern embryos are utilizing an appropriate amount of yolk under lower incubation temperatures. O'Dea (2004) did not find a difference in percent yolk sac at hatch between modern broiler strains and an unimproved 1978 female broiler line. Unincubated eggs of this study had a higher percentage of yolk weight in the 1978 line, but these eggs were weighed after they were covered in desiccant, remained in a desiccator for 9 d, and stored in a cooler for as much as 16 wks. Future work can determine if yolk utilization differs between the ACRB and modern broilers.

The smaller ACRB eggs had greater percentage of moisture loss than the larger Cobb eggs even after adjusting for surface area. This finding disagrees with Christensen et al. (1995) who found that ACRB eggs and AA eggs had similar conductance constants when analyzing conductance adjusted for egg weight. O'Dea et al. (2004) also did not find a difference in percent moisture loss between smaller 1978 female line broiler eggs and modern broiler strain eggs. Eggs should lose 12% of their initial egg weight prior to external pipping (Tullett, 1981; Cobb-Vantress, 2008). Our percent moisture loss is lower than 12% in both strains. Our Cobb

moisture loss is near the values obtained by Christensen et al. (1995) and our ACRB moisture loss is closer to the moisture loss found by O'Dea et al. (2004). Future work should incubate the ACRB and Cobb eggs at lower incubation temperatures and determine if moisture loss agrees with past research.

The greater percentage of early embryonic mortality in the ACRB either shows that genetic selection has increased the survival of today's early embryos or is the result of the longer storage of ACRB eggs compared to the Cobb 500 eggs. Since our eggs were stored at most 7 d and hatchability significantly decreases with greater early embryonic mortality only when eggs are stored beyond 7d, our greater early embryonic mortality in the ACRB is most likely due to genetics (Landauer, 1948; Walsh et al., 1995; Fasenko et al., 2001).

In conclusion, the modern broiler embryo has changed over the past 50 years. Current breeders lay 23% larger eggs which result in larger embryos and chicks, but as a percentage of the egg weight, the broiler chick remains unchanged. The modern broiler does not have a greater eggshell temperature than the past broiler during incubation. At the temperatures used in this study, modern broilers hatch earlier, lose less moisture as a percentage of egg weight, hatch with greater dry yolk weight as a percentage of chick weight, and experience greater late mortality which may be due to the longer time in the hatcher after hatch. The broiler embryo of 50 years ago appears to have a greater amount of early embryonic mortality. Further research is needed to clarify differences in modern broilers compared to the ACRB.

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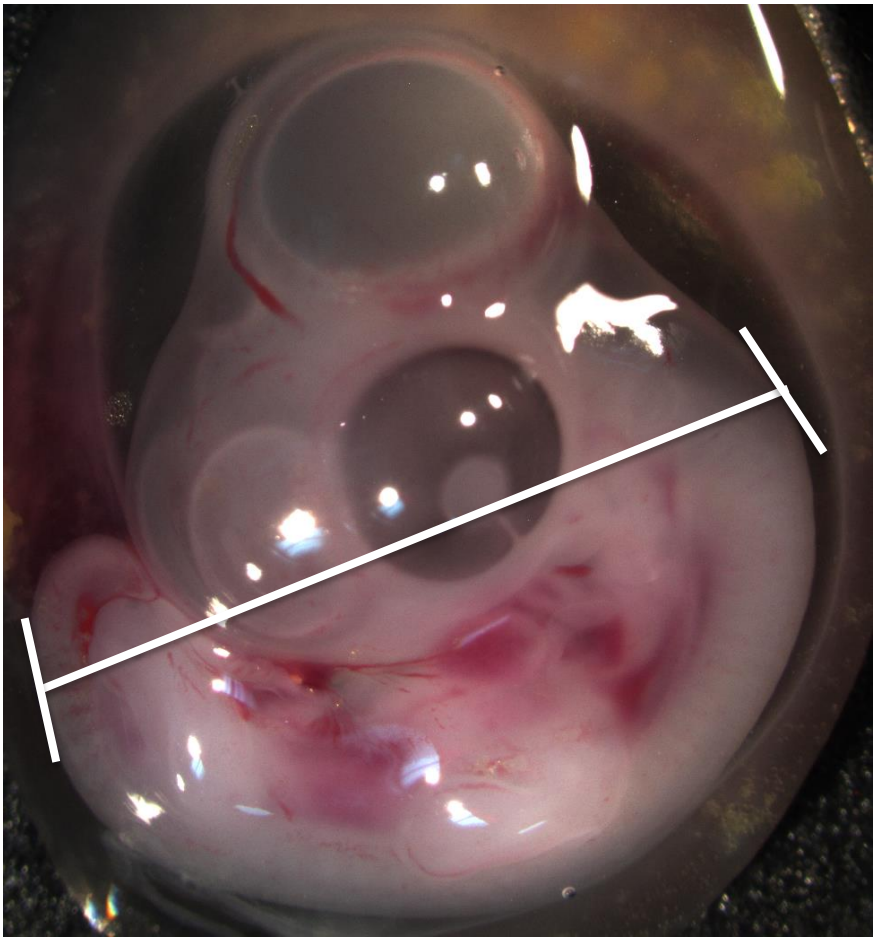


Figure 3.1. Demonstration of the natural measurement of a curled early embryo from the outermost portion of the curled tail to the outermost part of the curved neck. A 5 day old Cobb 500 broiler embryo is pictured. The white line indicates the length area that was measured.

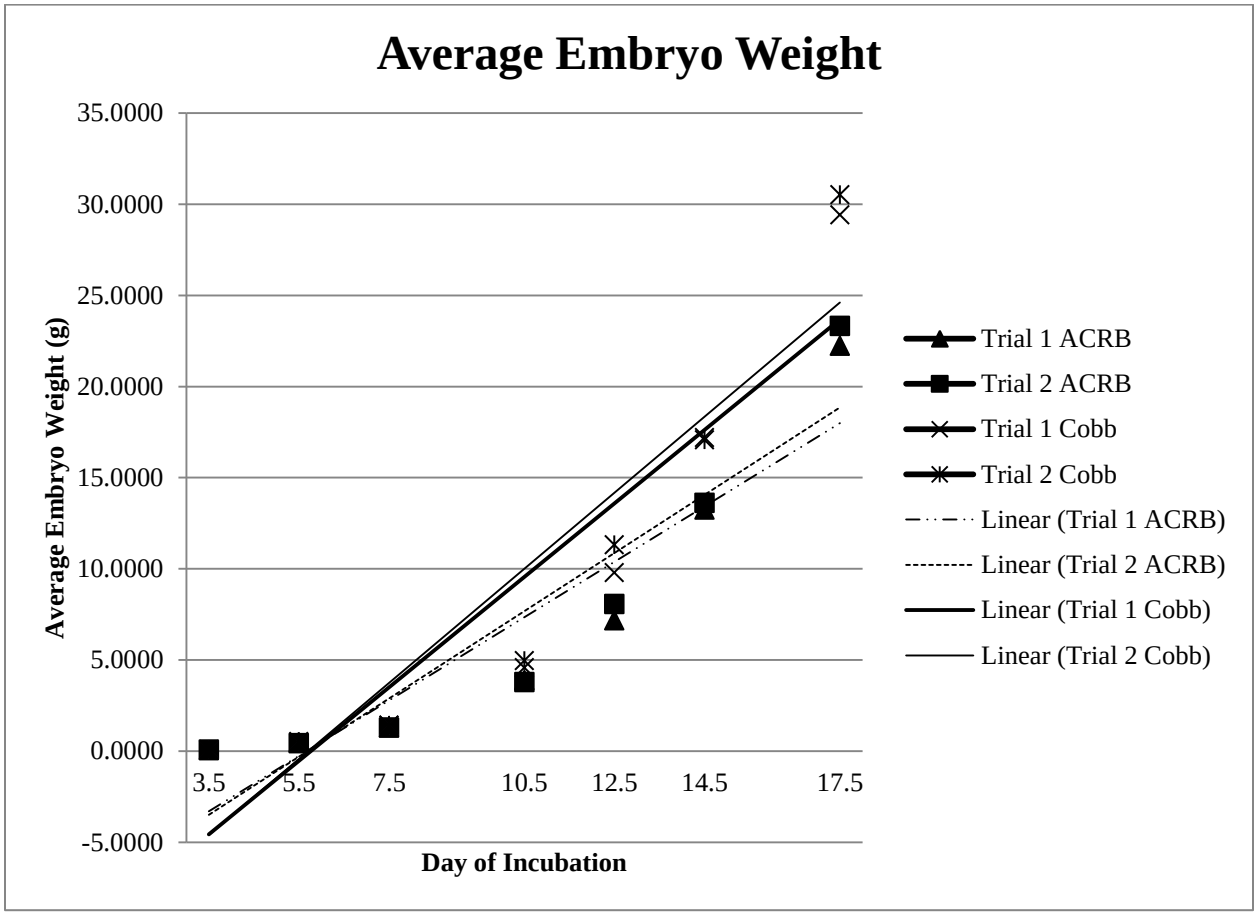


Figure 3.2. Average embryo weights comparing the Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb). Average embryo weights are the average of three embryos per strain on days 3.5-14.5. On day 17.5, eight embryos per strain were weighed in trial 1 and seven embryos per strain were weighed in trial 2.

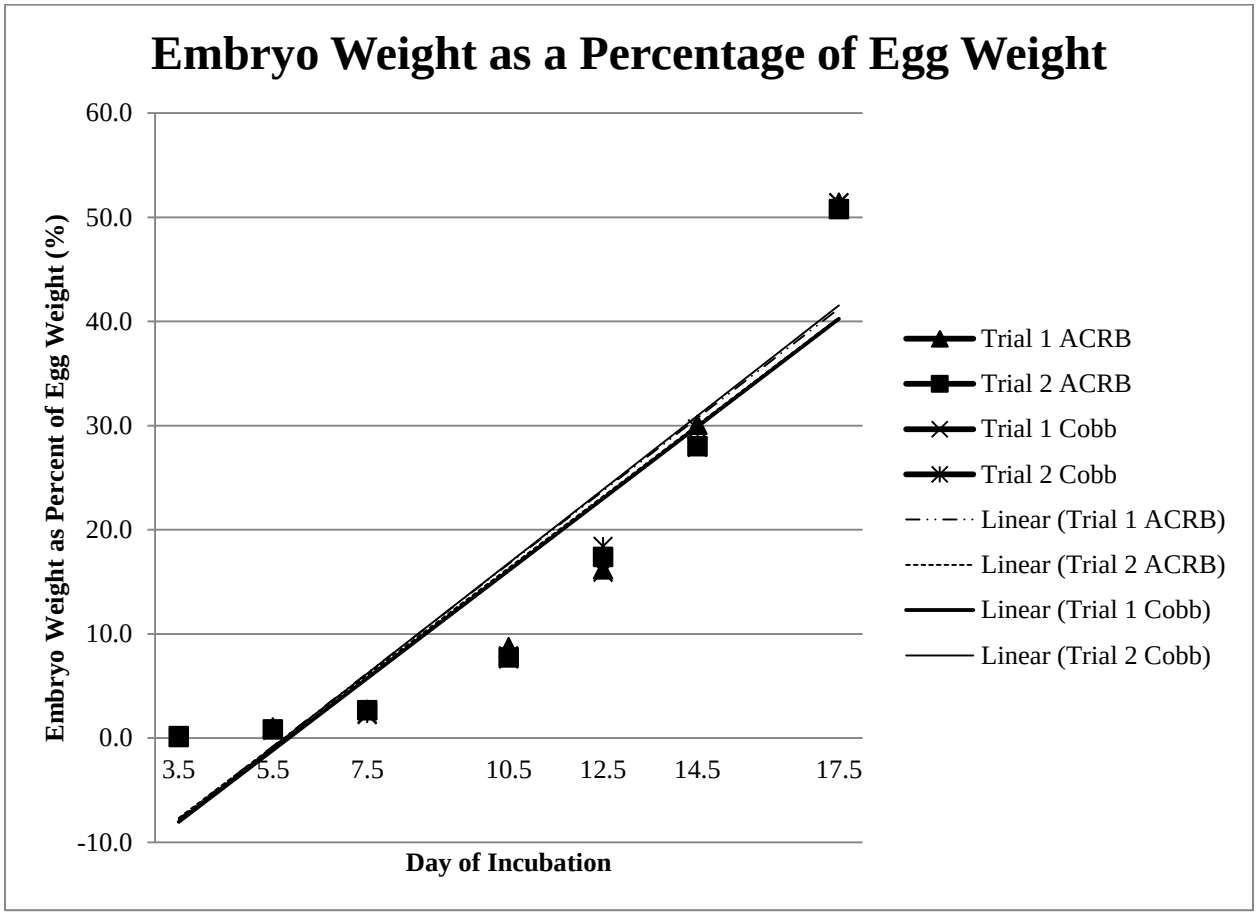


Figure 3.3. Average embryo weights as a percentage of initial egg weight comparing the Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb). Average embryo weights are the average of three embryos per strain on days 3.5-14.5. On day 17.5, eight embryos per strain were weighed in trial 1 and seven embryos per strain were weighed in trial 2.

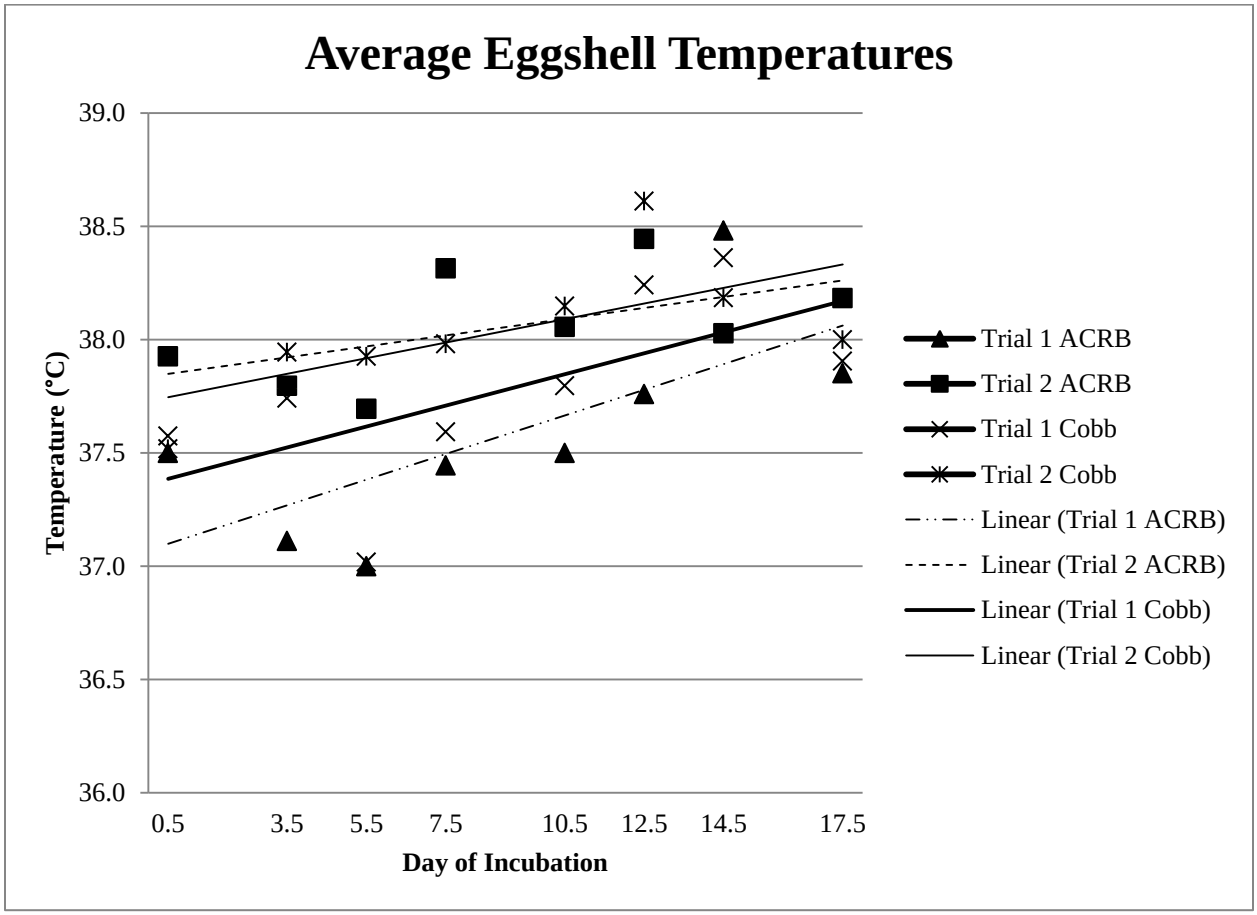
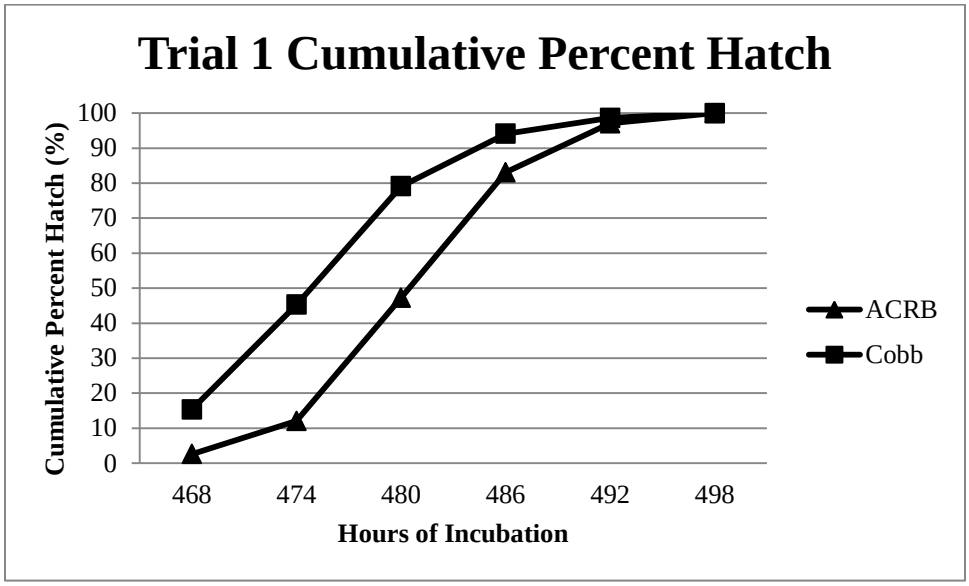


Figure 3.4. Average eggshell temperatures on 8 sample days taken using an Exergen temporal artery thermometer. Days 0.5-14.5 are the average eggshell temperatures of three eggs per strain. On day 17.5, eight eggs per strain were scanned in trial 1 and seven eggs per strain were scanned in trial 2. Any infertile or dead embryo eggs were not included.

ACRB=Athens Canadian Random Bred, 1950s meat chicken

Cobb=2012 Cobb 500



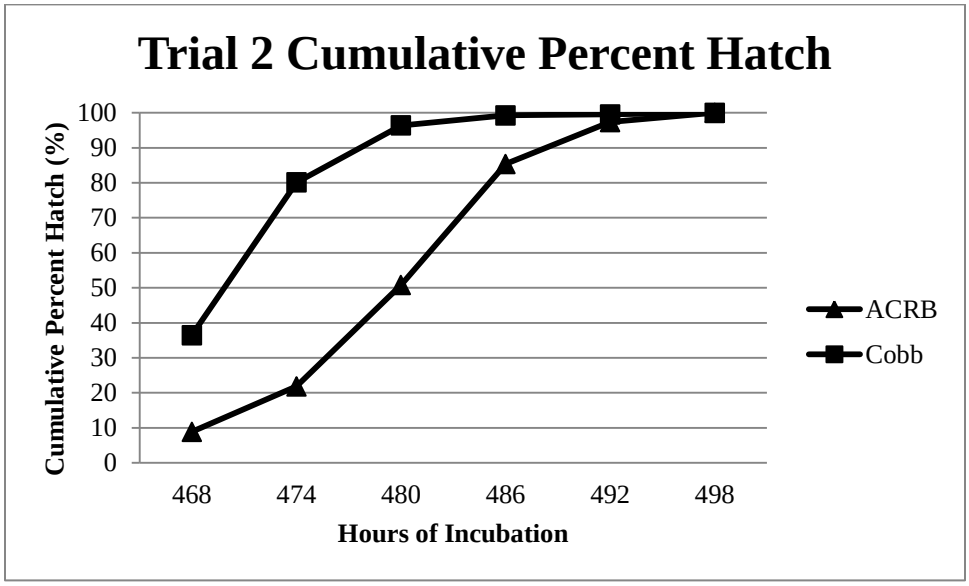


Figure 3.5 Cumulative percent hatch recorded every 6 hours from 468-498 hours of incubation in trial 1 and trial 2.

ACRB=Athens Canadian Random Bred, 1950s meat chicken

Cobb=2012 Cobb 500

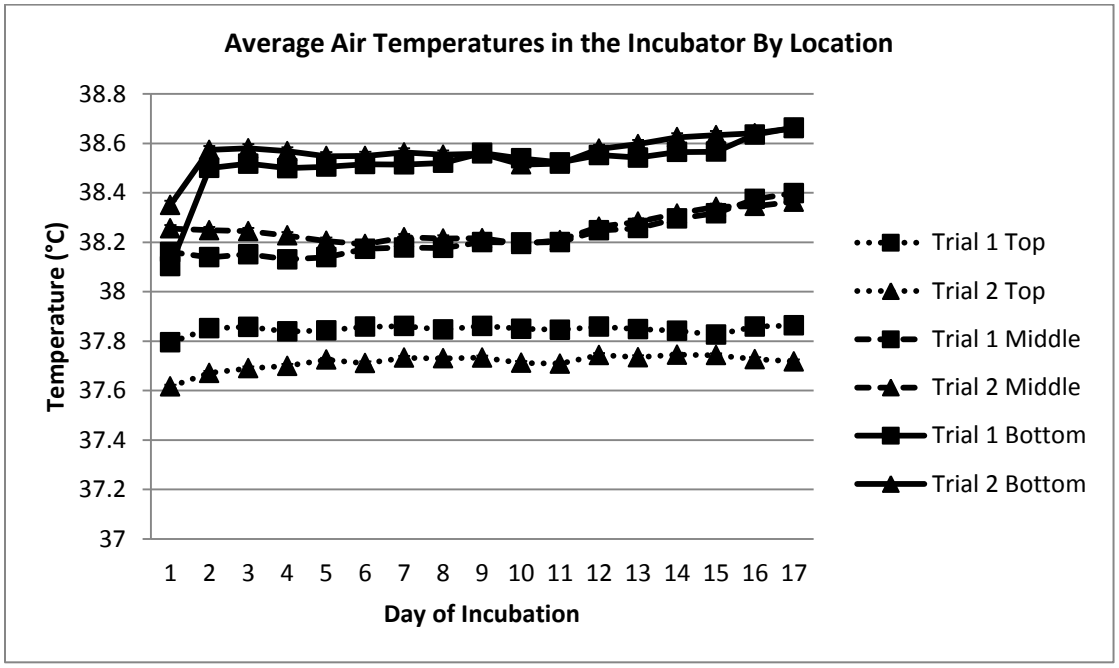


Figure 3.6. Average air temperatures in the setter during both trials logged every 15 minutes by location by HOBO temperature data loggers hanging in the machine on days 1-17 of incubation. The top probe was located next to the incubator's temperature probe, the middle probe was located in the top of the egg mass where the Athens Canadian Random Bred (ACRB) eggs were located, and the bottom probe was located in the bottom of the egg mass near the Cobb 500 eggs. There was a significant interaction of trial, location, and day of incubation ($p<.0001$). The bottom probe had highest temperature in both trials followed by the middle, and the top probe achieved the lowest temperature closest to the set temperature of 37.7°C. The top temperature was higher in trial 1 for all days. The middle temperature was higher during trial 2 on days 1-5. Trial 2 had a higher temperature in the bottom of the egg mass on days 1-5, 7, and 13-15.

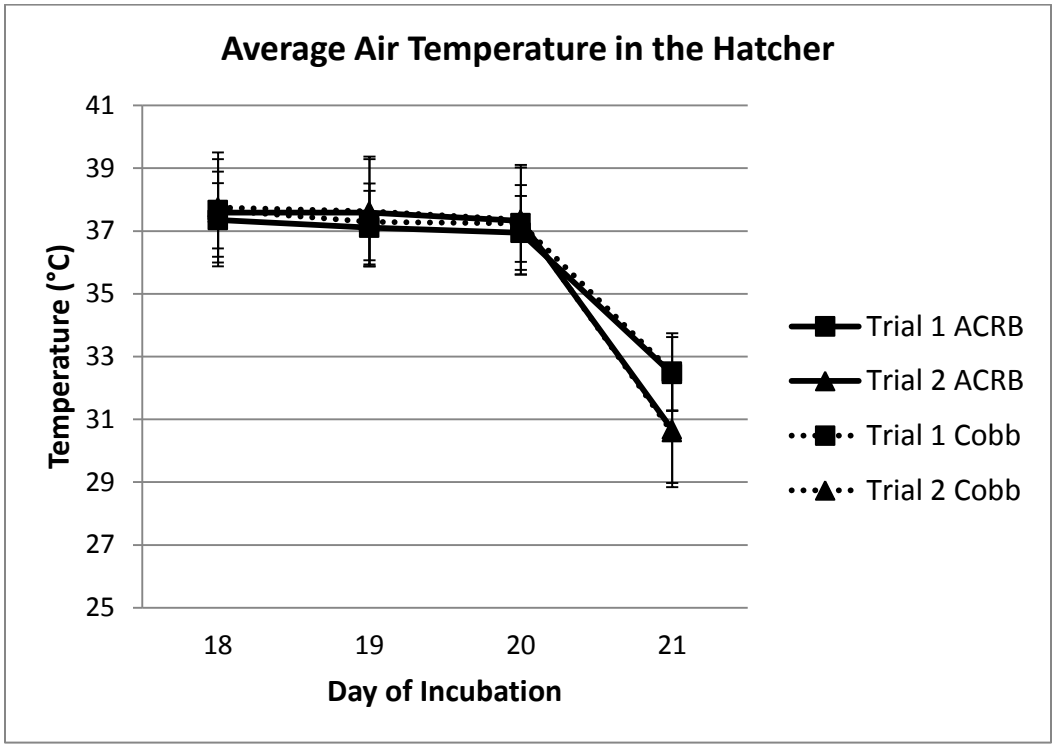


Figure 3.7. Average Chickmaster egglogger air temperatures in the hatcher by strain. Two eggloggers per strain were placed in hatching baskets with the eggs. The air temperatures did not differ between the strains while in the hatcher (p=0.7460). Eggs were transferred into the hatcher on day 18.5. The temperature decreases on day 21 since chicks were pulled from the hatcher at 498 hours of incubation (20.75 days of incubation). The machine was opened every 6 hours starting at 468 hours of incubation (19.5 days).

Table 3.1. Average Egg Measurements Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)

	Trial 1		Trial 2		Overall	
Measurement	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
Egg Weight (g)[1]	47.7±4.3	62.8±4.0	50.0±3.5	64.0±3.7	48.9±4.1	63.4±3.9
Moisture Loss (%)[1]	11.2±2.0	8.9±1.2	10.8±1.4	8.8±1.8	11.0±1.7	8.9±1.5
Moisture Loss corrected for egg surface area[1,3]	0.85±0.17	0.56±0.09	0.80±0.11	0.55±0.11	0.83±0.15	0.55±0.10
Chick Weight (g)[2]	32.9±3.1	42.7±3.3	35.1±2.8	43.5±3.2	34.0±3.1	43.1±3.3
Yolk-free Chick Weight (g)[2]	29.6±2.6	37.0±2.6	31.3±2.2	38.3±2.7	30.5±2.5	37.7±2.7
Dry Yolk (%)[2,4]	5.1±1.8	6.5±1.6	5.8±1.6	6.4±2.0	5.5±1.7	6.5±1.8
Yolk Moisture (%)[2,5]	49.9±3.2	51.4±2.6	45.9±3.4	46.9±5.8	47.9±3.9	49.2±5.0

P Values	**Strain**	**Trial**	**Strain*Trial**
Egg Weight (g)[1]	<0.0001*	0.0144*	0.4632
Moisture Loss (%)[1]	<0.0001*	0.4773	0.6992
Moisture Loss corrected for egg surface area[1,3]	<0.0001*	0.1487	0.4209
Chick Weight (g)[2]	<0.0001*	0.0007*	0.1148
Yolk-free Chick Weight (g)[2]	<0.0001*	<0.0001*	0.4806
Dry Yolk (%)[2,4]	<0.0001*	0.1997	0.1026
Yolk Moisture (%)[2,5]	<0.0169*	<0.0001*	0.6635

Means ± standard deviation

[1]n = 30 eggs per strain in each trial

[2]n = 57 ACRB and 56 Cobb in trial 1 and 62 ACRB and 56 Cobb in trial 2

[3]Moisture Loss corrected for egg surface area = moisture loss of the egg/initial weight of the egg$^{2/3}$

[4]Yolk sac of the chick dried for 72 h at 80°C

[5](Wet yolk sac weight – dry yolk sac weight) * 100

Table 3.2. Average Heart Weight (g) Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
7.5	0.0737	0.0749	0.0126	0.0134	0.0432	0.0442
10.5	0.0440	0.0441	0.0283	0.0395	0.0362	0.0418
12.5	0.0642	0.0782	0.1561	0.2158	0.1102	0.1470
14.5	0.0924	0.1262	0.0995	0.1301	0.0960	0.1282
17.5	0.1148	0.1738	0.1356	0.1960	0.1252	0.1849
P values						
Trial	0.0293*					
Day of incubation	<0.0001*					
Strain	<0.0001*					
Trial*Day	<0.0001*					
Trial*Strain	0.4389					
Day*Strain	0.0008*					
Trial*Day*Strain	0.5734					

[1]Average embryo heart weights are the average of three embryos per strain on days 7.5-14.5. On day 17.5, eight embryos per strain were weighed in trial 1 and seven embryos per strain were weighed in trial 2.

Table 3.3. Average Percentage Heart Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
7.5	5.8	5.4	1.0	0.9	3.4	3.2
10.5	1.2	1.0	0.8	0.8	1.0	0.9
12.5	0.9	0.8	2.0	2.0	1.5	1.4
14.5	0.7	0.7	0.7	0.8	0.7	0.8
17.5	0.5	0.6	0.6	0.6	0.6	0.6
P values						
Trial	<0.0001*					
Day of incubation	<0.0001*					
Strain	0.4446					
Trial*Day	<0.0001*					
Trial*Strain	0.3734					
Day*Strain	0.5317					
Trial*Day*Strain	0.7866					

[1]Average embryo percentage heart weights of embryo weights are the average of three embryos per strain on days 7.5-14.5. On day 17.5, eight embryos per strain were weighed in trial 1 and seven embryos per strain were weighed in trial 2.

Table 3.4. Average Intestine Weight (g) Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
12.5	0.0835	0.1238	0.0578	0.0769	0.0707	0.1004
14.5	0.2159	0.2717	0.2589	0.3358	0.2374	0.3038
17.5	0.4311	0.5818	0.4799	0.7227	0.4555	0.6523
P values						
Trial	0.0898					
Day of incubation	<0.0001*					
Strain	<0.0001*					
Trial*Day	0.0495*					
Trial*Strain	0.2709					
Day*Strain	0.0021					
Trial*Day*Strain	0.4645					

[1]Average embryo intestine weights are the average of three embryos per strain on days 12.5 and 14.5. On day 17.5, eight embryos per strain were weighed in trial 1 and seven embryos per strain were weighed in trial 2.

Table 3.5. Average Percentage Intestine Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
12.5	1.2	1.3	0.7	0.7	1.0	1.0
14.5	1.6	1.6	1.9	2.0	1.8	1.8
17.5	2.0	2.0	2.1	2.4	2.1	2.2
P values						
Trial	0.5156					
Day of incubation	<0.0001*					
Strain	0.2013					
Trial*Day	0.0003*					
Trial*Strain	0.3643					
Day*Strain	0.6574					
Trial*Day*Strain	0.4892					

[1]Average embryo percentage intestine weights of embryo weights are the average of three embryos per strain on days 12.5 and 14.5. On day 17.5, eight embryos per strain were weighed in trial 1 and seven embryos per strain were weighed in trial 2.

Table 3.6. Average Natural Curled Embryo Diameter Length (mm) Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
3.5	10.63	11.37	7.79	7.80	9.21	9.59
5.5	16.65	16.69	11.81	12.33	14.23	14.51
7.5	19.08	20.14	18.19	19.06	18.64	19.60
10.5	29.72	30.90	31.13	32.72	30.43	31.81
12.5	34.59	42.09	36.56	39.92	35.58	41.01
14.5	45.32	46.11	44.35	45.32	44.84	45.72
17.5	51.96	52.47	46.46	51.94	49.21	52.21
P values						
Trial	<0.0001*					
Day of incubation	<0.0001*					
Strain	<0.0001*					
Trial*Day	0.0130*					
Trial*Strain	0.3951					
Day*Strain	0.0623					
Trial*Day*Strain	0.1581					

[1]Average embryo lengths are the average of three embryos per strain on days 3.5-14.5. On day 17.5, eight embryos per strain were measured in trial 1 and seven embryos per strain were measured in trial 2.

Table 3.7. Average Stretched Embryo Length (mm) Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
7.5	NA	NA	29.39	30.65	29.39	30.65
10.5	45.75	48.03	56.06	60.76	50.91	54.40
12.5	80.45	82.28	71.87	87.67	76.16	84.98
14.5	96.48	95.14	102.93	108.10	99.71	101.62
17.5	123.33	132.40	127.00	136.80	125.17	134.60
P values						
Trial	0.1433					
Day of incubation	<0.0001*					
Strain	<0.0001*					
Trial*Day	0.0250*					
Trial*Strain	0.1225					
Day*Strain	0.1936					
Trial*Day*Strain	0.3810					

[1]Average embryo lengths are the average of three embryos per strain on days 7.5-14.5. On day 17.5, eight embryos per strain were measured in trial 1 and seven embryos per strain were measured in trial 2.

Table 3.8. Average Embryo Wing Length (mm) Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
5.5	1.83	1.39	1.40	1.66	1.62	1.53
7.5	3.47	3.12	3.04	3.41	3.26	3.27
10.5	6.11	5.92	5.60	5.96	5.86	5.94
12.5	7.70	8.25	7.39	8.78	7.55	8.52
14.5	9.11	11.44	12.08	11.23	10.60	11.34
17.5	12.57	13.39	12.07	13.26	12.32	13.33
P values						
Trial	0.9558					
Day of incubation	<0.0001*					
Strain	<0.0001*					
Trial*Day	0.0098*					
Trial*Strain	0.6369					
Day*Strain	0.0400*					
Trial*Day*Strain	0.0023*					

[1]Average embryo lengths are the average of three embryos per strain on days 5.5-14.5. On day 17.5, eight embryos per strain were measured in trial 1 and seven embryos per strain were measured in trial 2.

Table 3.9. Average Embryo Leg Length (mm) Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
5.5	2.33	1.99	1.71	1.46	2.02	1.73
7.5	3.06	2.91	2.93	3.23	3.00	3.07
10.5	6.94	6.40	6.80	7.54	6.87	6.97
12.5	9.92	10.58	10.46	10.98	10.19	10.78
14.5	12.75	14.19	13.66	14.95	13.21	14.57
17.5	19.00	20.89	18.79	23.30	18.90	22.10
P values						
Trial	0.1201					
Day of incubation	<0.0001*					
Strain	<0.0001*					
Trial*Day	0.2545					
Trial*Strain	0.0188*					
Day*Strain	<0.0001*					
Trial*Day*Strain	0.1628					

[1]Average embryo lengths are the average of three embryos per strain on days 5.5-14.5. On day 17.5, eight embryos per strain were measured in trial 1 and seven embryos per strain were measured in trial 2.

Table 3.10. Average Embryo Third Toe Without the Nail Length (mm) Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
12.5	8.62	8.94	8.21	9.59	8.42	9.27
14.5	12.01	12.65	10.87	11.89	11.44	12.27
17.5	16.55	18.12	16.47	17.53	16.51	17.83
P values						
Trial	0.0178*					
Day of incubation	<0.0001*					
Strain	<0.0001*					
Trial*Day	0.2084					
Trial*Strain	0.8659					
Day*Strain	0.5260					
Trial*Day*Strain	0.3010					

[1]Average embryo lengths are the average of three embryos per strain on days 12.5 and 14.5. On day 17.5, eight embryos per strain were measured in trial 1 and seven embryos per strain were measured in trial 2.

Table 3.11. Average Embryo Third Toe With the Nail Length (mm) Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
7.5	2.18	2.23	2.71	2.59	2.45	2.41
10.5	6.39	6.52	6.56	7.31	6.48	6.92
12.5	9.94	10.38	9.77	11.09	9.86	10.74
14.5	13.37	15.12	13.38	14.28	13.38	14.70
17.5	19.65	21.20	19.14	22.83	19.40	22.02
P values						
Trial	0.0073*					
Day of incubation	<0.0001*					
Strain	<0.0001*					
Trial*Day	0.0896					
Trial*Strain	0.7290					
Day*Strain	0.0081*					
Trial*Day*Strain	0.5924					

[1]Average embryo lengths are the average of three embryos per strain on days 7.5-14.5. On day 17.5, eight embryos per strain were measured in trial 1 and seven embryos per strain were measured in trial 2.

Table 3.12. Average Embryo Beak Length (mm) Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
7.5	1.59	2.03	1.27	1.54	1.43	1.79
10.5	4.94	4.63	2.36	2.80	3.65	3.72
12.5	5.48	5.65	3.36	3.91	4.42	4.78
14.5	6.27	6.37	3.92	4.19	5.10	5.28
17.5	7.30	8.01	5.09	5.65	6.20	6.83
P values						
Trial	<0.0001*					
Day of incubation	<0.0001*					
Strain	<0.0001*					
Trial*Day	<0.0001*					
Trial*Strain	0.4994					
Day*Strain	0.1652					
Trial*Day*Strain	0.3500					

[1]Average embryo lengths are the average of three embryos per strain on days 7.5-14.5. On day 17.5, eight embryos per strain were measured in trial 1 and seven embryos per strain were measured in trial 2.

Table 3.13. Average Embryo Third Toe Nail Length (mm) Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
12.5	1.3	1.4	1.6	1.5	1.45	1.45
14.5	1.4	2.5	2.5	2.4	1.95	2.45
17.5	3.1	3.1	2.7	5.3	2.90	4.20
P values						
Trial	0.6907					
Day of incubation	<0.0001*					
Strain	0.0600					
Trial*Day	0.2143					
Trial*Strain	0.8926					
Day*Strain	0.5943					
Trial*Day*Strain	0.0738					

[1]Average embryo lengths are the average of three embryos per strain on days 12.5 and 14.5. On day 17.5, eight embryos per strain were measured in trial 1 and seven embryos per strain were measured in trial 2.

Table 3.14. Average Hamburger and Hamilton Stages Comparing the 2012 Athens Canadian Random Bred (ACRB) to the 2012 Cobb 500 (Cobb)[1]

	Trial 1		Trial 2		Overall	
Day of Incubation	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**
0.5	4.0	3.3	3.5	4.7	3.8	4.0
3.5	22.0	22.0	23.0	23.0	22.5	22.5
5.5	27.0	26.7	27.0	27.3	27.0	27.0
7.5	33.0	32.0	32.0	32.0	32.5	32.0
10.5	37.0	37.3	37.8	38.0	37.4	37.7
12.5	39.0	39.0	39.0	39.5	39.0	39.3
14.5	40.0	40.0	40.0	40.0	40.0	40.0
17.5	42.1	42.7	41.9	42.7	42.0	42.7
P values						
Trial	0.0061*					
Day of incubation	<0.0001*					
Strain	0.0253*					
Trial*Day	0.0003*					
Trial*Strain	0.0106*					
Day*Strain	0.0113*					
Trial*Day*Strain	0.1141					

[1]Average embryo stages are the average of three embryos per strain on days 0.5-14.5. On day 17.5, eight embryos per strain were staged in trial 1 and seven embryos per strain were staged in trial 2.

Table 3.15. Residue Egg Analysis Comparison of the 2012 Athens Canadian Random Bred (ACRB) and the 2012 Cobb 500 (Cobb) Evaluated After Hatch

	Trial 1		Trial 2		Overall		P values		
Measurement (%)[1]	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**ACRB**	**Cobb**	**Strain**	**Trial**	**Strain* Trial**
Hatchability[2]	88.0	81.4	83.9	83.3	86.0	82.4	0.1432	0.5872	0.1635
Infertile[3]	5.4	4.0	7.3	5.9	6.4	5.0	0.4433	0.3070	0.9988
Early Dead[4]	4.3	2.3	4.1	3.5	4.2	2.9	0.0218*	0.3162	0.1662
Middle Dead[4]	0.0	0.3	0.2	0.2	0.1	0.3	0.5712	0.7733	0.5036
Late Dead[4]	1.4	6.6	2.7	3.3	2.1	5.0	0.0069*	0.2329	0.0137*
Live Pip[5]	0.0	2.9	1.2	0.4	0.6	1.7	0.0728	0.1187	0.0001*
Dead Pip[5]	0.6	1.4	0.0	0.4	0.3	0.9	0.0468*	0.0113*	0.4263
Dead Chick[6]	0.0	0.0	0.0	1.0	0.0	0.5	0.0244*	0.0529	0.0529
Cull Chicks[7]	0.0	0.6	0.4	0.4	0.2	0.5	0.4913	0.7002	0.3882
Set Cracks[8]	0.0	0.3	0.0	0.0	0.0	0.2	0.3196	0.2413	0.2413
T. Cracks[9]	0.3	0.3	0.2	0.6	0.3	0.5	0.3820	0.6552	0.4588
Ct. Eggs[10]	0.0	0.0	0.0	1.0	0.0	0.5	0.0655	0.1152	0.1152

[1]Each measurement represents the mean percent of 5 trays of 70 eggs each per strain in trial 1 and 7 trays of 70 eggs each per strain in trial 2

[2]Percentage of chicks hatched from the total number of eggs set (70 per tray)

[3]Eggs with no fertilized blastoderm visible

[4]Eggs with no external broken eggshell containing embryos that were either early (0-7 d), middle (8-14 d), or late (15-21 d) age embryonic mortality

[5]Eggs with a chick that had externally pipped or broken through the eggshell; live and dead were designated if the chick was still alive and breathing at the time the egg was opened or if the chick was dead

[6]Chicks fully hatched and dead when the hatch was pulled

[7]Chicks fully hatched, alive, and strikingly unable to survive, such as protruding internal organs

[8]Crack present in the eggshell and the internal contents of the egg dried down to a low region of the egg

[9]Crack present in the eggshell with a white hardened shell membrane that the chick was unable to penetrate; T = Transfer

[10]Eggs that had a black, blue, or red color inside with a noticeable odor and/or eggs that popped when the eggshell was broken; Ct = Contaminated

CHAPTER 4

NO EVIDENCE OF TEMPERATURE-DEPENDENT SEX DETERMINATION OR SEX-BIASED EMBRYO MORTALITY IN THE CHICKEN[1]

[1]K.E. Collins, B.J. Jordan, B.L. McLendon, K.J. Navara, R.B. Beckstead, and J.L. Wilson. To be submitted to Poultry Science.

ABSTRACT

Skewing the sex ratio at hatch in commercial poultry would be economically beneficial to the poultry industry. The existence of temperature-dependent sex determination is uncertain in birds. This experiment investigated if incubation temperatures skew offspring sex ratios produced by commercial broilers. We used Latin Square design in which three incubators were each set at a hot (38.3°C), standard (37.5°C), or cool (36.7°C) single-stage incubation temperature profile once over three trials. Sex ratios of hatched and dead embryos were monitored. In one trial, embryo weights and 14 d post hatch chick weights were evaluated. The percentages of male hatched chicks did not differ based on incubation temperature (P=0.3883; 49.5% in the hot treatment, 51.4% at standard temperature, and 49.8% in the cool treatment). The percent hatch of eggs set was significantly lower in the hot treatment (83.6%) than the standard (93.5%) and cool (91.6%) treatments (P<0.0001) with significantly greater late embryonic mortality in the hot treatment (P<0.0001); however, the sex ratio of dead embryos did not differ among treatments (P=0.0915). Embryos from the hot treatment were significantly heavier than those from the standard treatment by day 14 of incubation and were heavier than the embryos from the cool treatment by day 9 of incubation (P<0.0001). After 14 d of post hatch growth, the broilers from the standard treatment were heavier and more uniform than those from the cool and hot treatments (P<0.0001). Standard incubation broilers at 14 d post hatch weighed 480g (CV=8.9%), cool treatment broilers weighed 453g (CV=9.8%), and hot treatment broilers weighed 454g (CV=10.0%). These data indicate that incubation temperature affects embryonic mortality as well as embryonic and post hatch growth rate, but does not affect the sex ratio of broiler chickens.

Keywords: broiler, temperature-dependent sex determination, sex, embryo, polymerase chain reaction

INTRODUCTION

In fishes, amphibians, and reptiles (lizards, turtles, and crocodylidae) temperature-dependent sex determination (TSD) mechanisms exist, and in some circumstances, these mechanisms can override genetic mechanisms of sex determination (Shoemaker and Crews, 2009). In birds, the ability to skew the offspring sex ratio based on incubation temperature is uncertain. TSD has potential financial implications for the poultry industry especially with its probable ease of application by simply changing the incubation temperature (Halverson and Dvorak, 1993). The ability of birds to bias sexes based on temperature is uncertain since they have heteromorphic sex chromosomes instead of homomorphic, which is thought to be necessary for TSD, and birds also have a narrow range of acceptable incubation temperatures compared with reptiles (Webb, 1987; Deeming and Ferguson, 1991; Janzen and Paukstis, 1991).

Deeming and Ferguson (1991) and Ferguson (1996) cited a Russian article (Shubina, Zhmurin and Vedeneeva, 1972) that incubated chicken eggs at 37.5°C, and after 72 h of incubation, reduced the temperature to 22°C for 7-8 h. These authors reported Shubina et al. (1972) hatched 150 males for every 100 females under this treatment regime.

Ferguson (1996) stated that pulsing chicken eggs with a high or low incubation temperature altered the embryo's sex with low mortality (<9% at 10 d of incubation). On day 3 of incubation (at Hamburger-Hamilton stage 18/19), he pulsed Rhode Island Red chicken eggs for 24-36 h at 22°C to produce more males and for 18-96 h at 36°C to produce more females. However, this is not peer-reviewed work and there are no other published reports that duplicate these findings.

Sex ratio skews have been documented in response to incubation temperature in Australian brush-turkeys (Göth and Booth, 2005). During both artificial incubation (31, 36, or 34°C) and natural incubation in the soil/leaf mounds (range in temperatures 27-37.3°C) maintained by the male brush-turkey, 77% males hatched at lower temperatures and 29% males hatched at higher temperatures with equal amounts (16/32) of both sexes at the intermediate temperature. Investigating the cause of these sex ratio differences, Eiby et al. (2008) repeated the artificial incubation experiments of Göth and Booth (2005) and sexed all chicks and dead embryos. They concluded that the reason for the sex skew was that the lower incubation temperature treatment had higher mortality rates for female embryos and the higher incubation temperature had higher mortality rates for male embryos, while the intermediate temperature had similar embryonic mortality for both sexes. This was the first report of temperature-dependent sex-biased embryo mortality in birds.

Sex-biased embryo mortality has been found in chickens at standard incubation temperatures. Li et al. (2008) incubated five breeds of chickens at 37.5°C and reported a bias for greater female mortality during the first week of incubation. Previous studies with Rhode Island Red chickens also found that, as hatchability decreased, the percentage of males sexed at 8 weeks post hatch increased (Hays, 1949; Hays and Spear, 1950, 1952; Hays, 1951, 1952).

In a Japanese quail experiment, Yılmaz et al. (2011) concluded that incubation temperature of a parent generation may alter the sex of the next generation, and that grandparent age may also play a role in offspring sex for birds. Japanese quail eggs from grandparents of three different ages (ranging 8-24 weeks) were incubated at five different temperatures (ranging 36.7-38.7°C) and separately reared and bred. Eggs from these temperature-treated birds (15-18 weeks old) were incubated at a standard temperature (37.7°C), and sex ratios for the resulting

offspring (F2 generation) were examined. Sex skews were observed only in the F2 generation from the oldest age grandparents (22-24 wk). Eggs collected from the parents who were incubated at lower temperatures (36.7°C and 37.2°C) had 61.8% and 63.3% males at 6 wk post hatch. Eggs collected from parents who had themselves incubated at higher temperatures (37.7°C-38.7°C) had 42.86% and 44.87% males at 6 wk post hatch. Previous research with White Leghorns and Rhode Island Red chickens (Jull, 1932; Hays, 1954) has shown that the ability to skew offspring sex ratios is not an inherited trait. These studies bred White Leghorn and Rhode Island Red hens and cocks, noting the sex ratio of the family from which the parents hatched, and examined the sex ratio of the hatched chicks from these crosses.

The current study investigates whether incubation temperature can skew chicken sex ratios at hatch and/or cause mortality in a particular embryonic sex in a modern broiler strain.

MATERIALS AND METHODS

Three single-stage NatureForm NMC2000 incubators were set to one of three temperatures (cool, standard, and hot). Over three trials, each machine was set at each of the temperature treatments once as a Latin Square Design. The treatments were set at starting temperatures of 36.7°C for the cool treatment, 37.5°C for the standard, and 38.3°C for the hot treatment. These temperatures were then gradually decreased (0.1-0.3°C per day of incubation) to 36.7°C by day 18 of incubation as a single stage profile based off the manufacturer instructions (Table 4.1). The cool treatment maintained a constant temperature throughout the duration of incubation. On day 18 of incubation, the eggs were transferred to hatching baskets in two Natureform NMC2000 incubators at 36.7°C. Two trays (90 eggs per tray) of different temperature treatments where placed in a single 76.2cm width, 71.1cm length, and 11.4cm deep

hatching basket with a divider placed in the middle of the hatching basket between the treatments. The treatments were randomly and equally interspersed throughout both hatchers. Relative humidity was set at 53% in the incubators and the hatchers. All incubators were calibrated prior to each trial. Single stage temperature profiles were applied since the highest temperature occurs during early incubation. Past work indicates that the bipotential gonad of the chick begins to differentiate into either sex between 5-8 d of incubation (Romanoff, 1933, 1960), and TSD acts on sex determination prior to the start of morphological sex differentiation in reptiles (Shoemaker and Crews, 2009). If TSD exists in chickens, manipulation of incubation temperature early in embryonic development (<8 d) would presumably have an effect on sex ratio.

Ross 708 hatching eggs were obtained from commercial broiler breeder flocks in all three trials. The eggs were collected from a 34 wk old Ross 708 breeder flock in trial 1, a 38 wk old flock in trial 2, and a 43 wk old flock in trial 3. Half the eggs incubated in trial 2 were collected from a 36 wk old Cobb 500 breeder flock. In trial 1, nine trays of eggs (n=810) were incubated for each temperature treatment. Eight trays of eggs (n=720) per each treatment were incubated for trials 2 and 3.

The incubator door was opened to record eggshell temperatures along the bottom half of the egg for a sample of eggs in each treatment using an infrared thermometer (Exergen Temporal Artery Thermometer Home Model 2000C, Exergen Corporation, Watertown, M.A.) while in the incubator. In trial 1, eggshell temperatures were recorded on days 2, 4, 7, 9, 11, 14, 16 and 18 (n=6 Ross 708 eggs per treatment each sample day). In trial 2, eggshell temperatures were taken with four eggs of each strain (n=4 Cobb 500 and n=4 Ross 708 eggs) per treatment on days 2, 5, 7, 9, 12, 14, and 16. In trial 3, eggshell temperatures were recorded for eight Ross 708 eggs per

treatment on days 4-18 of incubation. Only in trial 1, three eggs per treatment were opened, the embryo was removed, humanely euthanized by decapitation and weighed on days 4-16 of incubation. Also only in trial 1, hatched chicks from two trays per treatment (n=477) were weighed at hatch and raised to 14 d and weighed. The chicks were weighed as an entire tray at hatch. These birds were placed by sex and tray so there were 12 total pens of 6 female and 6 male pens in a closed sided house. The pens were 1.5m by 1.5m with an average of 40 birds per pen with a maximum of 47 and a minimum of 29 birds. The birds were reared under full (not dimmed) lighting conditions during the entire 14 d. One hour of dark was provided at placement until 1 week of age whereupon the birds were provided 4 hours of dark. A starter diet was provided the entire 14 d, and the room temperature decreased from 30.6°C at placement to 28.1°C at 14 d. The 14 d old birds were individually weighed.

After 21.5 d of incubation, the chicks were removed from the incubator and counted. The same professional vent sexer sexed all chicks from the three trials. The numbers of male and female chicks were recorded. Subsamples of each sex were necropsied to determine the accuracy of the vent sexer for each temperature treatment. The total 477 14 d old broilers (two trays of hatched chicks per treatment) were necropsied to check the vent sexer's accuracy in trial 1, 60 hatched chicks were necropsied in trial 2, and 30 hatched chicks were necropsied in trial 3. For two trays per each of the three treatments, all embryonic mortality was sexed by gross necropsy or PCR. Sex can be determined by necropsy with the naked eye at hatch as male chicks have two visible testes and females have only a left ovary (Romanoff, 1933). A residue breakout was done for all trays to determine fertility and embryonic mortality (Wilson, 2010).

Tissue samples for PCR were taken from dead embryos that were unable to be sexed by necropsy. The earliest embryo age sampled was 24 hours of incubation with an enlarged

blastoderm. DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit Spin Column Protocol for animal tissues. Primer sequences were designed by Haunshi et al (2008) to amplify a 481 bp portion from the W chromosome from nucleotide positions 135-615 and a 256 bp portion of the 18S ribosomal gene from nucleotide positions 1267-1522. Primers were diluted to a 5pm/µl concentration. The total 25µl PCR reaction volume consisted of 1µl of the SaC-Forward primer, SaC-F primer 5' TAACACGCTTCACTCACA 3', 1µl of the SaC-Reverse primer, SaC-R primer 5' ATGTTTGGACAGAGGTGC 3', 1µl of the 18S Ribosome-Forward primer, 18S R-F primer 5' AGCTCTTTCTCGATTCCGTG 3', and 1µl of the 18 S Ribosome-Reverse primer, 18S R-R primer 5' GGGTAGACACAAGCTGAGCC 3', 12.5µl of DreamTaq Green PCR Master Mix (Fermentas-Life Sciences), 500 ng/µl solution of extracted DNA when possible, and molecular biology grade water. Early dead samples had very low DNA concentrations (< 20 ng/µl) and for these cases 8.5µl of extracted DNA was added with no water. Cycling parameters were 94°C for 2 min, 25 cycles of 94°C for 15 s, 54°C for 15 s, 72°C for 30 s, then 72°C for 7 min. PCR products were visualized by gel electrophoresis using a 1% agarose gel stained with 5µl Ethidium Bromide and visualized with ultraviolet light. Adult rooster and hen liver samples were extracted as controls and a 100bp ladder (ready to use GeneRuler Fermentas-Life Sciences) was run to compare band lengths.

Statistical Analysis

The data were analyzed using the general linear model procedure of SAS (Version 9.2) as a Latin Square design to determine if the temperature treatment and the incubator affected the percentages of each sex that hatched, percent hatch of eggs set, percentages of embryonic mortality, sex percentages of embryonic mortality, embryonic weights, and 14 day posthatch weights. The experimental unit was the tray (90 eggs per tray), so percentages were calculated

for each tray. Sex percentage data were arcsine transformed for analysis. Any P value less than 0.05 were regarded as significant. Significant differences were compared using Fisher's Least Significant Difference.

RESULTS

The eggshell temperatures correctly mirrored each temperature treatment (Figure 4.1, 4.2, and 4.3). The hot treatment was significantly higher temperature than the standard which was significantly higher temperature than the cool treatment in all trials. The standard temperatures did not differ between trials 2 and 3, but trial 1's standard temperature was 0.5°C higher. The cool temperature treatment was 0.3°C higher in trial 2 and trials 1 and 3 did not differ. The hot treatment's eggshell temperatures were not different between trials 1 and 3, but trial 2's hot eggshell temperature was 0.8°C lower than the other trials. Trials 1 and 2's high eggshell temperature in the standard and cool treatment occurred in the same incubator and could be due to an incubator effect.

Percentages of hatched chicks that were male did not differ by temperature treatment (P=0.3883, Table 4.2; 49.5% in the hot treatment, 51.4% at standard temperature, and 49.8% in the cool treatment). The vent sexer was found to be 99.6% accurate in trial 1 and 100% accurate in trials 2 and 3. Since only 60 and 30 chicks were necropsied in trials 2 and 3, these estimates to confirm the vent sexer's chick sex determinations are not as accurate as trial 1's 477 broiler necropsies.

A total of 133 dead embryos were sexed to determine the embryonic mortality sex ratio from two trays per treatment in each trial. Overall our sample of 133 dead embryos from the total 414 dead embryos across the trials is an adequate sample size. The overall percentage of dead

male embryos was not significantly different between the temperature treatments (P=0.0978; hot 45.5%, standard 43.5%, cool 45.5%, Table 4.2). Within each embryonic mortality age range (early: days 0-7 of incubation, middle: days 8-14 of incubation, and late: days 15-21 of incubation), there was no difference in male percentages among treatments (P=0.1586 early, P=0.6224 middle, P=0.1314 late). Within each embryo age range, only the early dead embryos had a sufficient sample size with 50 early dead embryos out of a total 175 needing at least 43 embryos to detect a significant difference. Late embryonic mortality comes close with 66 sampled out of a total 190 late dead embryonic mortality needing at least 85 to detect a significant difference. Thus, for the middle and late dead embryonic mortality, greater sample sizes would be needed to confirm our finding. Since no treatment effect was found for sex percentages of dead embryos, if all embryo mortality data from each temperature treatment are pooled, these data can determine if any sex-biased embryo mortality occurred in this study. Analyzing the pooled data of 133 embryos as a chi-square goodness-of-fit test compared to the expected 50:50 sex percentage does not indicate any sex biased mortality with an embryonic mortality sex ratio (female/male) of 1.22:1 ($\chi 2$=1.27; P=0.2596). Out of the total 133 embryos sexed in this study, 50 embryos were early dead (first week of incubation) with a sex ratio of 0.92:1 ($\chi 2$=0.08; P=0.7773). Analyzing our late dead embryo mortalities (days 15-21 of incubation, n=66), more female mortality occurred with a sex ratio of 1.75:1 ($\chi 2$=4.91; P=0.0267); however, analyzing all late mortality (late dead, live and dead pips, and dead chicks; n=79) no longer shows a significant female mortality bias at 1.55:1 ($\chi 2$=3.66; P=0.0558). Again, the late dead mortality alone is not a sufficient sample size, but our sample size of all late mortality (late dead, live and dead pips, and dead chicks) is a sufficient sample size to detect significant differences.

The percent hatch of eggs set was significantly lower in the hot treatment (83.6%) than the standard (93.5%) and cool (91.6%) temperature treatments (P<.0001). The hatch residue breakout showed a significantly (P<0.0001) higher percentage of late dead embryos in the hot treatment (7.9%) than the standard (1.4%) and cool (1.1%) incubation treatments which did not differ from one another. The hot treatment also had a significantly greater percentage of dead pips (P<0.0001; hot 0.9%, standard 0.05% and cool 0.2%) and dead chicks (P=0.0014; hot 0.7%, standard 0.05%, cool 0%) than the other treatments.

There was no incubator effect for male chick (0.1079) or male embryo (0.5048) sex percentages. The percent hatch of eggs set had no incubator differences in the least squares mean comparison. All hatch residue breakout parameters had no incubator effect.

Embryo weights from trial 1 were significantly different among the treatments (P<0.0001; Figure 4.4) with the hot incubation treatment heavier than the cool treatment by day 9 of incubation and heavier than the standard treatment by day 14 of incubation. The standard treatment became heavier than the cool treatment by day 11 of incubation. Chicks weights at hatch weighed as a tray (maximum 90 chicks per tray with 9 trays per treatment) were not different among incubation temperature treatments (P=0.2564). Individual broiler weights at 14 d post hatch were significantly (P<0.0001) greater in the standard treatment (480g); broilers from the cool (453g) and hot (454g) treatments did not differ. The coefficient of variation for 14 d body weights was 10.0% for the hot, 8.9% for the standard and 9.8% for the cool treatments. Female weights averaged 433g in the cool treatment, 469g in the standard, and 449g in the hot treatment. Male weights averaged 470g in the cool treatment, 491g in the standard, and 460g in the hot treatment. Strain comparisons in trial 2 yielded no difference between the strains in eggshell temperatures, egg weights, percentage hatch of eggs set, or sex ratio differences.

DISCUSSION

The incubation temperatures applied in this study are near the edge of plausible and applicable temperature ranges for commercial chicken incubation since lower temperatures delay hatches and higher temperatures increase mortality (Barrott, 1937; Romanoff et al., 1938; Suarez et al., 1996; Willemsen et al., 2010). The timing for sex determination of the bipotential gonad of the chick is thought to occur during the first week of incubation (Romanoff, 1960). The temperature profile in this study applied the highest temperatures during early incubation. Even so, these data provide no evidence for temperature-dependent sex determination or sex-biased embryo mortality.

Our results agree with older research that reported chick sex ratios at hatch were near 50% males and 50% females, as expected of genetic sex determination (Jull, 1932; Hays, 1945). These findings disagree with other studies that found sex skews in birds due to incubation temperature in Japanese quail and the Australian brush turkey (Göth and Booth, 2005; Eiby et al. 2008; Yılmaz et al., 2011). The Australian brush turkey has homomorphic sex chromosomes which may be the reason for temperature causing sex skews in this bird when compared to the heteromorphic sex chromosomes of chickens without incubation temperature causing sex skews (Deeming and Ferguson, 1991; Janzen and Paukstis, 1991). Japanese quail are more closely related to chickens (both are in the family Phasianidae) and quail also have heteromorphic sex chromosomes (Shibusawa et al., 2001; Kayang et al., 2004). The Japanese quail experiment found sex percentage skews of up to 23.6% more males due to incubation temperature only from temperature treated eggs of the oldest grandparent flock (22-24 week old). Further work would need to evaluate if older broiler breeder flocks could produce a sex skew due to incubation

temperature. The flock ages of our study (34 wk, 36 wk, 38 wk, and 43 wk) did not cause a difference in sex percentages between treatments.

Our study did not find any sex-biased mortality due to incubation temperature; however, previous studies have found higher female embryo mortality throughout incubation at standard incubation temperature in Rhode Island Red chickens (Hays, 1949; Hays and Spear, 1950, 1952; Hays, 1951, 1952). Wu et al. (2012) found greater female embryonic mortality throughout incubation in Hubei and Zhusi chickens but not in Ai-jiao, Xing-Xing, or Hy-line chickens. Our pooled data does not indicate any female biased embryo mortality in Cobb 500 and Ross 708 broilers. Li et al. (2008) and Wu et al. (2012) found greater female mortality during the first week of incubation in Hubei, Zhusi, and Hy-line Brown chickens at 37.5°C but not in Yellow or Aijiaohuang chickens. From our early embryonic mortality data, we did not find predominately more female embryo mortality during the first week of incubation. We conclude that commercial broilers do not experience sex-biased mortality even when incubated outside of optimum incubation temperatures. Future experiments could further investigate if only particular breeds of chickens experience sex biased embryo mortality.

The higher temperatures of this study did cause larger embryos but this difference was not maintained during growout which agrees with past research (Henderson, 1930; Barott, 1937; Hulet et al., 2007). The lowered percent of eggs that hatched and higher embryonic mortality late in development in the hot treatment also agrees with past research finding that high eggshell temperatures reduces hatchability and increases the number of late dead embryos (Lourens, 2001; Willemsen et al., 2010). These references state that eggshell temperatures above 39°C cause these effects, and in this study, we found decreased hatch and elevated late mortality with eggshell temperatures near 38.5°C.

In conclusion, within the temperature range and breeder flock ages of this study we found no evidence for temperature-dependent sex determination or sex-biased embryo mortality in broiler chickens. Additionally, the temperature ranges of this study had detrimental effects on embryonic and post hatch growth rates and embryo mortality rates.

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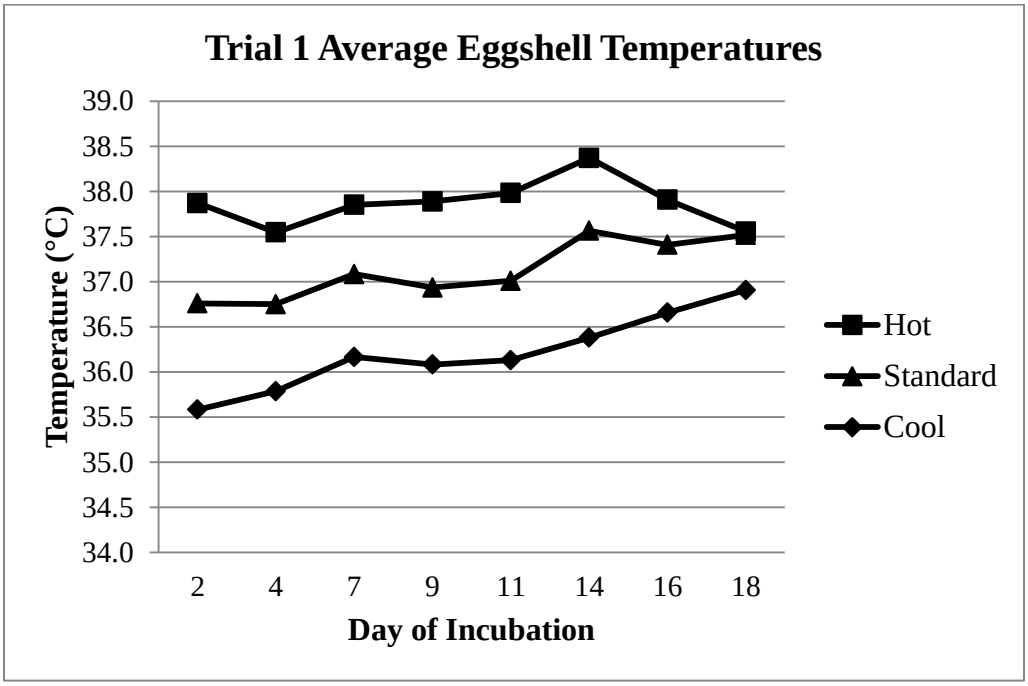

Figure 4.1. Average eggshell temperatures (°C) in three trials at a hot (38.3°C), standard (37.5°C), and cool (36.7°C) incubation temperature treatment. Trial 1 eggshell temperatures were recorded on days 2, 4, 7, 9, 11, 14, 16 and 18 (n=6 Ross 708 eggs per treatment).

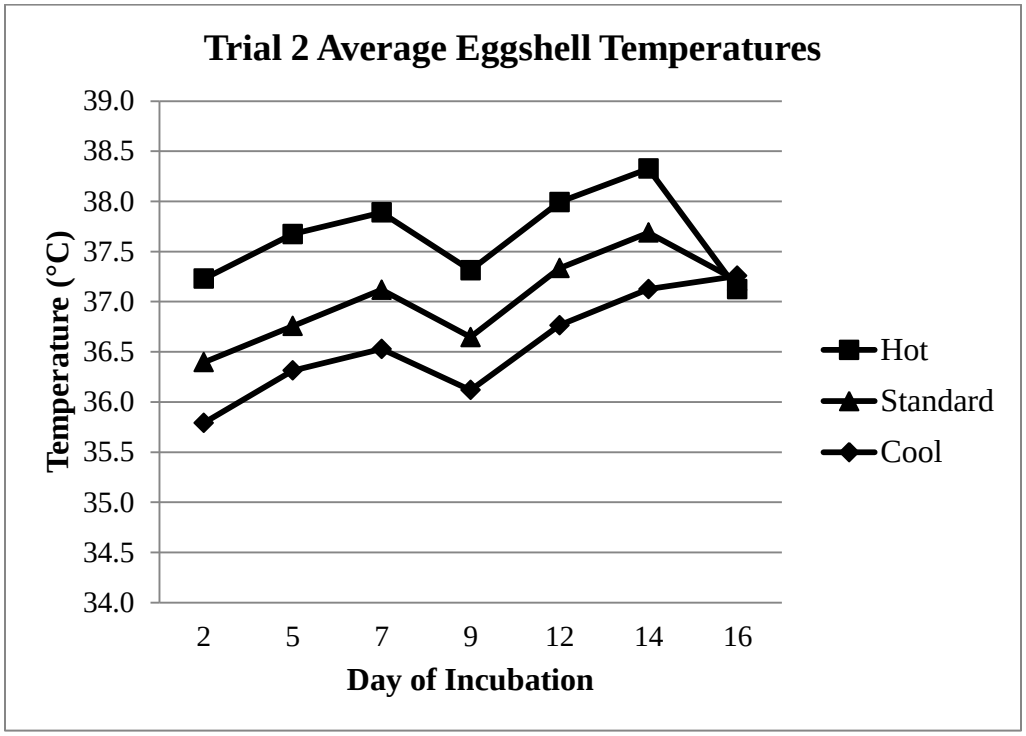

Figure 4.2. Average eggshell temperatures (°C) in three trials at a hot (38.3°C), standard (37.5°C), and cool (36.7°C) incubation temperature treatment. Trial 2 eggshell temperatures were recorded on days 2, 5, 7, 9, 12, 14, and 16 (n=4 Cobb 500 and n=4 Ross 708 eggs per treatment).

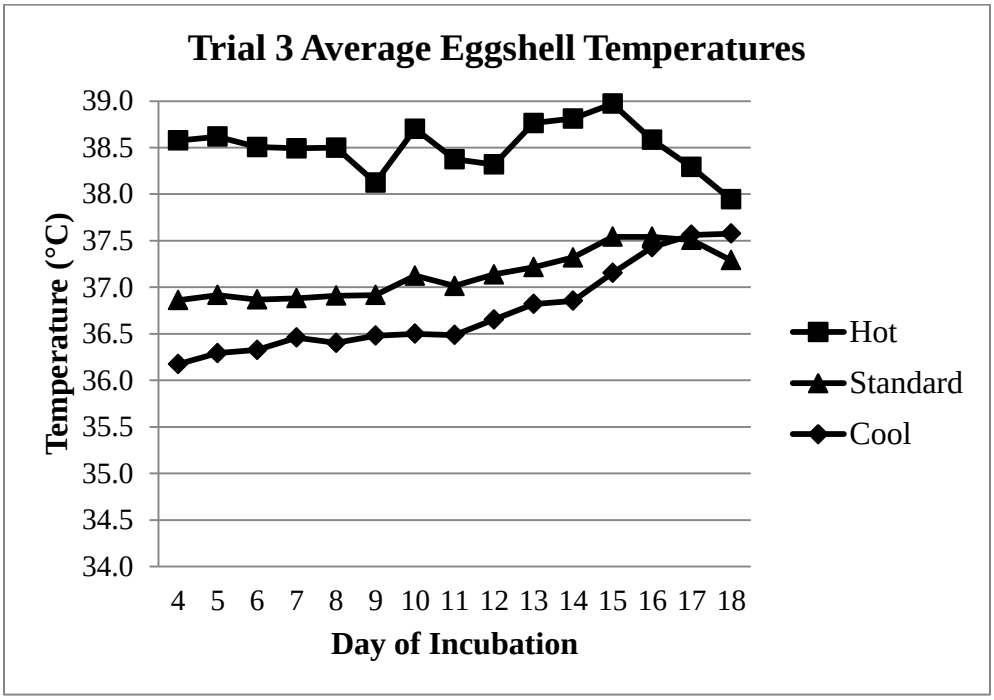

Figure 4.3. Average eggshell temperatures (°C) in three trials at a hot (38.3°C), standard (37.5°C), and cool (36.7°C) incubation temperature treatment. Trial 3, eggshell temperatures were recorded on days 4-18 of incubation (n=8 Ross 708 eggs per treatment).

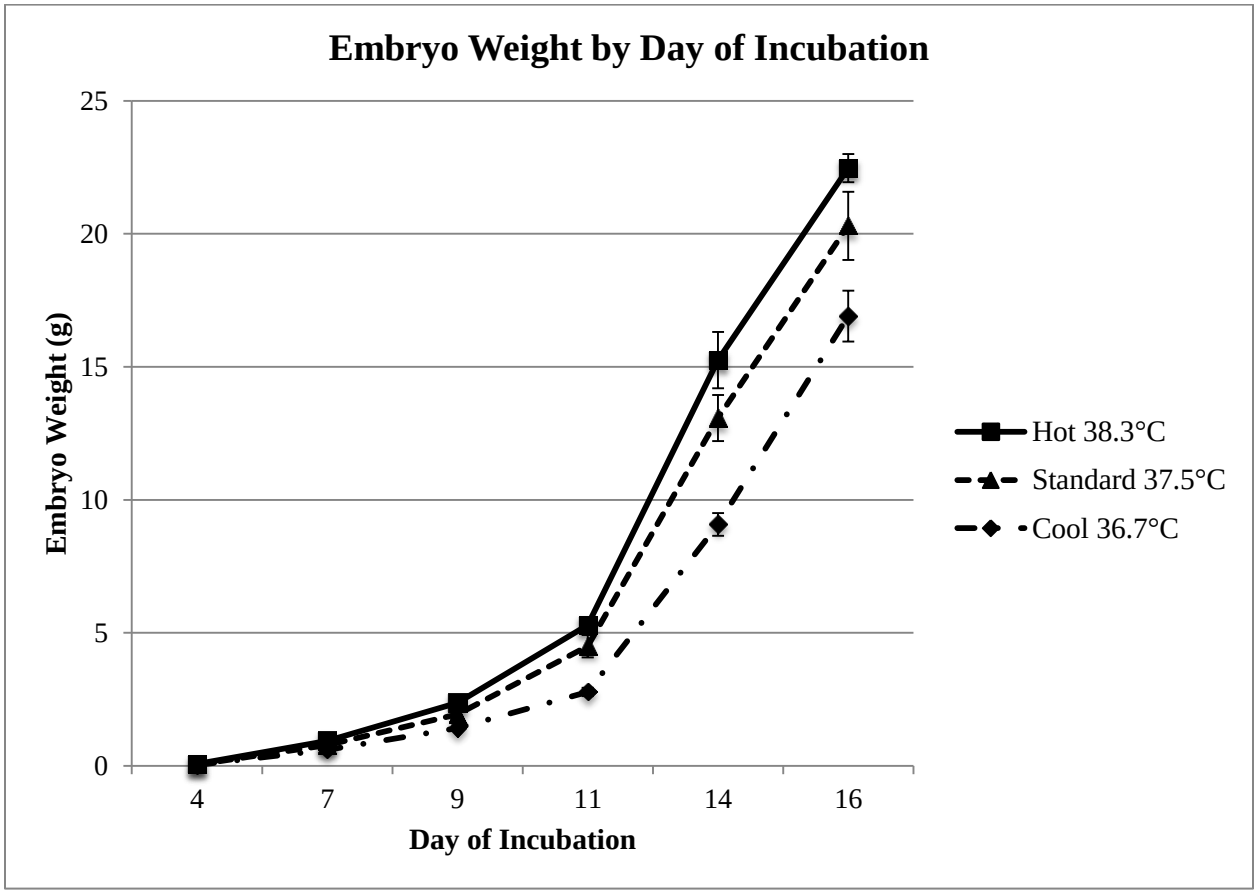

Figure 4.4. Embryo weight (g) during incubation at a hot, standard, and cool incubation temperature. One Ross 708 embryo was sampled from the top, middle and bottom tray locations of each incubator (n=3 embryos per treatment) on each day.

Table 4.1. Single Stage Set Incubation Temperatures (°C)

	Treatment		
Day of Incubation	**Hot**	**Standard**	**Cool**
1	38.3	37.5	36.7
2	38.2	37.4	36.7
5	38.1	37.4	36.7
7	38.0	37.3	36.7
10	37.8	37.2	36.7
14	37.6	37.1	36.7
15	37.3	37.0	36.7
16	37.1	36.9	36.7
17	36.9	36.8	36.7
18	36.7	36.7	36.7
Hatcher	36.7	36.7	36.7

Table 4.2. Male Broiler Percentages Based on Incubation Temperature (%)

	Incubation Temperature			
	Hot (38.3°C)	**Standard (37.5°C)**	**Cool (36.7°C)**	**P Value**
Hatched Chicks[1]	49.5	51.4	49.8	0.3882
Embryonic Mortality[2]	45.5	43.5	45.5	0.0978

[1]Based on a total 5735 hatched chicks from 73 trays of 90 eggs each
[2]Based on a total 133 embryos from 6 trays from each incubation temperature treatment

CHAPTER 5

CONCLUSIONS

A modern commercial broiler, the 2012 Cobb 500, was compared to a 1950s broiler, the Athens Canadian Random Bred (ACRB) as an embryo. Cobb eggs were an average 14.5g heavier than ACRB eggs and had heavier embryos starting on day 12.5 of incubation. When embryo weights were taken as a percentage of egg weight, the strains were not different. Similar results were found for heart and intestine weights. Yolk-free chick weights at hatch were an average 7.2g heavier for the Cobb 500 than the ACRB. These results indicate that while modern broiler eggs are larger, with heavier embryo and chick weights, proportionally, the modern broiler embryo is not different from the 1950s embryo. No difference was found in eggshell temperature during incubation between the strains. Moisture loss was 2.2% greater for ACRB eggs which may be due to egg size. There was no great difference (<1 Hamburger and Hamilton, 1951 stage) in developmental pace between the strains from 0.5-17.5 days of incubation, yet Cobb chicks hatched 6-12 hours earlier than ACRB chicks. This finding may indicate a difference in developmental pace in the hatcher or an earlier cue to hatch in the modern broilers. The ACRB experienced greater early embryonic mortality and the Cobb had greater late embryonic mortality. The ACRB early mortality may be due to genetics. The percentage of dry residual yolk at hatch was 1% greater in Cobb 500 chicks than ACRB chicks. The earlier hatch, greater late embryo mortality, and greater residual yolk at hatch may indicate that the incubation temperature of this study (37.7°C in the incubator and 37.5°C in the hatcher at 53% relative humidity) is too high for modern broiler embryos. Future work can determine if

these differences in hatch time and residual yolk are eliminated when modern broiler embryos and ACRB embryos are incubated at a lower temperature.

Modern broiler strains were also incubated at a high (38.3°C), standard (37.5°C), and cool (36.7°C) incubation temperature to determine if these various temperatures could result in a sex skew at hatch. All hatched sex percentages were near 50% as expected of genetic sex determination. Incubation temperature also did not cause sex-biased mortality. Pooled data of all embryonic mortality did not show any female biased mortality. These data do not provide evidence that commercial broiler sex can be skewed by incubation temperature. Future studies could determine if only particular breeds of poultry experience sex biased mortality. Investigating if broiler breeder flock age along with incubation temperature could cause sex skews would also be of interest.

APPENDICES

Appendix A: RECORDING EGGSHELL TEMPERATURE

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Eggshell temperatures are higher than air temperatures within an incubator and are considered a closer measurement of the temperature experienced by the embryo (French, 1997; Lourens et al., 2005). Commercial incubators monitor eggshell temperatures (Jameway, 2013). Eggshell temperatures have typically been measured using an infrared ear thermometer such as a Braun Thermoscan (Tullett, 2009; Molenaar et al., 2011; Nangsuay et al., 2013). Forehead thermometers have recently been developed as another noninvasive infrared thermometer. Studies on humans have conflicting results about the accuracy of the temporal artery thermometer. Some studies indicate that professional model forehead thermometers (Exergen Model 5000) record a lower temperature than professional rectal and ear thermometer temperatures (Penning et al., 2011; Hamilton et al., 2013). However, other studies have found no significant difference between rectal temperatures and temporal artery thermometer temperatures (Carr et al., 2011; Bahorski et al., 2012). For the purpose of measuring poultry eggshell temperatures during incubation, future work should evaluate how ear and forehead thermometer measurements compare on an embryonated egg.

Ear thermometers and forehead thermometers cover different areas of the egg. The ear thermometer remains stationary while recording an eggshell temperature, and forehead thermometers are scanned over a larger area of the eggshell. Another important distinction to consider when using an ear thermometer compared with a forehead thermometer involves the cap. Ear thermometers require a lens filter in order to record a measurement. Forehead

thermometers also have a protective cap, yet this cap must be removed before taking an eggshell measurement. Values obtained with the cap on the temporal artery scanner are hugely inaccurate such as recording a 25.8°C eggshell temperature in an incubator at a 38.2°C air temperature.

Citations state that eggshell temperatures should be taken on the midline of the egg and not the blunt end of the egg where the air cell is located (Aviagen, 2012; Tullett, 2009). This recommendation is due to the fact that the blood vessels located along the inner shell membrane are closer to the eggshell at the midline of the egg than at the air cell of the egg (Rahn et al., 1980). Eggshell temperatures (unpublished data) are lower when recorded at the air cell location than along the equator of the egg (Figure A.1).

In conclusion, eggshell temperatures can be recorded using infrared thermometers. Ear and forehead thermometers have distinct differences in use and how these measurements differ when recording eggshell temperatures is currently unknown. When using either device, measurements should be taken along the midline or equator of the egg.

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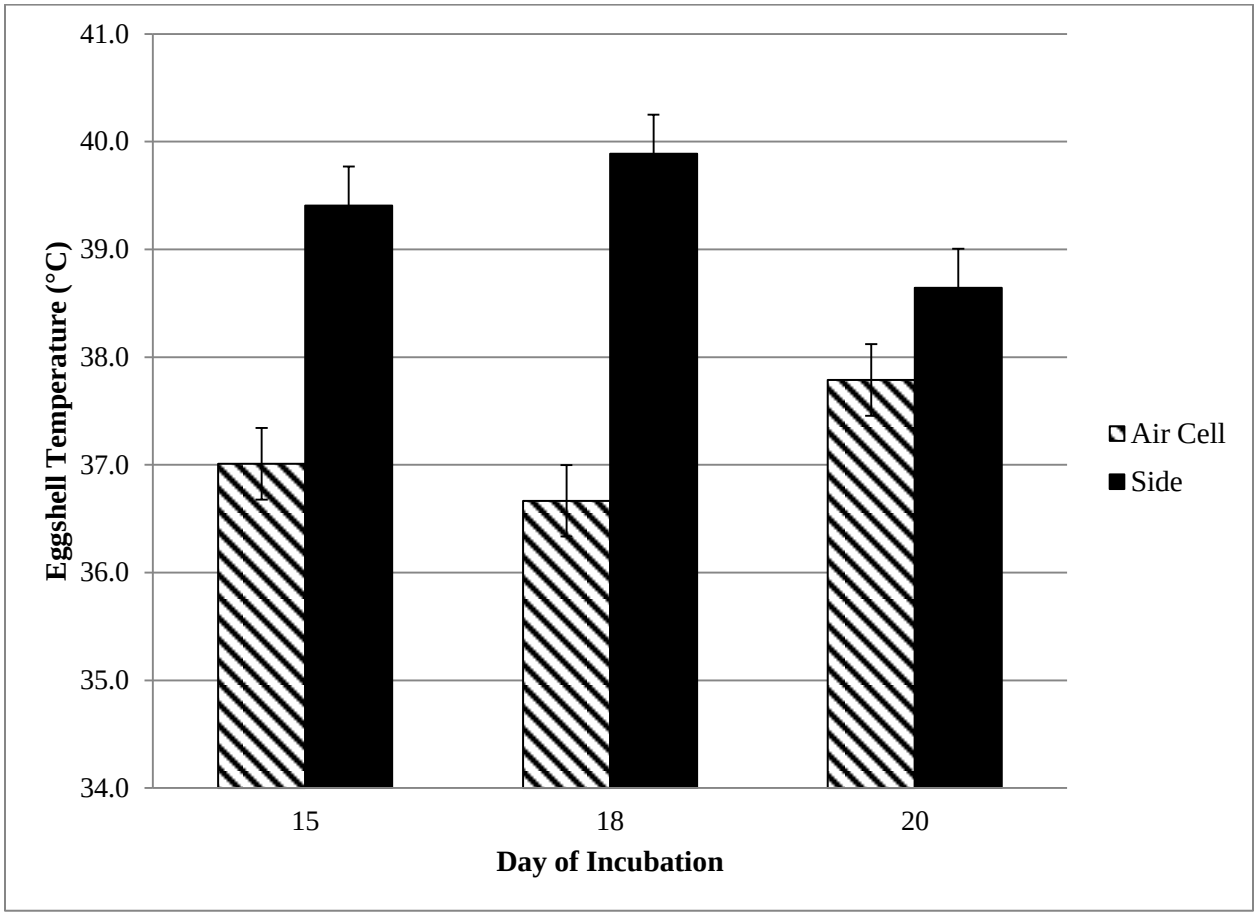

Figure A.1. Comparison of average eggshell temperatures when recorded at the location of the air cell/blunt end of the egg and when recorded on the side or midline of the egg on three days during late incubation in broiler hatching eggs. (n=6 eggs on day 15, n=3 eggs on day 18 and n=5 eggs on day 20).

Appendix B: HISTORY OF THE ATHENS CANADIAN RANDOM BRED CONTROL POPULATION

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In science a control is required to determine an experiment's effects. In the same manner, control populations of poultry have been widely used over the years and considered very helpful in quantifying changes in poultry genetics and other factors. The maintenance and use of unselected control strains in poultry only developed around the 1950s (King et al., 1959). Using a White Leghorn control strain, Gowe et al. (1959a) showed the importance of a control strain for a selection study is to provide a way to differentiate between genetic and environmental effects on traits in a population of poultry so that specific selection traits can be adjusted for the environment's influence. The authors concluded that without a control, environment effects can obscure results and conclusions from selection programs (Gowe et al., 1959a). Random mating is considered the ideal method to maintain a control flock (King et al., 1959). Control flocks may be reproduced either as pedigreed, "each member of the flock contributes equally to the next generation," or non-pedigreed, non-identified matings with "eggs being taken at random from the flock over a limited period" (Gowe et al., 1959b). Pedigreed flocks are considered more ideal producing a lower rate of inbreeding, an increased effective number of parents, and minimize natural selection between families (Gowe et al., 1959b). In either form of reproduction in a control flock, artificial insemination is considered more ideal than natural mating by reducing genetic drift, possibly caused by the social order of a few males producing more offspring than other males (Gowe et al., 1959a,b).

After World War II, there was an interest in applying the research collaboration idea used for the creation of the atomic bomb toward poultry breeding and genetics, also described as "the creation of the Ninth Wonder of the World-the modern-day chicken" (Quisenberry, 1959). From this interest, the Southwestern and Southeastern Projects were founded in 1946 and then combined into the Southern Regional Project in 1947 to focus on research pertaining to breeding meat-type chickens among agriculture experiment stations (Quisenberry, 1959). Athens, Georgia was selected as a central testing station where a then $75,000 facility was dedicated on May 12, 1955 as the Southern Regional Poultry Testing Station (Quisenberry, 1959). The name was changed in 1958 to the Southern Regional Poultry Genetics Laboratory (Quisenberry, 1959). At this location, to accomplish the 3rd objective of the Southern Regional Poultry Breeding Project: "To develop adequate methods of measuring genetic change in populations under selection, such as the development and use of reliable control stocks," two populations of control meat-type chickens were developed and maintained: the Athens Randombreds (ARB) and the Athens-Canadian Randombreds (ACRB) (Quisenberry, 1959; Hess, 1962).

The ARB has "a much broader genetic base than the AC[RB]" with its population originating from broilers from 10 commercial sources in 1956 consisting of "most of the major broiler stocks commercially available in 1956" (Hess, 1962). These breedings came from eight male and eight female lines (Hess, 1962; Marks, 1971b). Known breeds in the male lines included Plymouth Rock, White Cornish and New Hampshire, and the female lines breeds included Rhode Island Red, Barred and White Plymouth Rock, New Hampshire, Single Comb White Leghorn and Cornish (Hess, 1962). Hess (1962) described the ARBs as "single and pea comb birds and the plumage color ranges from white to black, with most known plumage color patterns appearing." Traits in the ARB population were monitored from 1961-1969 with the

only changes found were a decrease in 300 day old body weight and an increase in egg production which also occurred in the ACRB population during the same time period, indicating environmental effects instead of genetic changes (Marks, 1971b, Marks and Siegel, 1971).

The ACRB population arrived in Athens in 1958 from the Canada Department of Agriculture's Research Branch as a reproduction of the Ottawa Meat Control Strain (OC) (Hess, 1962). According to Hess (1962), the birds used to develop the OC "included White Wyandotte and three synthetic populations…of wide genetic backgrounds." Merritt (1968) stated that the OC was developed from three commercial and one experimental strain of meat-type chickens in 1955. The strains selected were all dominant for white plumage (Merritt and Gowe, 1962). The three commercial strains included two with Cornish background (pea and single combs) and one strain of White Wyandottes (rose combs) (Merritt and Gowe, 1962). The experimental strain added into the OC was the Ottawa Broad Breasted White which had single combs and also originated from the Cornish breed (Merritt and Gowe, 1962). Crawford (1961) also states that the OC consists of White Wyandotte, White Cornish, and Broad Breasted White with four different comb types of single, pea, rose and walnut but terms the other commercial strain as Peachblow. The start of the ACRB strain arrived as pedigreed eggs which hatched 1806 chicks March 30, 1958; birds from the same families present in the Canada population were selected as breeders to keep the two populations as similar as possible from the start (Hess, 1962; Marks and Siegel, 1971). Production traits of the ACRB population were monitored from 1958-1968 both in Athens and in samples reared in Virginia with most traits showing no significant changes over time (Marks and Siegel, 1971). Traits that did change varied with location and included adult (300 day old) body weight, egg production, egg weight, and comb type (rose comb frequency increased) (Marks and Siegel, 1971). From 1959-1965 the ACRB population traits were

compared with the Canada OC population with most traits remaining similar between the two populations except for the difference in pea and rose combs frequencies (Marks, 1971a). These comparisons allowed the author to conclude that the egg production increase and adult body weight decrease in the Athens population were the result of environmental effects and not genetic especially, as mentioned, since these patterns were also seen in the ARB population (Marks, 1971a; Marks, 1971b). The comb type difference is thought to be an inappropriate trait to determine any genetic changes in the population since rose and pea loci are associated with fitness traits and are shown to have "abnormal segregation ratios" (Marks, 1971a). The ACRB, like the OC, is described as being "dominant white with occasionally some black and frequently red showing in the white plumage" with the same comb types as the OC: single, pea, rose and walnut (Crawford, 1961; Hess, 1962).

Both the ARB and the ACRB populations are maintained today in Athens, Georgia at the University of Georgia. Due to budget restrictions, both populations of birds were downsized and moved in 1998 from the Southern Regional Poultry Genetics Laboratory to the University of Georgia's Poultry Research Center. The ARB's population size in 1962 was maintained at a minimum of 64 males and 384 females (Hess, 1962); today's (2012) population is 15 roosters and 50-60 hens. The 1962 ACRB population's minimum size was 60 males and 300 females with today's (2012) population at 108 roosters and 150 hens (Hess, 1962). The ARBs were mated one male to eight females for 60 males from 1961-1972 then recorded as "mass mated" with 15 males and 150 females in 1988 (Somes, 1988). The ARBs are currently randomly mated by artificial insemination with pooled semen and are not pedigreed. The ACRBs remain as pedigreed birds and are artificially inseminated with matings currently designed by Dr. Samuel Aggrey, professor at the University of Georgia. The matings of the ACRB are designed to mate

one rooster with several sister hens while avoiding full sibling matings. This technique helps reduce inbreeding while not technically adhering to the textbook definition of random mating which is that "all members of a given population have an equal chance of producing offspring and…any given ovum has an equal chance of being fertilized by the sperm from any male" (Lerner, 1950). The OC was non-pedigreed starting in 1974 (Somes, 1988) indicating that the ACRB are the oldest pedigreed control commercial meat-type chicken strain still in existence today.

Research using ACRBs as a control have been useful in declaring changes in the commercial broiler over time. Research dates from 1961 to the present day. More recent research has utilized the ACRB to investigate genetic selection for nutrient use and absorption (Sethi et al., 2008; Ankra-Badu et al., 2010), and has been used for a past broiler comparison of intestinal development and microflora (Lumpkins et al., 2010) and retained yolk sacs (Buhr et al., 2006). North Carolina's Poultry Science Department has used the ACRB to chart the genetic and nutritional changes for the broiler in 1991 and then again in 2001. In these studies, the ACRB was compared to the 1991 Arbor Acres feather-sexable broiler and then to the 2001 Ross 308 broiler (Havenstein et al., 1994a; Havenstein et al., 2003a). Both the Arbor Acres (AA) and the Ross broilers well outgrew the ACRBs on both a 1957 diet and a modern diet (Havenstein et al., 1994a; Havenstein et al., 2003a). When reared on their respective diets, at 6 weeks of age, the AA average body weight was 2,132g, the Ross was 2,672g, and the ACRB weighed in at 508g in 1991 and 539g in 2001 (Havenstein et al., 1994a; Havenstein et al., 2003a). The final market weight of the ACRB at 12 weeks only reached 1,400g in 1991 and 1,430g in 2001 (Havenstein et al., 1994a; Havenstein et al., 2003a). The ACRB's similar weights in both studies were not found to differ from earlier reports (Marks, 1971a and Marks and Siegel, 1971) and

were concluded to have remained unchanged over the years (Havenstein et al., 1994a; Havenstein et al., 2003a). Havenstein et al. (1994a; 2003a) concluded the obvious changes in body weight between the 1950s and the more modern strains were 10-15% due to nutrition changes and 85-90% by genetic changes. Although very different values were obtained for mortality between the two time spans, thought due to management changes, the ACRBs had a lower mortality than the modern strains, 3.3% vs. 9.1% in 1991 and 1.8% vs. 3.6% in 2001, and the modern strains experienced higher late mortality when reared beyond 6 weeks (Havenstein et al., 1994a; Havenstein et al., 2003a). The lower mortalities of the 2003 study saw no difference in mortality between the strains until 70-84 days of rearing (Havenstein et al., 2003a). The modern strains also had better feed conversion at 2.04 in 1991 and 1.63 in 2001; the ACRBs respectively had a reported feed conversion of 3.00 and 2.34 with a more limited feed wastage issue in 2001 (Havenstein et al., 1994a; Havenstein et al., 2003a). Carcasses of the modern strains had higher yield (hot carcass without giblets), but no difference was found between the ACRB and the modern strains for rack (rib cage, sternum, thoracic vertebrae, and back muscles and skin) expressed as percentages of live body weight after feed removal for 10 hours (Havenstein et al., 1994b; Havenstein et al., 2003b). The percentage yield for leg, saddle and breast muscles were higher in the modern strains; the AA breast yield was 4.4% higher than the ACRB in 1991 and increased to nearly 10% more breast yield for the Ross compared to the ACRB, which the authors attributed to the development of the high-yielding broiler (Havenstein et al., 1994b; Havenstein et al., 2003b). The ACRB had higher percentage wings than both modern broiler strains (Havenstein et al., 1994b; Havenstein et al., 2003b). The modern strains had greater percentage fat in their carcass when compared to ACRBs of the same age, yet the 43 day old modern strains and the 85 day old ACRBs fat levels were not different: meaning at

market age for each, the percentage of fat was the same (Havenstein et al., 1994b; Havenstein et al., 2003b). The amount of water taken up by the carcass during chilling was lower for the modern strains than the ACRB due to carcass size (Havenstein et al., 1994b; Havenstein et al., 2003b). Both the percentage heart and lungs were found to be significantly smaller in the modern strains than the ACRB (Havenstein et al., 1994b; Havenstein et al., 2003b). The studies also examined immune function for the modern and ACRB broilers. On a percentage basis, ACRBs had a greater bursa, spleen, and cecal tonsil weights relative to body weight (Cheema et al., 2003). Studies from both years concluded that the ACRBs had a better humoral immune system; the cell-mediated immune system was found to be the same between AA and ACRBs whereas the Ross had a better cell-mediated immune and inflammatory response system (Qureshi and Havenstein, 1994; Cheema et al., 2003).

In addition to these growout comparisons, the ACRB was also compared to the 1991 AA during the last four days of incubation (from 17 d of incubation to hatch) by Christensen et al. (1995). This comparison found that AA embryos were an average 13g heavier than ACRB embryos during these days of incubation. The percentage liver weight was greater for the ACRB from 19 d of incubation to hatch. The percentage heart was greater for the ACRB on days 17-19, but percentage heart was greater for AA on day 20 and at hatch. There was no difference in conductance constants adjusted for egg weight between the ACRB and AA. These embryos also exhibited differences in metabolism with higher heart glycogen in the ACRB, increased liver glycogen on day 19 of incubation in the ACRB, increased blood glucose on day 19 of incubation for the AA, and higher T3 concentration for the AA on day 20 at external pipping. These chicks had similar hatch time and hatchability values were 89% for the AA and 93% for the ACRB.

In conclusion, the University of Georgia still maintains two control chicken populations: the ARB and the ACRB. The ACRB in particular is a true treasure as the oldest pedigreed meat-type chicken control strain still in existence today. These birds have fulfilled their purpose as a control in the past for studies ranging from incubation to post hatch growth to selection studies and will continue to be a unique and valuable comparison tool in the future.

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