Abstract

CUTCHIN EVANS, HEATHER RENEE. Effect of Angle of Turning and Shaking Agitation During Incubation on Embryo Development and Hatchability. (Under the direction of Michael J. Wineland.)

Successful incubation is the result of many factors, including turning the eggs to a 45° angle once an hour through day 18 or 19 of incubation. Not turning eggs at all during incubation leads to high percentages of embryonic mortality throughout incubation as well as several distinct extra-embryonic abnormalities, such as a decrease in sub-embryonic fluid formation, an increase in the amount of residual albumen after d18, and others which can be harmful to chick weight at hatch. However, turning eggs creates hot spots in the machine, therefore developing a way to increase air flow by turning egg less or not turning at all but still providing change in orientation of the egg would increase the hatchability as well as provide a more even chick quality across the machine.

Three trials were performed to examine the effects of turning at reduced angles of 15°, 30°, 35° and 40° once an hour as well as increased frequency of turning at a reduced angle of 15° three times an hour. Eggs were obtained from prime age broiler breeder flocks and stored for 1-3 days prior to incubation to imitate industry ideal conditions. Sub-embryonic fluid was sampled at d6 of incubation and embryos were sampled at d18. At hatch, embryonic day of death was noted as well as any abnormalities such as residual albumen, malpositions or excessive urates. Overall it was determined that turning at 15° was not suitable for hatchability and results were noted similar to those reported by other authors that did not turn eggs during incubation. Turning 30° is not as harmful as turning 15°, but not as adequate as turning 45°. Increasing the frequency of turning 15° to three times an

hour alleviated some of the detrimental effects of turning 15° once an hour, but was still not as successful as 45°. Turning 35° and 40° did not affect hatchability and embryonic mortality significantly.

Shaking, or agitation, of the egg was attempted in a series of trials where the speed, frequency, and duration of shaking were manipulated to determine a combination that would be suitable for incubation in the industry. Eggs from prime age broiler breeder flocks were used for the four trials. Two similar machines were utilized; one that turns eggs conventionally (turns) and a modified incubator that moves eggs through a 4.5cm distance (shakes). Eggs were shaken intermittently either the entire incubation period or during parts and then turned. A control group of turned eggs were included in each trial for comparison. Overall, it was determined that shaking agitation did not produce suitable for hatch of fertile for industry use. Turning eggs at least d1-3 of incubation provides a better hatch of fertile than shaking eggs throughout incubation. Shaking eggs leads to higher percentages of early and late mortality, residual albumen and malpositions. Interestingly, the chicks that do hatch from shaken eggs are similar in quality to those from turned eggs, as indicated by their yolk free body mass.

In conclusion, reduced turning angles of 35° and 40° do not seem harmful to embryonic development, though 30°, 15° once an hour and 15° three times an hour do. Shaking agitation has been determined to not be advantageous for hatch of fertile, however further research is warranted on combinations of speed, frequency, and duration of shaking that may prove more favorable.

Effect of Angle of Turning and Shaking Agitation During Incubation on Embryo Development and Hatchability

by
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Dedication

To my son, Graham Darwin Cutchin Evans, may you be able to follow your dreams, achieve great things and experience wonderful opportunities as I have.

Biography

Heather Renee Cutchin Evans was born on November 12th, 1983 to Mike and Pam Cutchin. She grew up in Columbus County, North Carolina with the desire to become a veterinarian. While attending West Columbus High School, Heather was very involved in the FFA chapter as well as the theatre club. Through FFA she began working at a veterinary clinic and has continued to work at various clinics since. Involvement in the FFA and in theatre taught her to be confident and comfortable with public speaking, working in teams and being a leader. She obtained her Bachelor's of Science in Poultry Science at North Carolina State University in 2006. While obtaining her degree she was active in CERES Women's Agricultural Fraternity and the Poultry Science Club. While an undergraduate, Heather worked for Dr. Ken Anderson in the Poultry Extension office and realized she has a passion for research. After completing her degree she began working for Dr. Mike Wineland, also in Poultry Extension, concentrating on incubation and hatchery management. While working on her M.S. degree she was elected as President of the University Graduate Student Association, the governing body for the graduate students at NC State, and served for almost a year before stepping down to have a son, Graham, with her husband, Matt. After completion of the MS degree Heather plans to be a wonderful mom and let life's opportunities take her where they will!

Acknowledgements

I would like to sincerely thank Dr. Mike Wineland for giving me the chance to achieve so many things, for introducing me to the poultry industry and for helping me get to Australia! I would also like to thank the other members of my committee, Dr. Ken Anderson, Dr. John Barnes and Dr. Vern Christensen for their "always open doors" and support.

Thank you to everyone in the Poultry Science Department, but especially Mike Mann, Debbie Ort and Lynn Strother for all of the help freely given, the advice that was greatly needed and being the shoulders for me to cry on when things got rough.

My deepest gratitude is given to my parents for their never-ending support of all of my crazy ideas. Without them I would not have made it this far in my education or in life.

Finally, I appreciate my husband's patience, help and support in all that I do, but especially his unwavering love for me.

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Chapter I: Literature Review

Introduction

Turning of avian eggs during incubation affects various physiological and physical aspects of the embryo and extra-embryonic membranes including formation of sub embryonic fluid, utilization of albumen, and embryonic growth. An embryo begins development even before the egg leaves the hen's body and with the optimum environmental factors will continue development. There are numerous factors that influence embryonic development throughout incubation, including environmental temperature, humidity, air movement, and turning of the egg. These factors are altered depending on the number of eggs set, the age of the egg at set, the age of the hen at lay, and the breed of the hen. Turning is one of those factors that can have detrimental and beneficial effects and will be discussed in more detail in this thesis. Air movement through the incubator is essential for removal of metabolic heat and carbon dioxide from around the egg and turning alters that air movement, causing varying microclimates deviating from set point in the air around some of the eggs. This can lead to a wider hatch window and a variance in chick quality. The angle and frequency of turning, orientation of the egg, and age of the embryo during which the egg is turned during incubation all influence embryo growth and normal development. This review will discuss embryonic development and significant research on turning and its effects on embryonic and extra-embryonic development. Prior to discussing the effects of turning, an understanding of embryonic development is needed.

Pre incubational development

Fertilization occurs in the oviduct prior to albumen and shell formation. The egg is telolecithal with a small disc of cytoplasm sitting on top of the large yolk, and the eggs undergo discoidal meroblastic cleavage. Cleavage only occurs in the blastodisc. Equatorial and vertical cleavages divide the blastoderm into a tissue five to six cell layers thick and these cells become linked together by tight junctions (Gilbert, 2006). Numerous cell divisions occur while the egg is in the shell gland, at first just laterally so that all cells are in contact with the yolk, but then division begins to increase in depth. Near the end of the fifth hour in the shell gland, or ten to thirteen hours after fertilization, the undersurface of the embryo begins to be separated from the yolk by subgerminal fluid (Eyal-Giladi and Kochav, 1976). The subgerminal cavity is created when the blastoderm cells absorb water from the albumen and secrete fluid between themselves and the yolk. For the next 13 to 16 hours, the cells at the top of the blastoderm continue to divide and those underneath are shed into the yolk. This shedding, in combination with the subgerminal cavity filling with fluid, causes the central area of the embryo to appear translucent giving rise to the term “area pellucida”. Surrounding the area pellucida is the area opaca that remains in intimate contact with the yolk (Etches, 1996). The marginal zone is a thin layer of cells between the area pellucida and the area opaca. The embryo already has started to form the second of three primary germ layers as the egg is reaching the end of structural completion in the oviduct (Romanoff, 1960).

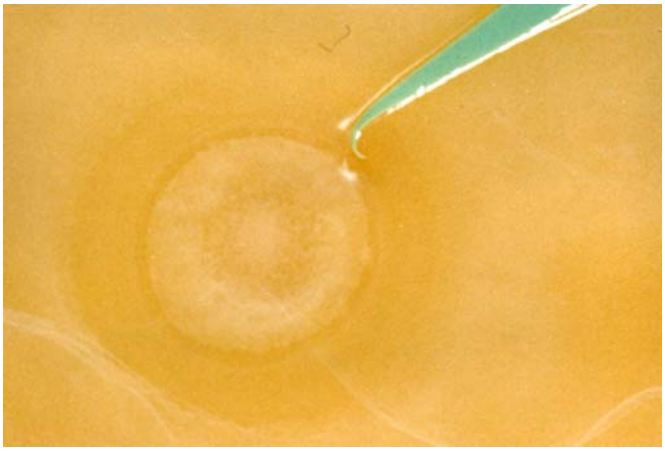

Figure 1: Fertile egg: An unincubated, fertile egg demonstrating the area opaca, area pellucida and marginal zone.

Egg storage after oviposition

Development immediately following oviposition is highly dependent on temperature and humidity of the environment (Etches, 1996). Eggs are typically collected several times a day from houses and placed in a cooler at 10-15°C with 80% relative humidity and can be held there for several days (Fasenko, 1996). This holding period can slow embryonic growth while allowing some needed degradation of the albumen (Brake *et al*., 1997). There are also logistical reasons for storage of eggs, such as limited incubator space or a decreased demand for eggs in certain times of the year. Temperature is a primary factor in embryonic development immediately following oviposition. The terms “physiological zero” and “cold torpor” have been used to describe the extremely slowed development of embryos when between temperatures of 10 and 15.5°C (Fasenko, 1996). Temperature will continue to play a major role throughout incubation.

Incubational development

By the time the egg is laid the blastoderm contains about 20,000 cells and most of the cells remain at the surface forming the epiblast, while other area pellucida cells delaminate and migrate into the subgerminal cavity to form the polyinvagination islands (primary hypoblast). A sheet of cells from the posterior margin of the blastoderm migrates and pushes the primary hypoblast cells anteriorly, forming the secondary hypoblast (endoblast), which is distinguished from other regions of the margin by Koller's sickle. The epiblast and hypoblast are joined at the marginal zone of the area opaca and the space between forms the blastocoel. The avian embryo comes entirely from the epiblast while the hypoblast cells form portions of the external membranes, such as the yolk sac and the stalk linking the yolk mass to the endodermal digestive tube. The three germ layers (endoderm, mesoderm and ectoderm) are formed solely from the epiblast (Gilbert, 2006).

The primitive streak is the major structure in avian, reptiles, and mammals during gastrulation which arises within the posterior marginal region, and cells from other areas of the embryo are not involved in its formation. Cells of the primitive streak digest away the extracellular matrix underlying them so that they can intercalate mediolaterally and undergo convergent extension. The convergent extension is responsible for progression of the streak, and the cells that initiated streak formation migrate anteriorly and may constitute an unchanging cell population that directs the movement of epiblast cells into the streak. The primitive groove forms within the primitive streak and serves as an opening through which migrating cells pass into the blastocoel. The head of the embryo will form at the end of the primitive streak closest to the center of the blastoderm, while the long axis of the body will

form along the streak. Hensen's node is a regional thickening of cells at the anterior end of the streak and is considered the "organizer" (Gilbert, 2006).

Endodermal precursors from the epiblast are the first cells that ingress through the primitive streak and these undergo epithelial to mesenchymal transformation and the basal lamina beneath them breaks down. The secondary hypoblast, or endoblast, cells migrate anteriorly from the posterior margin of the blastoderm, elongating and directing the movement of the primitive streak. The streak defines the axes of the avian embryo. Extending from posterior to anterior; migrating cells enter through the dorsal side and move to its ventral side, separating its left from the right. Prechordal mesoderm, the notochord, and the anterior somites come from Hensen's node, while the heart and kidneys are from cells that ingress through the middle of the streak. Lateral plate and extraembryonic mesoderm are from cells that become the posterior portion of the streak. Cells closest to the streak after ingression of the mesoderm will form the medial structures while those farther from it form the lateral structures. Endoderm and mesoderm are formed as cells enter the primitive streak (Gilbert, 2006).

Between the 16th and 30th hour of incubation the primitive streak will reach full development (Romanoff, 1960). Regression of the primitive streak begins as mesodermal ingression continues, moving Hensen's node from near the center of the area pellucida to a more posterior region. As Hensen's node moves caudally the posterior region of the notochord is laid down and the node finally regresses to its posterior position, forming the anal region (Gilbert, 2006).

There is a distinct anterior to posterior gradient of developmental maturity with cells

of the anterior region forming organs while the posterior cells are still undergoing gastrulation. Between the 36th and 49th hour of incubation the embryonic body begins to form as the primitive streak regresses and forms the tail bud at the 20 somite stage. The head fold forms slightly earlier than the tail bud (Romanoff, 1960).

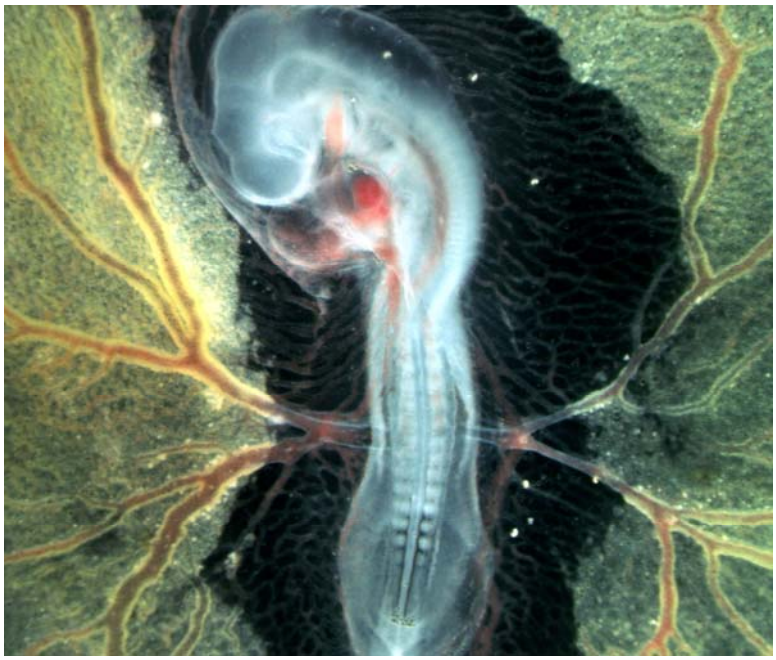

Figure 2: 72 hour embryo: Normally incubated embryo at 72 hours to show the somites in the medial and caudal portions. The head is flexed and the heart tube can be seen folding. Also, vessels of the area vasculosa can be seen extending from the embryo out.
(Photo taken by H.R.C. Evans)

Embryonic Nutrition

Even during the first few days of embryonic growth, nutrition is extremely important. Glucose is absolutely indispensable; the embryo will die immediately without glucose as well as essential amino acids and vitamins (Romanoff, 1960). The embryo obtains its nutrition during incubation from the albumen and yolk and requires oxygen to convert the lipids, proteins, and water from egg contents into chick tissue. This conversion produces carbon

dioxide and metabolic heat. During the first 9 days of incubation, production of metabolic heat is insignificant due to the low growth rate; however, during the last 10 days of incubation, daily production of metabolic heat rises steadily due to exponential growth of the embryo. It is important to monitor the temperature of the incubator during this time, as after the 10th day of incubation, the egg temperature can be as much as 1.0-1.5°C above incubator air temperature (Leksrisompong *et al.*, 2007).

When the sero-amniotic connection ruptures at the junction of the amnion and chorion and albumen is transferred to the amnion for the embryo to imbibe, a connection opens from the yolk directly into the amniotic sac, and some of the immunoglobulin molecules are transferred to the embryo. It is believed that these molecules go directly to the gut, where they are absorbed into the vascular system without modification, therefore providing the chick with maternal immunity (Etches, 1996).

Organ system development

The nervous system develops first. The neural tube begins to form near the 20th hour of incubation, then the brain begins formation in the first day, and the formative phase goes through until the 4th or 5th day of incubation. On the 5th or 6th day, differentiation and proliferation of nervous elements within the brain substance begins. The major features of the adult brain are present by the 12th day (Romanoff, 1960).

Before the head process is fully grown, or the first somite stage, blood islands of Wolff may become visible in the area opaca. Around 25 hours of incubation, the first angioblasts, which represent the future endocardium, appear. In the 48-hour embryo, blood islands gradually disintegrate into their individual elements and form the first blood cells.

During the early part of the embryonic period, initial blood formation occurs around the yolk sac, where red blood cell formation predominates over white blood cell formation. Blood vessel development in the yolk sac intensifies on the chick's 4th day of incubation, reaching its peak between the 10th and 14th days. At the start of the third week of incubation hematopoiesis slows in the yolk sac and intensifies in the liver, spleen and bone marrow of the chick (Romanoff, 1960). Presumably, vascular growth is the least reliable process in embryonic development as most of the early dead are found with the blood islands formed, but lack of vessel formation (Etches, 1996).

During the first 10 hours of incubation, as the extra-embryonic and intra embryonic blood vessels are developing, so is the heart. Around 6 to 8 days of incubation the pericardial sac is forming and the heart resembles the adult form. During embryonic development, the heart is the first organ to function, even before it is completely developed. The heart starts beating around day 2 of incubation (Romanoff, 1960). Heart rate is stimulated by turning as early as the 15th day of incubation (Vince *et al*., 1979).

The portion of the digestive system that is involved in the process of digesting and assimilating food forms from the endoderm. The fore-gut is the first to appear and has the most derivatives, or other organs, originating from it, including the esophagus, crop, proventriculus, gizzard, most of the oral cavity and duodenum. Also originating from the fore-gut that have both digestive and other functions are the liver, gall bladder, and pancreas.

The mid-gut gives rise to the small intestine except for the duodenum and is continuous with the yolk sac at the umbilicus. From the hind gut the cloaca, rectum, large intestine, and caecal appendages originate. The fore gut is considered complete by the 27

somite stage, which is the end of the 2nd day of incubation or early in the 3rd day. During the last week of incubation the length of the proventriculus and stomach complex increases in size by about 50% (Romanoff, 1960).

Extra-embryonic membrane development

Extra-embryonic membranes such as the yolk sac membrane, the amnion, the chorion, and the allantois (which fuse together to form the chorioallantoic membrane) and the albumen sac are temporary appendages to the embryo which provide it with a means of nourishment, protection, respiration, and segregation of waste products.

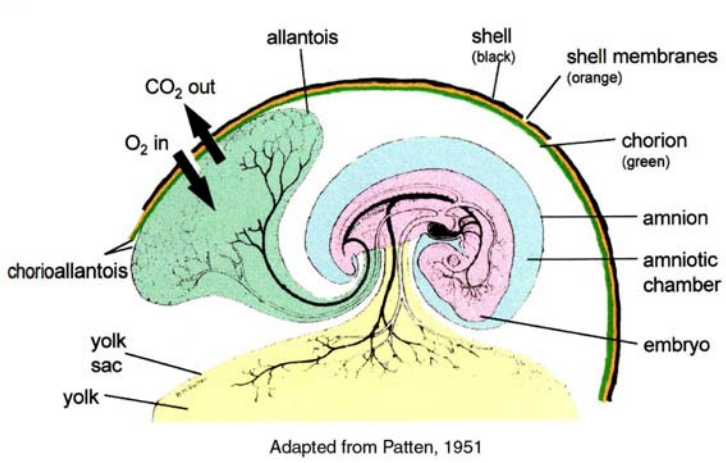

Figure 3: Extra-embryonic membranes: A schematic of the extra-embryonic membranes surrounding the embryo. (Patten, 1951, modified by Christensen)

One of the first extra-embryonic membranes to arise is the yolk sac, which is known as the area vasculosa (AV) early in incubation. The AV is a highly vascularized region that completely surrounds the embryo by the 3rd day of incubation. The area vitellina, which previously held the yolk contents in place, is pushed down by the AV. The AV grows most rapidly between the 3rd and 5th days of incubation from its marginal portion, or leading edge,

instead of its central portion. Oxygen concentration and blood circulation contribute to the stimulation of growth of the AV (Romanoff, 1960). The AV will grow until the 14th or 15th days of incubation (Romanoff, 1960), or the 17th day (Baggot, 2001).

The AV is the first major respiratory surface available to the embryo (Deeming, 1989a) as well as the site of blood formation through most of incubation (Romanoff, 1960). A reduced AV may limit oxygenation of the blood and restrict embryo growth. The rate at which nutrients can be taken up from the yolk is determined by the size of the AV, as it is a transporter of nutrients from the yolk to the embryo (Romanoff, 1960). The endoderm of the yolk sac (the AV is now called the yolk sac as it has grown considerably over the yolk) contains the enzymes necessary to convert proteins and lipids stored in the yolk and albumen into the amino acids and energy required for embryonic development and growth (Etches, 1996). A yolk stalk is formed that attaches the yolk sac to the intestine and allows vitelline blood vessels to reach the embryo. The yolk sac and its remaining contents begin to enter the embryo's body on or after the 19th day of incubation, chiefly through movements of the abdominal muscles with considerable assistance from respiratory movements after the chick has pipped into the air cell (Romanoff, 1960).

The amnion is a thin, elastic, liquid filled sac that surrounds the embryo and provides protection as well as prevents the embryo from becoming dehydrated. It originates in the primitive streak from epiblast and mesoblast, initially growing as a single undifferentiated membrane. This membrane grows up and over the embryo, forming a dome above it, then folds over on itself, giving rise to both amnion and chorion. The inner membrane, closest to the embryo, is the amnion, while the outer membrane forms the chorion. Primitive muscle

fibers develop in the amnion and it is believed that these fibers' contractions help to prevent adhesions of the growing tissues to the shell membrane (Romanoff, 1960; Etches, 1996).

The chorion, which has grown very close to the inner shell membrane, initially has no major function until the allantois invades it and together they form the chorioallantoic membrane (CAM). The allantois is the last extra-embryonic membrane to appear, beginning development around the 2nd day of incubation, and develops as an appendage of the hind gut. The allantois is a double walled membrane, with one of its walls forming the allantoic sac that accumulates kidney secretions and the other grows within the chorion to aid in respiration. The allantois that accumulates kidney secretions grows as it fills, if the flow of secretions is hindered experimentally, the sac stops growing (Romanoff, 1960). The allantois that fuses with the chorion continues to grow until the 11th or 12th day of incubation. Around day 9 the chorioallantoic membrane folds around the albumen that is left in the bottom of the egg to form the albumen sac. The allantois is a highly vascularized membrane and once fused with the chorion transports oxygen to the embryo and carbon dioxide and metabolic water out of the egg. As the embryo is growing, the yolk sac is decreasing in proximity to the shell surface, therefore carrying less oxygen to the embryo. The CAM makes up for this decrease in oxygen uptake by the yolk sac membrane (Romanoff, 1960; Etches 1996).

Turning during incubation

Turning of eggs during incubation dates back to the Egyptian era of artificial incubation (Buhr, 1989). The process of turning can adversely or favorably influence embryonic and extra-embryonic membrane development. Turning is the rotation of the egg, usually on the vertical axis in a 45 degree arc from vertical. The frequency of turning,

orientation of the egg, angle of turning, and age of the embryo during which the egg is turned all influence embryo growth and development.

Many workers believe in a "Critical Period" for turning eggs, i.e. a period that eggs must be turned or the embryo mortality will increase, while other times during incubation turning is not as important. One of the causes of early embryonic death is adherence of the embryo to the shell membrane (Eycleshymer, 1906). The amnion that surrounds the embryo has primitive muscle fibers that regularly contract. During the 5^{th} through 9^{th} days of incubation, the amnion contracts vigorously and in doing so, pushes the yolk sac down, and then as it relaxes, the less dense yolk sac (compared to the albumen) begins to rise again. This contraction prevents the embryo at the top of the yolk from adhering to the shell membranes (Romanoff, 1960). It may be that before the 5^{th} day of incubation, the amnion is not contracting as vigorously or is not large enough to push the yolk down with its contractions, and without turning the egg, the embryo is allowed to adhere to the shell membranes. Late in incubation, the allantois can adhere to the yolk sac in unturned eggs, rupturing the yolk sac and preventing utilization of the yolk sac nutrients by the embryo (Eycleshymer, 1906).

Failure to turn eggs significantly reduces expansion of the AV by the 5^{th} day of incubation and thereafter, though turning does not seem to affect the AV prior to day 3. Turning days 3 through 7 seems to significantly improve the growth of the AV compared to unturned eggs, but growth is not as advanced as it is in eggs turned from day 1. The AV possibly expands due to generation of localized increases in blood pressure in peripheral blood vessels caused by turning the egg (Deeming, 1989a). The AV also expands with the

circulation of blood; if the embryo dies during the 1st through 5th days of incubation the AV will continue to expand, but only half as quickly as if the embryo was still alive (Romanoff, 1960). Vince *et al*. (1979) noted that by day 15 the pulse of the embryo may be stimulated by turning. It is possible that turning also could influence the blood pressure in the AV.

The AV plays a major role in production of sub-embryonic fluid (SEF) during incubation. SEF is located directly beneath the embryo inside the yolk sac membrane. Sub-germinal fluid was discussed in the initial stages of embryonic development and is the fluid that fills the sub blastodermal cavity. It first occurs while the embryo is still in the oviduct, around the 5th hour after fertilization, and separates the embryo from the yolk (Etches, 1996). Once incubation begins, sub-germinal fluid is called sub-embryonic fluid. During days 1-7 of incubation, SEF is formed from active and/or passive transport of water and sodium ions from the albumen to the yolk sac through specialized cells of the AV (Baggott, 2001; Baggott *et al*., 2002). Deeming *et al*. (1987) reported that SEF increases rapidly through the end of the first week, then decreases in amount while amniotic and allantoic fluids are forming.

The function of the SEF is postulated by New (1956) to assist in respiration early in incubation while the allantois is still small and underdeveloped. As the albumen loses water to the SEF its specific gravity becomes greater and it falls to the bottom of the egg, displacing the yolk more toward the top of the egg. This allows the AV's highly vascularized system to be in closer contact with the inner shell membrane, where it is better able to transport oxygen, as well as placing the chorion in a desirable position for fusion with the allantois at the closest source of oxygen. It is possible that SEF plays a role in dilution of urea produced by the embryo prior to development of the allantoic sac (New, 1956).

Absence of turning eggs from day 3 to day 7 reduces the size of the AV and therefore the volume and ion concentration of SEF (Deeming *et al*., 1987). Having less SEF can prevent the embryo from growing properly. It is possible that static incubation causes "unstirred layers" in the albumen, which would result in a depletion of sodium ions in the albumen adjacent to the yolk sac. Fewer ions would prevent transport of water iso-osmatically into the yolk, therefore decreasing the amount of SEF (Deeming *et al*., 1987; Baggott *et al*., 2002). Also, a deficiency in SEF will not allow the AV to get as close to the shell membrane, thus decreasing oxygen uptake by that structure.

At oviposition through day 2 of incubation, albumen is observed throughout the egg and as water is transported to the SEF layer from the albumen, it becomes denser than the rest of the egg contents. By the end of day 2, the albumen has settled to the bottom of the egg. During the 9^{th} day of incubation, the CAM should start to form the albumen sac and reduplicate itself around the albumen. Closure occurs around day 12 or 13 and this formation seems to be delayed when the egg is unturned. Just prior to closure, the sero-amniotic connection is perforated and albumen begins to enter the amniotic sac to be utilized by the embryo (Romanoff, 1960).

Tullet and Deeming (1987) have noted that the amount of residual albumen was related to embryo size. The largest embryos were from turned eggs with no residual albumen after day 19. Albumen was measured arbitrarily on day 19 by opening the eggs and scoring the amount of albumen present on a range of 0 (none) to 5 (excessive). By not turning eggs, abnormal orientation of the embryo is observed related to the yolk and albumen sacs and a decreased development of the sero-amniotic connection can occur. This abnormal

orientation restricts transfer of albumen into the amniotic fluid and denies the embryo a valuable source of nutrition, the cause for retarded embryonic growth during days 13 through 18 when the embryo should be imbibing the albumen and amniotic fluid mixture (Romanoff, 1960, Deeming 1991). Turning eggs allows the egg components (yolk sac, albumen sac, and amniotic sac) to be correctly oriented to maximize the movements of albumen through the sero-amniotic connection (Deeming, 1991). Excess viscous albumen could also produce a mechanical obstacle preventing normal movement of the embryo within the egg, leading to malpositions and mortality (Tazawa, 1980).

Kuo (1932) reported that throughout the first two weeks of incubation, the embryo and yolk sac are moving in the egg, and by the 14th day the yolk sac will have come over the ventral side of the embryo. He also indicated that when residual albumen is in the bottom of the egg, the yolk sac cannot be squeezed from behind the embryo, preventing it from being brought into the embryo's abdomen. If the embryo does manage to pip through the shell, it will have an unhealed navel.

Any position of the embryo that is different from the natural hatching position of head under the right wing in the large end of the egg is considered to be a malposition. There are certain times during incubation when a specific position will be normal, such as head between the legs through day 18 of incubation, while at hatch this would be a malposition. Kuo (1932) reported that the embryo must be in proper orientation in the egg (head in the blunt end) by day 9 of incubation or it will not hatch. According to Waters (1935) the positions are as follows: I: normal hatching position, with head under the right wing, II: head buried between the thighs (normal through day 18), III: head in the small end of the egg, IV:

head turned to the left (above or below the wing), V: head rotated away from the air cell, VI: feet over head (not very common), VII: normal, but beak above right wing. Malposition II can be lethal if it occurs after day 19 of incubation as the embryo cannot pip, but before then is normal (Waters, 1935; Munday, 1952). Malposition III can be lethal if the embryo does not pip fast enough.

New (1957) indicated that premature adhesion of the chorion to the inner shell membrane is prevented during the first 7 days by turning the eggs. Abnormal adhesion of the chorion and shell membrane during the first week of incubation is correlated with high embryonic mortality. During the first week of incubation, the chorion and allantois begin adhering to each other to form the CAM and it is normal during the second week of incubation for the CAM to firmly adhere to the shell membrane to better facilitate gaseous exchange in the egg. Premature adherence of the chorion prior to day 6 to the shell membrane impedes development of the CAM, but after the 6^{th} day it would actually be part of normal development. Turning helps to prevent this adhesion by allowing albumen to be washed over the dry regions of the membranes.

Tazawa (1980) noted that the CAM is vital to the circulation of oxygen to embryonic tissues, and failure to turn eggs retards blood oxygenation through the chorioallantoic capillary plexus. This may be caused by excess albumen becoming interposed between the CAM and shell membrane which lowers the diffusion of gases across the CAM, and by the slowed growth of the blood vessels in the CAM. Tullet and Deeming (1987) have shown that hatchability in unturned eggs was related to the area of the CAM. There was a marked decrease in hatchability for eggs in which the CAM failed to line the entire interior of the egg

shell. They measured the CAM on the 19th day by candling the eggs and determining area of the CAM as a percentage of total shell area. Retarded growth may be explained by examining the development of the CAM and AV. As the egg is turned, the blood is pulled by gravity towards the bottom of the egg on the side that is facing down. To accommodate this unequal sharing of blood, the opposite side of the vascular network may collapse and the localized increase of blood pressure in the down side may lead to differentiation of peripheral blood vessels. As the egg is turned back the other way, this process happens again in another region of the egg, causing the AV and CAM to spread evenly throughout. When the egg is unturned, this process does not happen, and it is harder for the AV and the CAM to grow at the same rate as turned eggs (Deeming, 1989a).

In their natural habitat, hens will move eggs approximately 96 times per day (New, 1957, quoting Olsen, 1930). Hens turn their eggs in a hit-and-miss fashion instead of a certain angle each time. Eggs are set horizontal in the nest (Takeshita and McDaniel, 1981) and the hen will turn, jostle, or move her eggs up through hatching time, actually increasing the frequency of movement towards the last few days of incubation (Impekoven, 1976).

Modern hatcheries have tried to combine what the hen does naturally with the mechanical efficiency of their incubators. Turning eggs 96 times per day has been shown to be the optimum rate; however, due to wear and tear on the machines, it is typical to only turn 24 times per day (Robertson, 1961). However, Elibol and Brake (2006b) demonstrated that the increased frequency only helps older flocks' hatchability. Clark (1933) turned eggs 6 times a day versus 2 times a day and noted heavier embryos in the first week of incubation, but this difference disappeared during the second half of incubation. It was suggested that multiple turnings produce a more even distribution of heat to the embryo.

According to Funk and Forward (1960) eggs should be turned through a 90 degree arc, 45 degrees from their vertical axis each way to achieve the best hatchability. If eggs are turned at a reduced angle of 35° from vertical, there is an increased incidence of malpositioned embryos. However, this can be ameliorated by increasing the frequency of turning to 96 times per day, as shown by Elibol and Brake (2006a).

Buhr (1989) examined the effects of tilting eggs instead of turning them. In modern hatcheries, eggs are turned to the 45 degree angle and kept there for an hour, then they are turned in the opposite direction, a full 90 degree arc, and kept in that position for an hour. During the later stages of incubation some hatcheries keep eggs in the vertical position for 5 minutes every hour, but the majority of their time is spent in a 45 degree fashion. Buhr proposed that tilting the eggs would increase air flow and remove more metabolic heat from around the eggs. Tilting the eggs involves moving them to the 45 degree angle and bringing them back up to vertical immediately, then in the other direction 45 degrees and finally back to vertical for the remainder of the hour. Unfortunately, tilting was found to not be a viable alternative to turning.

Eggs are placed vertically to increase the number that can be placed in the incubator and they are turned from their vertical axis (Buhr, 1989). Hatchability is better when eggs are set large end up instead of small end up (Takeshita and McDaniel, 1981).

The critical period for turning eggs has been established to be the first week of incubation (New, 1957; Deeming, 1989b; Elibol and Brake, 2004; and others) though Deeming (1989b) argues that days 3 to 7 are most critical while Elibol and Brake (2004) believe days 0 to 2 are just as critical. Most hatcheries stop turning and transfer eggs from

the setter to the hatcher at day 18 or 19 of incubation as this is best for the *in ovo* vaccination schedule. Proudfoot *et al*., (1981) have studied transfer times, and determined that eggs may be transferred on the 16^{th}, 17^{th}, 18^{th} or 19^{th} day without any significant effect on hatchability.

Shaking agitation

The process of turning, though necessary, presents problems of its own. When eggs are turned there is restricted air flow over parts of the egg. This restricted air flow removes less metabolic heat and can create "hot spots" in the incubator. Having an alternative to turning eggs that will still allow normal development of the extra-embryonic membranes, as well as proper distribution of albumen, nutrients, and gases, but lessen restrictions to air flow would possibly induce higher hatchability and reduce production costs. Shaking agitation is a possible alternative and has been suggested by Randles and Romanoff (1954). Shaking agitation is a method of incubating eggs that would reduce air flow restrictions by letting eggs stay in one orientation while the tray goes through a number of oscillations per minute. Displacement of the tray through the oscillation would be small. The increased air flow would allow more metabolic heat to be removed in a more even fashion, depleting hot spots, and creating a better environment for the developing embryo.

Randles and Romanoff (1954) modified an incubator to induce shaking agitation at various oscillations with different durations. Their results show that none of the eggs subjected to shaking hatched as well as those turned through a 90 degree arc, but they did hatch better than those left unturned. Their best results came with 216 oscillations per minute for 1 to 3 seconds with a displacement of 1.27cm (72 and 82% hatchability).

Table 1: Randles and Romanoff, 1954 data on Shaking Agitation

	0.5 sec[1]	1.0 sec[1]	1.5 sec[1]	2-3 sec[2]
Control, turned every 2 hours	89.6	69.7	93.1	93.0
Unturned	41.8	44.7	48.3	39.8
115 osc/min	56.5	59.7	63.2	64.0
216 osc/min	63.5	81.8	63.3	72.4
345 osc/min	4.5	-	1.6	0
431 osc/min	-	-	-	0

Hatchability, expressed as a percentage of fertile, trials "F, G, H and I".
[1]Eggs were shaken every hour
[2]Eggs were shaken every 2 hours

The type of tray that the eggs are set in can influence how well the shaking agitation works. Randles and Romanoff (1954) discussed the use of a tray with "parallel, shallow, crescentric grooves in the tray bottom at right angles to the direction of movement" in order to keep the eggs from hitting each other. In their experiment, they used trays with flat bottoms and the eggs were allowed to move freely within these trays. They noted that the eggs would clump in the center and would not move around as freely as those that were on the periphery.

Olsen and Byerly (1938) experimented with shaking eggs parallel to their long axis and short axis. They subjected a group of eggs each day during incubation to this shaking agitation for 7 minutes (229 oscillations per minute through a 3 inch distance), and then noted the number of deaths 72 hours later. Eggs shaken parallel to their short axis were more severely affected and had much lower hatchability than eggs shaken parallel to their long axis. Highest mortality due to shaking was in eggs shaken parallel to their short axis on day 7 of incubation.

From this review several questions are raised about incubation practices and possible means of improvement. Research on various turning angles is limited and further research

on different angles and effects on embryonic and extra-embryonic development are needed. Also, the research on shaking agitation is narrow, especially as to the effects on extra-embryonic development. Therefore we conducted a series of trials where the turning angle was reduced as well as the frequency of turning was increased with a reduced angle to determine the effects on embryonic and extra-embryonic development. We also performed a series of trials with shaking agitation where the frequency, duration, and velocity of shaking were manipulated to determine if shaking would be a feasible alternative to turning.

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Chapter II: Embryonic Development When Eggs are Turned at Reduced Angles During Incubation

Abstract

Not turning eggs during incubation can reduce the incidence of hot spots in machines, lessening the chance of embryonic mortality due to overheating, and improve hatchling quality. Not turning eggs can also allow for more eggs to be placed in an incubator by designing the machine with trays closer together. However, eggs that are not turned during incubation exhibit a higher frequency of embryonic developmental deficiencies and a reduced hatch than those in turned eggs. Two trials were conducted to determine the effects of a reduced turning angle and the effects of a reduced angle in combination with increased turning frequency. In the first trial, turning eggs 15° (15) caused an increase in the number of embryos malpositioned, embryonic mortality d4-10 and d17-21, pipped embryos and overall decrease in hatch. Turning eggs 30° (30) decreased the hatch of fertile as compared to 45° (45), though the embryonic deficiencies/abnormalities were not as severe as 15. In the second trial, eggs turned 15° 3x an hour (15_{3x}) and 15° 1x an hour (15_{1x}) also demonstrated elevated early and late embryonic mortality and a higher incidence of malpositions than those turned 45° 1x an hour (45_{1x}). Overall, it was determined that a reduced turning angle decreases hatch of fertile and causes multiple embryonic developmental abnormalities.

Introduction

Typical industry practice is turning eggs in the incubators once an hour through approximately a 45° angle from perpendicular, which is optimum for hatchability (Funk and Forward, 1960) while still economical for the hatchery. Reducing hot spots in the machine

by alleviating resistance to airflow can help to increase hatch and quality of hatchlings. This can be accomplished by turning eggs at a decreased angle or not turning eggs at all (Buhr, 1989). However, not turning eggs, especially during certain periods of embryonic development, can cause abnormal development when compared to turning eggs such as: reduced area vasculosa (AV) (Deeming, 1989a), reduced sub embryonic fluid (SEF) (Deeming, 1989b) and reduced chorioallantoic membrane (CAM) development (Tullet and Deeming, 1987). Not turning eggs also can indirectly cause malpositions (Randles and Romanoff, 1950) and improper adhesions of the allantois to the yolk membrane or the embryo to the shell membrane (Eycleshymer, 1906; New, 1957), all of which can lead to embryonic mortality.

Frequency of egg turning can influence hatch. Wilson (1991) concluded remarks from many researchers and determined that while maximum hatchability is obtained by turning eggs 96 times per day, it is more practical to turn 24 times per day. Elibol and Brake (2003) saw an increased hatch of fertile when they turned eggs 96 times per day from d3-11 of incubation. Proudfoot (1981) demonstrated that eggs can be transferred to the hatching trays (i.e. cease turning) as early as d13 of incubation with no serious ill effects. Funk and Forward (1960) examined the hatch of fertile of eggs turned various angles, but did not report any embryonic developmental deficiencies.

While researchers have studied the effects of a reduced turning angle on hatch of fertile and have examined embryonic developmental abnormalities in unturned eggs, to our knowledge the effects of reduced turning angles on embryo and extra-embryonic development have not been observed. Therefore, the objective of this project was to

determine the effects of turning angles of 15°, 30° and 45° from the perpendicular plane, as well as a combination of decreased turning angle and increased turning frequency, on embryonic and extra-embryonic membrane development.

Materials and Methods

Eggs

Research for both trials was conducted at the Piedmont Research Station, Poultry Unit, North Carolina Department of Agriculture and Consumer Services in Salisbury, North Carolina. For the first trial, 2160 eggs from 48-week-old broiler breeders were divided between three incubators (Natureform I-14) with turning angles of 15, 30, and 45 degrees. 200 eggs from each treatment were numbered and weighed and used for sampling throughout incubation. 520 additional eggs from each treatment were divided between 6 trays to serve as replicates. Eggs were turned once per hour through day 18 of incubation then were transferred into a single hatcher. Machines were calibrated, and the temperature and relative humidity profiles for each incubator were identical. Temperature and humidity profiles for the machines were typical for single stage incubators used in industry. Eggs were stored 1-3 days before set.

For the second trial, 2,490 eggs from 50-week-old broiler breeders were divided between the three machines with turning angles of 15_{1x}, 15_{3x}, and 45_{1x}. 15_{1x} and 45_{1x} were turned once an hour, while 15_{3x} was turned 3 times an hour to evaluate the effect of frequency of turning when turning at a reduced angle. 200 eggs from each treatment were numbered and weighed and used for sampling throughout incubation. An additional 630 eggs from each treatment were divided among 7 trays as replicates. Eggs were turned

through day 18 of incubation then placed into a single hatcher. Machines were calibrated; temperature and humidity profiles were identical between the machines and were the same as in trial 1. In both trials, dataloggers were placed in the machines to record temperature and humidity. Eggs were stored 3-4 days prior to set. In both trials, the experimental unit for area vasculosa, sub embryonic fluid, and embryo weight is the egg. The experimental unit for amount of residual albumen, hatch of fertile, and malpositions is the tray.

Sampling

Eggs were weighed to the nearest 0.01g. Samples of 20 eggs were removed and weighed at each sampling (described below). Area vasculosa was measured on d3 using digital calipers taking two measurements perpendicular to each other across the embryo. Volume of SEF was determined on d6 by freezing the eggs overnight at -7°C. The shell was carefully removed, and the SEF layer was separated, allowed to thaw then measured (with a 20cc syringe in trial one and with a pipette in trial two to more accurately measure the SEF).

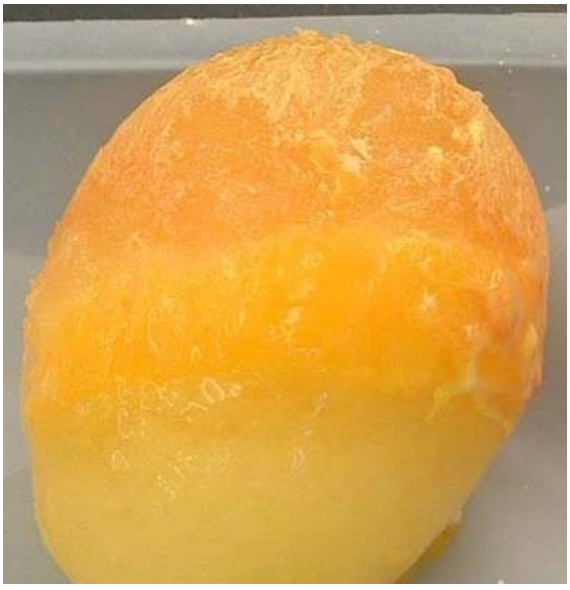

Figure 4: Frozen Egg. Egg incubated for 6 days and then frozen to separate SEF from yolk and albumen. (Photo taken by H.R.C. Evans)

Embryo weight without yolk sac was determined at d18. Hatched chicks were killed by cervical dislocation; the residual yolk sac was removed and weighed and the chick without yolk sac was weighed. The residual yolk sac and chick without yolk sac were dried in an oven (Blue M) at 70°C for a week, and then re-weighed to determine percent moisture. Hatch residue was examined for day of death as well as presence of other abnormal characteristics of the embryo and extra-embryonic membranes. Heart rate was measured in trial two at d13 using a Buddy Digital Egg Monitor (Avitronics) which was inside a tabletop incubator at a temperature of 37.5°C. Hearts were removed at hatch and were weighed to the nearest 0.001g.

Statistical analysis

The trials were statistically analyzed separately. Data analysis for this paper was generated using SAS software, Version 8.02 of the SAS system for Windows (SAS, 2001). Copyright 2001 SAS Institute Inc. SAS and all other SAS Institute, Inc product or service names are registered trademarks or trademarks of SAS Institute, Inc, Cary, NC, USA. Hatch residue data was transformed with arc sine before analysis.

Results and Discussion

Trial one

There were no significant differences between treatments when comparing AV on d3 (data not shown). At d6 of incubation but not before, a decreased AV has been noted in unturned eggs when compared to those turned (Deeming, 1989a). It is possible that turning does not influence the AV until d5 or 6 of incubation. The eggs turned 15° demonstrated significantly less SEF and percentage of SEF to initial egg volume on d6 (Table 1).

Decreased SEF results agree with Deeming *et al*., (1987) who demonstrated that unturned eggs resulted in a significant decrease in the volume of SEF by d7 of incubation. It is possible that the AV, which plays a role in formation of SEF, in 15 has not continued to develop normally after d3 of incubation, limiting formation of SEF. Embryo mass as a percentage of initial egg mass was significantly different at d18 with 30 having the heavier embryos than 15; 45 was intermediate (Table 1).

Hatch of fertile was significantly different with 15<30<45. Embryos in 15 also demonstrated a significantly higher incidence of early dead (d4-10), late dead (d17-21) and live and dead pipped embryos (Table 2). Between d12 and 14 of incubation the albumen should be transported from the albumen sac through the sero-amniotic duct into the amnion and by d17 all of the albumen should have been consumed by the embryo (Randles and Romanoff, 1950). In this trial, a significantly greater number of unhatched eggs with embryos at least attaining d18 contained RA in 15, therefore these embryos were not utilizing the albumen normally (Table 3). Additionally, the RA could have contributed to a decrease in oxygen uptake by preventing the CAM from coming into contact with the inner shell membrane in the small end of the egg. Tazawa (1980) reported that the interposition of RA between the CAM and shell membrane will lower the gas diffusive conductance of the CAM by decreasing the area of the CAM in contact with the shell membrane. This reduction in oxygen consumption hinders the growth of the embryo.

On examination of hatch residue 15 showed a significantly higher percentage of malpositions under left wing (ULW), small end embryo (SEE), and head between legs (HBL) (Table 3). Randles and Romanoff (1950) suggest that RA in the bottom of the egg could

serve as a mechanical obstruction to the embryo. Hatch of fertile was negatively influenced in 15 due to the high incidence of pipped chicks (live and dead), early to mid dead, late dead, malpositions and RA.

The period of d4-10 of incubation is characterized by rapid growth and differentiation of the embryos organs and extra-embryonic membranes (Deeming, 1989c). Turning during this period will prevent adhesion of the embryo to the shell membrane, adhesion of the yolk sac to the allantois (Eycleshymer, 1906), and premature adhesion of the chorion to the inner shell membrane (New, 1957). Deeming *et al*., (1987) proposed that turning eggs during the first week of incubation will stir up the yolk protein to be made more available to the embryo, and that in unturned eggs the proteins do not make it near enough to the SEF to be transferred to the embryo. It is possible that the embryo, during the last week of incubation, may not be completely developed due to lack of adequate turning during the first week and will die.

Trial two

The AV was not significantly different in size on d3 (data not shown). On d6, the ratio of SEF to initial egg weight was significantly less in eggs turned 15_{1x} and 15_{3x} than those turned 45_{1x} (Table 4). These results agree with the first trial. On d13 of incubation, 15_{1x} had significantly higher heart rates than 15_{3x} and 45_{1x} and also significantly heavier embryos as a percentage of initial egg weight (relative embryo) (Table 4). Possibly there was better airflow over the eggs in 15_{1x}, resulting in a more even incubator temperature, which was beneficial for those embryos in utilizing nutrients to develop chick mass. There were no significant differences in d18 relative embryo (data not shown).

At hatch heart weight was significantly heavier in 15_{3x} and 45_{1x} than 15_{1x}, and heart weight as a percentage of chick weight was significantly greatest in 45_{1x}, lowest in 15_{1x} and intermediate in 15_{3x} (Table 4). 15_{3x} and 45_{1x} had significantly heavier hatched chicks (without yolk sacs) than those in 15_{1x}. 45_{1x} demonstrated significantly smaller residual yolk sacs than those in 15_{3x} while 15_{1x} was intermediate. This suggests that the chicks in 15_{1x} were not able to utilize their yolk sac nutrients for growth as well as 45_{1x}. There were no significant differences in the dried chicks without yolk sac or the yolk sac, or in percent moisture of the chick or the yolk (Table 5).

Hatch of fertile was significant with $45_{1x}>15_{3x}>15_{1x}$. There was no significant difference in fertility. 15_{1x} and 15_{3x} demonstrated the greatest percent of mortality d1-3 and pipped chicks. Embryonic mortality between d17 and 21 was significantly greater in 15_{1x} than 15_{3x}, which was greater than those in 45_{1x} (Table 6). There was a higher incidence of SEE in 15_{1x} and 15_{3x} than 45_{1x} and a significantly increased incidence of over right wing (ORW) in 15_{1x} than 45_{1x}, with 15_{3x} being intermediate. There was a significantly lower incidence of RA at hatch in 45_{1x} than 15_{3x}, which had significantly less than 15_{1x} (Table 7).

These results are due to numerous factors throughout incubation. As the embryos approach 4 to 10d of incubation they are utilizing more of the nutrients from the yolk sac and relying on the AV for gas exchange (Deeming, 1989c). Deeming (1989a) noted a significant decrease in size of the AV of unturned eggs on d6 of incubation, but not before. While we did not note a difference by d3 in AV in these trials, it could be that after d3 there is an underdeveloped AV in 15_{1x}. It is also possible that with a decreased turning angle, the nutrients in the yolk sac become stratified (Deeming *et al*., 1987). This stratification of

nutrients combined with underdevelopment of the AV could contribute to decreased nutrient transport to the embryo. Also, an underdeveloped AV will decrease the oxygen supply available to the embryo early in incubation, prior to CAM formation. Turning could increase blood pressure and stimulate heart rate (Vince *et al*., 1979) and the lack of adequate turning in 15_{1x} could have caused the embryos to die of inadequate blood flow. Also, the higher heart rate in 15_{1x} could be due to the lack of adequate turning stimulating heart tissue growth, resulting in a smaller heart that had to work harder. There is increased RA in the bottom of the egg, which is detrimental to oxygen consumption, as well as decreased nutrition and water utilized by the embryo. The increased RA correlates with a decreased amount of SEF formation early in incubation, which limits the embryo's ability to use nutrients from SEF. The embryo may be in malposition and unable to internally pip due to being faced away from the air cell.

Those embryos externally pipped, either live or dead, were higher in 15_{1x} and 15_{3x} than 45_{1x} (Table 6). These embryos may have been oxygen deprived due to a decreased CAM, which reduced the available energy the embryo needed to escape the eggshell. Also, embryos may have started pipping in an abnormal area of the egg due to malposition. The increased incidence of malpositions could be related to the increased incidence of RA.

It is clear that increasing the frequency of turning with a reduced turning angle can help prevent the extreme of the embryonic developmental deficiencies and improve hatchability to a small degree. This is consistent with Elibol and Brake (2003) who demonstrated that increased frequency of turning from d3 to d11 of incubation improves hatchability when compared to turning once an hour.

Conclusions and Applications

Turning angles of 15 and 30 degrees cause multiple embryonic maladies including: decreased embryonic size, increased incidence of malpositions, and increased embryonic mortality compared to a turning angle of 45 degrees. Increasing the frequency of turning at 15 degrees significantly increased hatch of fertile, significantly decreased the incidence of residual albumen and late embryonic mortality, and decreased some of the malpositions noted although, still poorer than the control of 45.

Acknowledgements

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Tables

Table 1: Trial 1 sub-embryonic fluid volume and embryo mass

Turning angle	SEF[1] (cc)	SEF: egg volume[2]	d18 Embryo mass (g)	d18 % embryo mass: egg mass[3]
15	15[b]	24[b]	31.194	46.32[b]
30	17[a]	27[a]	31.149	48.92[a]
45	16[a]	27[a]	31.742	48.01[ab]
Mean ± SEM	16 ± 0.48	26 ± 0.66	31.36 ± 0.56	47.75 ± 0.70

[a] Significantly different at $p<0.05$

[1]Sub-embryonic fluid volume at d6 of incubation

[2] Sub-embryonic fluid as a percentage of initial egg volume.

[3]Embryo mass as a percentage of initial egg mass.

Table 2: Trial 1 fertility, hatchability and embryonic mortality

Turning angle	Fertility	Hatch of fertile	Early mortality[1]	Late mortality[2]	Pipped chicks[3]
15	93.63	58.20[C]	3.80[a]	21.01[A]	7.16[a]
30	95.31	83.40[B]	1.42[b]	6.88[B]	1.42[b]
45	97.38	91.07[A]	0.99[b]	1.34[C]	1.43[b]
Mean ± sem	95.44 ± 1.24	77.54 ± 2.60	2.07 ± 0.47	9.74 ± 1.43	3.34 ± 1.03

[a] Significantly different at $p<0.05$

[A] Significantly different at $p<0.0001$

[1]Embryonic mortality between days 4 and 10 of incubation as a percentage of fertile.

[2]Embryonic mortality between days 17 and 21 of incubation as a percentage of fertile; 21 noted by embryo being alive still with yolk sac completely in the abdomen, yet the embryo had not pipped the air cell.

[3]Chicks that had pipped through the egg shell and were either live or dead as a percentage of fertile.

Table 3: Trial 1 residual albumen and malpositions noted in hatch residue

Turning angle	ULW[1]	HBL[2]	SEE[3]	RA[4]
15	1.908[a]	0.495[a]	4.089[a]	18.344[A]
30	0.857[ab]	0.0[b]	0.722[b]	3.846[B]
45	0.189[b]	0.0[b]	0.142[b]	0.00[C]
Mean ± sem	0.99 ± 0.39	0.16 ± 0.14	1.65 ± 0.79	7.40 ± 1.61

[a] Significantly different at $p<0.05$
[A] Significantly different at $p<0.0001$
[1]Embryos with their head under their left wing as a percentage of fertile.
[2]Embryos with their head between their legs as a percentage of fertile.
[3]Embryos that were in the small end of the egg as a percentage of fertile.
[4]Embryos with residual albumen that had reached at least 18 days of age as a percentage of fertile.

Table 4: Trial 2 sub-embryonic fluid volume, d13 heart rate and embryo weight, and hatch heart weight

Turning angle	SEF: egg volume[1]	d13 Heart rate (bpm)[2]	d13 Relative embryo	Hatch heart weight (g)[3]	% Heart: Chick[4]
15_{1x}	24.79[b]	275[a]	16.44[a]	0.322[b]	0.738[b]
15_{3x}	23.63[b]	263[b]	14.69[b]	0.355[a]	0.793[ab]
45_{1x}	27.26[a]	262[b]	14.31[b]	0.383[a]	0.842[a]
Mean ± sem	25.23 ± 0.57	267 ± 3.69	15.14 ± 0.42	0.353 ± 0.011	0.79 ± 0.03

[a] Significantly different at $p<0.05$
[1]Sub-embryonic fluid as a percentage of egg volume at d6 of incubation.
[2]Heart rate in beats per minute at d13 of incubation.
[3]Heart weight at hatch in grams.
[4]Heart weight at hatch as a percentage of chick weight at hatch.

Table 5: Trial 2 hatched chick and yolk weights

Turning angle	Wet chick without yolk (g)	Wet yolk (g)	Dry chick without yolk (g)	Dry yolk (g)	Percent moisture chick	Percent moisture yolk
15_{1x}	36.67[b]	6.75[ab]	7.81	3.49	78.73	48.63
15_{3x}	38.27[a]	7.24[a]	7.99	3.79	79.07	47.76
45_{1x}	38.58[a]	5.98[b]	8.16	3.21	78.85	46.50
Mean ± sem	37.84 ± 0.54	6.66 ± 0.36	7.99 ± 0.14	3.50 ± 0.20	78.88 ± 0.28	47.63 ± 0.63

[a] Significantly different at $p<0.05$
[A] Significantly different at $p<0.0001$

Table 6: Trial 2 fertility, hatch of fertile and embryonic mortality

Turning angle	Fertility	Hatch of fertile	Early mortality[1]	Late mortality[2]	Pipped chicks[3]
15_{1x}	88.38	44.46[C]	11.18[A]	28.51[A]	7.59[A]
15_{3x}	89.48	64.09[B]	8.41[A]	15.28[B]	6.12[A]
45_{1x}	89.51	91.30[A]	1.77[B]	3.54[C]	0.51[B]
Mean ± sem	89.12 ± 1.19	66.62 ± 2.71	7.12 ± 1.14	15.78 ± 2.06	4.74 ± 0.87

[a] Significantly different at $p<0.05$
[A] Significantly different at $p<0.0001$
[1]Embryonic mortality between days 1 and 3 of incubation as a percentage of fertile.
[2]Embryonic mortality between days 17 and 21 of incubation.
[3]Chicks that had pipped through the shell and were either live or dead.

Table 7: Trial 2 residual albumen and malpositions noted in hatch residue

Turning angle	ULW[1]	SEE[2]	ORW[3]	RA[4]
15_{1x}	3.95[a]	5.07[a]	3.06[a]	24.04[A]
15_{3x}	1.61[b]	3.73[a]	1.23[ab]	15.51[B]
45_{1x}	0.86[b]	1.44[b]	0.00[b]	1.76[C]
Mean ± sem	2.14 ± 0.75	3.41 ± 0.69	1.43 ± 0.70	13.77 ± 1.60

[a] Significantly different at $p<0.05$

[A] Significantly different at $p<0.0001$

[1]Embryos with their head under their left wing as a percentage of fertile.

[2]Embryos that were in the small end of the egg as a percentage of fertile.

[3]Embryos with their head over their right wing as a percentage of fertile.

[4]Embryos with residual albumen that had reached at least 18 days of age as a percentage of fertile.

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Chapter III: Embryonic Development When Eggs are Turned 35°, 40° and 45° During Incubation

Abstract

We have shown that reduced turning angles of 15° and 30° are harmful to hatch of fertile and cause an increase in early and late mortality as well as malpositions compared to the industry standard turning angle of 45°. Also, increasing the frequency of turning at 15° to three times an hour did not alleviate the poor hatchability caused by turning 15° once an hour. The effect of turning at reduced angles of 35° (35) and 40° (40) compared to 45° (45) was studied in this experiment. Hatching eggs from a prime age broiler flock were subjected to reduced turning angles in order to determine the effect on hatchability, sub embryonic fluid (SEF) formation, embryo weight at d18 of incubation, heart and liver weights at hatch, and time of hatch. A sample of hatched chicks was grown to 6 weeks of age. The decreased turning angle did not significantly affect the hatch of fertile, though differences were seen at d6 in SEF and at hatch in heart glycogen. Chick quality, as defined by the yolk free body mass and percent moisture of the chick, was not significantly different. Body weights at 6 weeks of age were not significantly different. Overall, it is concluded that decreased turning angles of 35° and 40° are not drastically harmful in incubation.

Introduction

Effects of not turning eggs have been studied in detail by many authors and it was determined that not turning eggs is highly detrimental to hatch as well as embryonic and extra-embryonic development (Robertson, 1961; Tazawa, 1980; Deeming, 1989; Elibol and Brake 2006 and others). Specifically, not turning can reduce the volume of SEF (Deeming *et al.*, 1987). However, not turning eggs would allow for more air flow around the eggs,

removing more metabolic heat which could provide a more even chick quality throughout the machine (Buhr, 1989) and allow for more eggs to be placed in a machine which would reduce incubation cost per chick (Elibol and Brake, 2006).

Since not turning eggs is so detrimental to embryonic development and hatchability, researchers have examined turning eggs at a reduced angle which would allow for more air flow. Funk and Forward (1960) noted in a previous study that hatch was increased by 5 percent by turning eggs 45° versus 30°. Elbiol and Brake (2006) examined the effect of reduced turning angle on a specific malposition, small end embryo, and found that turning angle had no effect on hatchability, but a reduced turning angle did result in a higher incidence of small end embryo in hatch residue examined.. Cutchin *et al*., (2009) demonstrated that turning 15° and 30° were not satisfactory for hatchability and that increasing the frequency of turning 15° to three times an hour did not alleviate all of the detrimental effects of the decreased angle.

To the author's knowledge, the effects of a reduced turning angle on sub-embryonic fluid formation have not been examined. Our objective was to study the result of a reduced turning angle on sub-embryonic fluid formation, chick quality at hatch including heart and liver weights and glycogen, and bird weight up to 6 weeks of age.

Materials and Methods

Treatments and management:

Research was conducted at the Piedmont Research Station, Poultry Unit, North Carolina Department of Agriculture and Consumer Services, in Salisbury, North Carolina. 2160 eggs from a prime age broiler breeder flock were weighed, identified, and divided

between 3 (Natureform I-14 model) incubators with turning angles of 35°; 40° and 45°. Temperature and relative humidity profiles for each incubator were identical. Eggs were stored 3-4 days before they were set. Eggs were weighed (Mettler scale) to the nearest 0.01 gram at set and again at transfer. A sample of 20 eggs from each treatment was taken on d 6 and 18 and weighed (Mettler scale). Chicks were neck-tagged at hatch and randomly placed in pens to evaluate growth over a 6 week period. Sub-embryonic fluid was measured using the method described in Chapter II.

At d18 the embryo without the yolk sac was weighed to the nearest 0.001g. Numbered eggs were placed in ham wrap bags and tied at both ends to keep chicks with their eggs as they hatched. Eggs were transferred to a Natureform hatcher.

Time of Hatch at d19 through 21:

Numbered eggs were separated by treatment into three different baskets in the hatcher. Starting at 464 hours of incubation, eggs were examined every 2 hours until 508 hours of incubation had passed. Chicks that had hatched were identified and recorded, then placed on the left side of a wooden bar, which spanned the width of a basket.

Sampling at d21:

Chicks were killed by cervical dislocation. Residual yolk sac was removed and weighed as well as the chick without yolk sac to the nearest 0.001g. The yolk sac and chick without yolk sac were placed into a drying oven (Blue M at 70°C) and dried for 7 days. Samples were weighed back to the nearest 0.01g to determine percent moisture of the parts. The heart and liver were removed, placed immediately into 7% perchloric acid, and weighed to the nearest 0.001g. Tissues were then tested for glycogen (Dreiling, *et. al*., 1987).

Unhached eggs were examined to determine day of death as well as presence of other characteristics of the embryo and extra-embryonic membranes. This included malpositions, residual albumen, hemorrhage, or contamination.

Statistical Analysis:

Data analysis for this paper was generated using SAS software, Version 8.02 of the SAS system for Windows (SAS, 2001). Copyright 2001 SAS Institute Inc. SAS and all other SAS Institute, Inc product or service names are registered trademarks or trademarks of SAS Institute, Inc, Cary, NC, USA. Hatch residue was transformed with arc sine square root prior to analysis. P-values of <0.05 were considered significant.

Results and Discussion

The volume of SEF was not significantly different at d6, though when evaluated as a percentage of initial egg weight, a significant difference was noted with 45 having a higher volume than 40 or 35 (Table 1). This difference in volume could be due to less albumen coming in contact with the specialized cells of the area vasculosa to transport water from the albumen into the sub embryonic space. Deeming *et al*. (1987) noted a difference in SEF at d6 of incubation in unturned eggs compared to turned eggs. It appears that even reducing the turning angle slightly affects the SEF. At d18 embryos were weighed. There were no significant absolute weight differences between the treatments or relative differences when embryos were evaluated as a percentage of initial egg weight (Table 1).

Initial egg weight was not significantly different among the treatments; however, egg weight at transfer (d18) was significantly different with 35 having a heavier egg than 40 or 45. This difference was only 1.07g between the average of 35 and the average of 40.

Percent moisture loss was not significantly different at d18 (Table 2). Time of hatch was not significantly different between the treatments (data not shown) indicating that embryo development was not stalled during the latter portion of incubation.

Neither weight of wet yolk, dry yolk, wet chick, and dry chick as percentages of initial egg weight nor percent moisture of yolk or chick were significantly different (Table 3). Therefore the difference in turning angles does not influence the quality of the chick at hatch as defined by the amount of chick tissue present as a percentage of initial egg weight. Heart and liver weights were not significantly different, either as whole weights or as percentages of chick without yolk weights.

Liver glycogen and liver glycogen as a percentage of liver mass were not significantly different. Heart glycogen and heart glycogen as a percentage of heart mass were significantly different with 45 having the highest amount, 40 the lowest, and 35 having an intermediate amount (Table 4). The heart is a demand organ while the liver is a supply organ for glycogen. With the lack of significant differences in the liver it would be presumed that these embryos all had adequate access to oxygen and were able to aerobically metabolize lipids, not needing to utilize glycogen excessively. However, the significantly higher glycogen stores in the heart in 45 over 40 could indicate that the embryos in 40 struggled during the hatching process and utilized some of the glycogen from the heart. Possibly, prior to the embryo experiencing the plateau stage of oxygen consumption of incubation, 40 had a higher store of glycogen than 45 or 35, and when these embryos struggled to maintain metabolism their liver glycogen stores were utilized.

There were no differences in hatch of fertile or in fertility (Table 5) which is

consistent with findings of Elibol and Brake (2006) when they turned eggs 35°, 40° and 45° from vertical. Also, there were no differences in mortality early in incubation (d1-3), mid incubation (d4-10), or mid-late incubation (d11-16) (results not shown). However, there was a significant increase in late mortality (d17-20) in the 35 and 45 treatments over 40 (Table 5). There was also an increase in the amount of residual albumen seen at hatch in 35 and 45 over 40 as well as an increased amount of urates in 35 and 45 over 40 (Table 5). While these changes indicate an overheating of these embryos, the dataloggers reveal that the temperature and humidity of the three incubators were kept almost identical throughout incubation. Possibly 40 is a more optimum turning angle that provided embryos with the movement they needed during incubation better than 35 and better airflow than 45?

There were no significant differences in body weight among the treatments at 1 day of age or at 6 weeks. At 6 weeks, males and females were weighed separately, and no differences were seen within the treatments for either sex (Table 6).

Conclusion and Applications

A reduced angle of turning reduced the volume of SEF produced, however that did not affect the embryo mass or hatched chick quality. Also, the reduced turning angle did not adversely affect hatch of fertile or body weights at 6 weeks of age. Therefore it is concluded that the reduced turning angle could be utilized in a hatchery setting without adverse effects.

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Tables

Table 1: Sub-embryonic fluid and embryo mass

Turning angle	SEF[1] (cc)	SEF: egg mass[2]	d18 Embryo mass (g)	d18 % embryo mass: egg mass[3]
35	15.6	24.9[b]	30.58	48.11
40	16.3	25.7[b]	31.18	49.29
45	17.3	27.9[a]	30.62	50.49
Mean ± SEM	16.4 ± 0.6	26.1 ± 0.7	30.79 ± 0.53	49.30 ± 0.77

[a] significantly different at $p<0.05$

[1]Sub-embryonic fluid volume at d6 of incubation.

[2] sub-embryonic fluid as a percentage of initial egg mass.

[3]Embryo mass as a percentage of initial egg mass.

Table 2: Egg data

Turning angle	Initial egg weight (g)	Transfer egg weight (g)	Percent moisture loss
35	62.20	55.22[a]	11.14
40	61.26	54.15[b]	11.40
45	61.42	54.29[b]	11.60
Mean ± SEM	61.63 ± 0.30	54.26 ± 0.31	11.38 ± 0.18

[a] significantly different at $p<0.05$

Table 3: Chick data at hatch

Turning angle	Wet chick[1]	Dry Chick[1]	Percent moisture chick	Wet Yolk[1]	Dry Yolk[1]	Percent moisture yolk
35	61.34	12.44	79.74	9.74	4.95	49.68
40	61.38	12.33	79.92	10.17	5.19	49.49
45	61.26	12.26	79.99	10.11	5.11	49.64
Mean ± SEM	61.33 ± 0.42	12.34 ± 0.24	79.88 ± 0.32	10.01 ± 0.60	5.08 ± 0.36	49.60 ± 0.69

[1]As a percentage of initial egg weight

Table 4: Heart and liver data at hatch

Turning angle	Heart weight (g)	Heart glycogen (mg)	Heart glycogen / heart (mg/g)	Liver weight (g)	Liver glycogen (mg)	Liver glycogen / liver (mg/g)
35	0.406	0.879[ab]	2.182[ab]	0.971	3.43	3.58
40	0.396	0.808[b]	2.062[b]	0.970	3.63	3.73
45	0.404	1.058[a]	2.647[a]	0.996	3.82	3.83
Mean ± SEM	0.402 ± 0.014	0.915 ± 0.065	2.292 ± 0.167	0.979 ± 0.026	3.62 ± 0.33	3.71 ± 0.33

[a] significantly different at $p<0.05$

Table 5: Fertility, hatchability, and embryonic abnormalities

Turning angle	Fertility	Hatch of fertile	Late mortality[1]	Residual albumen[2]	Urates[3]
35	95.35	85.55	4.47[a]	1.56[a]	4.26[a]
40	94.62	89.64	1.37[b]	0.19[b]	0.97[b]
45	96.67	85.47	4.59[a]	1.71[a]	3.81[a]
Mean ± SEM	95.55 ± 0.86	86.88 ± 1.66	3.47 ± 0.85	1.15 ± 0.53	3.02 ± 0.86

[a] significantly different at $p<0.05$

[1]Embryonic mortality between days 17 and 20 of incubation

[2]Embryos with residual albumen, as a percentage of fertile, that had reached at least 18 days of age

[3]Embryos with excessive amounts of urates, as a percentage of fertile

Table 6: Body weight data

Turning angle	Average hatch weight[1](g)	6 week body weights of females[2] (g)	6 week body weights of males[2] (g)	Combined 6 week body weights[2] (g)
35	645.21	2536.69	2954.57	2745.63
40	642.92	2529.08	2987.79	2758.43
45	640.83	2534.51	3017.70	2776.11
Mean ± SEM	642.99 ± 3.03	2533.50 ± 24.01	2986.70 ± 28.05	2730.50 ± 18.40

[1]Average weight of 15 birds placed in a pen

[2]Average weight of all birds individually weighed

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Chapter IV: Embryonic Development When Eggs are Shaken During Different Periods of Incubation

Abstract

It has been determined that not turning eggs causes detrimental effects on embryonic and extra-embryonic membrane growth. We have shown that turning at a reduced angle causes a decrease in hatch of fertile and an increase in multiple embryonic and extra-embryonic abnormalities. Shaking has been discussed as a possible alternative to turning of eggs that would still allow for motion, which embryos obviously need, but allow for more air flow across the egg in order to improve removal of metabolic heat. Four trials were conducted to determine the effects of shaking agitation during various time periods of incubation on the growth of embryos and development of extra-embryonic membranes. The velocity, duration, and frequency of shaking were manipulated between the trials to develop an optimum combination. Included in the trials were treatments that were turned as well as shaken in order to determine an optimum time period for turning eggs. Eggs were kept in the vertical position similar to what is used in the industry. SEF was measured at d6 and embryo weights were obtained at d18 in trial one and d14 embryo weights obtained in trials three and four but not in other trials. In all trials embryonic mortality as well as embryonic and extra-embryonic abnormalities was noted at hatch. It was concluded that shaking is not a viable alternative to turning and that there are specific time periods during embryonic development in which turning is more critical.

Introduction

Tullet and Deeming (1987) demonstrated that not turning eggs during incubation can affect the growth of the CAM and have significant effects on hatchability. New (1957) reported that turning during the latter half of the first week of incubation is particularly important, while Elibol and Brake (2004) suggested d0-2 being the most critical 2 day period and the entire first week is significant. It has been shown that tilting eggs to simplify air flow patterns is not a practical alternative to turning eggs (Buhr, 1989).

Olsen and Byerly (1938) experimented with shaking eggs during incubation by oscillating eggs for 7 minutes at 229 oscillations per minute at various days during incubation. Eggs were shaken either in parallel to their short axis or their long axis. Eggs shaken at any point during the fourth to fifteenth days of incubation were severely affected, while mortality was lower in those shaken during the last week. Randles and Romanoff (1954) also tested shaking during incubation. Eggs in their trials were free in the bottom of trays which permitted some movement of the eggs while being shaken. One group was shaken at 121 oscillations per minute and another at 230 oscillations per minute three times a day for a short duration of time. The 121 osc/min group hatched 52%, while the 230 osc/min hatched 71%. Next, they tried shaking eggs for one minute every two hours with 100% mortality noted in the 230 osc/min treatment and 39% hatchability with the 121 osc/min treatment. Randles and Romanoff also tried reducing the duration of shaking to 5-6 seconds and increasing the frequency of shaking up to 431 osc/min. Higher shaking frequencies proved to be fatal and duration of shaking was not found to be a critical factor. Oscillations in these trials were over a 1.27cm distance.

From Randles and Romanoff's work we concluded that shaking frequencies and intervals between shaking periods, as well as the period of incubation in which eggs were shaken were all factors that could be examined in a series of trials. Hatchability, extra-embryonic and embryonic abnormalities, and embryonic mortality were aspects we measured to determine the effectiveness of shaking.

Materials and Methods

Treatments and Management

Research for all trials was conducted at North Carolina State University in Raleigh, North Carolina. Eggs from prime age broiler breeders were used and were stored 1-3 days prior to set. Eggs were completely randomized among the treatments, numbered on the small end, and weighed with a Mettler balance interfaced with a laptop to the nearest 0.01g. Two similar incubators were used; one that turned eggs conventionally (turn) and a modified one that oscillated the eggs (shake). Six treatment groups were used, Turn Control, Turn 3, Turn 7, Shake control, Shake 3, and Shake 7. The treatment groups were as follows: Turn control: turn d1-18, Turn 3: turn d1-3 then shake d3-18, Turn 7: turn d1-7 then shake d7-18**,** Shake control: shake d1-18, Shake 3: shake d1-3 then turn d3-18 and Shake 7: shake d1-7 then turn d7-18. The shake incubator was able to be manipulated so that the frequency, duration, and speed of shaking could be altered. All oscillations were over a 4.5cm distance. In all trials the same treatments were used, however the speed, duration, and frequency of shaking were altered. For the first two trials, 1600 eggs were used, for the second two trials 1440 eggs were used. For the first trial, shake was set at 1 oscillation per second for 20 seconds every 30 minutes. For the second trial, shake was set at 1 oscillation per second for 30 seconds

every 10 minutes. For the third trial, shake was set at 1.25 oscillations per second for 20 seconds every 20 minutes. For the fourth trial, shake was set at 1 oscillation per second for 20 seconds every 20 minutes. The turn incubator always turned eggs 45 degrees from vertical once an hour. The temperature and humidity in both machines were kept constant and monitored with data loggers inside the machines, next to the eggs. The set point for temperature was maintained at 99.5°F (37.5°C) and relative humidity at 55-57%. At d18 of incubation eggs were all transferred to a Jamesway 252 hatcher after candling was performed to remove infertile and early dead eggs (in trial four this was performed at d19). Those eggs were then examined for age of embryo death and any abnormalities. A sample of eggs were placed in individual pedigree bags so that the chick could be followed from its egg through hatch.

Sub-embryonic fluid (SEF)

In trial one, SEF was sampled at d6 of incubation, however since we were more concerned with embryonic development in these trials, it was not sampled in subsequent trials. We sampled SEF according to the method described in Chapter II.

Embryo weights throughout incubation

In the first trial embryo weights without yolk were determined at d18 and in the third and fourth trials they were determined at d14. At these two periods, eggs were removed from the incubator and weighed. The egg shell was carefully broken and embryo removed from the egg, then gently removed from its yolk sac. The embryo was killed by cervical dislocation. The embryo was blotted on a damp paper towel to remove excess moisture from the amniotic fluid and weighed to the nearest 0.01g.

Statistical Analysis

The design for all trials was a 2x3 factorial with factors shake and turn, and levels control, 3 and 7. Each trial was analyzed separately. Data analysis for this paper was generated using SAS software, Version 8.02 of the SAS system for Windows (SAS, 2001). Copyright 2001 SAS Institute Inc. SAS and all other SAS Institute, Inc. product or service names are registered trademarks or trademarks of SAS Institute, Inc. Cary, NC, USA. P-values of <0.05 were considered significant unless otherwise stated. Hatch residue abnormalities were transformed to arc sine square root before analysis.

Results and Discussion

Trial one

We began with a velocity of 1 oscillation per second for 20 seconds every 30 minutes in the first trial. There was a significant difference in the volume of SEF at d6 with turn control demonstrating the highest volume and shake control and turn 3 the lowest volume. Shake 3 was intermediate. As a percentage of initial egg weight, the volume of SEF was significantly greater in turn control than the rest of the treatments (Table 1). The greater volume of SEF was expected in turn control. As there were no significant differences between shake control, shake 3, and turn 3 when SEF was evaluated as a percentage of initial egg weight, turning during the first 6 days of incubation must be important to proper development of this fluid. Though the area vasculosa was not measured in this trial it is possible that not turning eggs the entire first 6 days of incubation inhibited development of area vasculosa in the eggs that were in shake for some period of time, decreasing the amount of SEF that formed. D18 embryonic weight as a percentage of initial egg weight was

significantly highest in turn control and lightest in shake control (Table 1). Possibly, eggs that are turned during incubation allow the embryo to better utilize their yolk sac and albumen nutrients (Deeming, 1989), as well as prevent the embryo from adhering to the shell membrane (Eycleshymer, 1906).

Overall, the shake groups had a significantly lower hatch of fertile than the turn groups (Table 2). This can be attributed to the significantly higher embryonic mortality in the shake groups d1-3 and d8-10 of incubation. Shake control also demonstrated a higher incidence of embryonic mortality d17-20 and chicks with residual albumen (RA) after d18 of incubation (Table 2). Lack of adequate turning in shake control could have influenced utilization of albumen, first as a contributor to SEF d1-6 and then through movement of nutrients through the sero-amniotic connection d12-15. This, coupled with a lack of nutrients from the yolk sac, could have caused the embryos in shake control to have not developed as well. This hatch was pulled too soon as noted by the high numbers of live pips in all treatments (Data not shown). Lack of significant differences in wet and dry chick and yolk weights and percent moisture of the chick and yolks indicates that chick quality among the groups was not impaired by being shaken (data not shown).

Trial two

The velocity was kept the same at 1 oscillation per second, however the duration was increased to 30 seconds and the frequency was increased to every 10 minutes in trial two. There was a significant interaction in initial egg weight with shake control having the heaviest eggs and turn control, turn 3 having the lightest eggs. All other groups were intermediate (data not shown). However, this significance is not concerning as the greatest

difference in mean egg weight was 1.68g and the eggs were completely randomized prior to set. There were no significant differences in percent moisture loss between the two machines and no interaction occurred among treatments. Percent moisture loss at d18 was overall low for the two machines with the mean for shake being 9.70 and for turn 9.91. Average air temperature within the egg mass recorded by the data loggers was 99.2-99.3°F and relative humidity was 51-52%.

Embryo without yolk as a percentage of initial egg weight was not significantly different at d14 in this trial. Also, chick mass without yolk as a percentage of initial egg weight was not significantly different (data not shown). Therefore chick quality is not considered to be different in those embryos shaken from turned.

Hatch of fertile was significantly highest in turn control and turn 7, lowest in shake control and intermediate in turn 3, shake 3, and shake 7 (Table 3). Late mortality (d14-20), as a percent of fertile, was significantly highest in shake control. Also, those embryos that pipped but could not make it out of the egg were also significantly highest in shake control (Table 3). This demonstrates that as long as eggs are turned at some point during incubation the late mortality will not be as great. Embryos that were turned at least through d3 were better able to utilize their albumen as evidenced by the decrease in RA at hatch noted in turn control and turn 7 (Table 3). Also, embryos in shake 3 were not significantly different from turn control and turn 7; therefore if embryos are turned after d3 they are able to utilize their albumen better than those shaken the entire incubation period (Table 3).

Trial three

Velocity of shaking was increased to 1.25 oscillations per second, the duration was lowered to 20 seconds, and the frequency was raised to every 20 minutes in the third trial. There were no significant differences in initial egg weight or percent moisture loss at d18. Embryonic weight as a percentage of initial egg weight at d14 was not significantly different (data not shown). There was a timing problem with this trial in that we pulled the hatch too early, causing a higher percentage of live pips and internal pips in some treatments.

Hatch of fertile was significantly greatest in turn 7 and lowest in shake control. Turn control, turn 3, and shake 3 were intermediate to shake 7 and turn 7 (Table 4). As no trays were moved from one incubator to the other by d3, the early embryonic mortality d1-3 can be explained as only main effects of shake and turn, with shake having the higher mortality (Table 4). This could be due to less area vasculosa development causing less SEF formation (Deeming, 1989) or by the embryo adhering to the side of the shell (Eycleshymer, 1906). Mortality during the middle of the incubation period was not very high in any group, consistent with industry trend. Late mortality was lowest in groups that were turned at least d3-7 as shown by the lower mortality in turn control, shake 3 and turn 7 (Table 4). Shake 7 did not have as high embryonic mortality late in incubation as shake control because it was turned after d7, however it was still not as low as shake 3. Late mortality could be due to stratification of nutrients in the yolk sac, less albumen uptake in the shake groups than the turn groups, or malpositions and decreased oxygen availability to the embryo due to decreased chorioallantoic membrane development.

RA was mistakenly not recorded for during breakout. The incidence of embryos with

their heads in the small end of the egg was highest in shake control and shake 7; turn 3 was intermediate and turn control, shake 7 and turn 7 were lowest (Table 4). It is probable that these embryos also had RA. It has been postulated that RA can cause a mechanical obstruction preventing the embryo from attaining proper position within the eggshell (Randles and Romanoff, 1950). Also, Elibol and Brake (2006) demonstrated that turning eggs at a reduced angle of 35° can increase the incidence of small end embryos; however the presence or absence of RA was not reported.

Trial four

It was obvious that increased velocity was detrimental so in the fourth trial we decreased the velocity back to 1 oscillation per second, and tried a duration of 20 seconds with a frequency of 20 minutes. Once again there was a significant interaction in initial egg weights, with turn control and turn three having the greatest egg weight, turn 7 and shake 3 the lightest, and shake control and shake 7 being intermediate (data not shown). However, the greatest difference in egg weight was 2.63g and eggs were completely randomized prior to set. There was no difference in percent moisture loss at d14, but there was at d19. At d19 of incubation, turn control and turn 7 had the greatest moisture loss, while shake control and turn 3 had the lowest, and shake 3 and shake 7 were intermediate (Table 5). The eggs that were in turn the greater period of time lost more moisture than those in shake. As the temperature and humidity of both machines was recorded by data loggers every 1.5 minutes and later analyzed to be constant between the machines, it is believed that the difference in moisture loss may be due to the actual turning of the eggs.

A main effect of machine was noted in percent embryo to initial egg weight at d14 of

incubation, with turn having the highest percentage embryo over shake (Table 5). This demonstrates that even when eggs are shaken for the first seven days of incubation, as long as they are turned thereafter, they will have heavier embryos than those shaken the entire time. Turn control and turn 7 had the highest hatch of fertile, shake control the lowest, and turn 3, shake 3, and shake 7 were intermediate (Table 5). Contributing to shake control's lower hatch of fertile was the higher incidence of late mortality (d17-20) (Table 5) and embryos that had pipped the shell but were unable to escape it (Table 6). Turn control and turn 7 were in turn for at least the first 7 days and this prevented mortality seen in the other groups. Shake 3, which was only shaken for the first 3 days of incubation and then turned, was intermediate in hatch of fertile and had decreased embryonic mortality. It is possible that being turned before d3 is not as critical as turning after d3. The incidence of RA and small end embryos were significantly higher in shake control (Table 6). As long as embryos were turned at some point during incubation, the incidence of RA was much less than those shaken the entire period. It seems most important to turn after d3 of incubation for albumen utilization, as those embryos that were shaken until d3 had much less RA than those shaken until d7. The lack of significant differences in chick weights and yolk weights, even as a percentage of initial egg weights, supports the conclusion that even though the shaken embryos are not hatching as well, those that do hatch are of the same quality as turned embryos (data not shown).

Conclusions and Applications

There is significant evidence that shaking embryos during the first 18d of incubation is harmful to development of the embryo proper and extra-embryonic structures as noted by

the decreased hatch of fertile and increased RA. If eggs are shaken, as long as they are also turned at least d1-3, then hatch of fertile will not be as poor. Increasing the velocity of shaking to 1.25 osc/sec was more harmful than 1.0 osc/sec. The increased frequency of shaking in trial two versus trial one was beneficial in preventing early embryonic mortality d1-3. Also, the increased frequency and duration in trial two versus trial four provided better hatchability with less early and late embryonic mortality. More research with shaking for 1 osc/sec for 30 seconds every 10 minutes as in trial two is warranted.

Tables

Table 1: Trial one sub-embryonic fluid and D18 embryo

Machine	Day moved	SEF volume[1]	SEF % to initial egg[2]	D18 Embryo[3]
Shake	18 (control)	15.06[b]	26.11[b]	40.76[b]
	3	15.76[ab]	27.01[b]	44.19[a]
	7	-	-	43.54[a]
Mean				
Turn	18 (control)	16.56[a]	28.47[a]	44.09[a]
	3	14.93[b]	25.78[b]	43.16[a]
	7	-	-	43.89[a]
Mean				
Machine		NS	NS	NS
Day moved		NS	NS	NS
Machine * day moved		0.0056	0.0008	0.0173

1 oscillation per second for 20 seconds every 30 minutes
[a-b]Columnar means with a different superscript differ significantly ($P < 0.05$)
[1]Sub-embryonic fluid volume
[2]Sub-embryonic fluid volume as a percentage of initial egg weight
[3]Embryo weight without yolk at day 18 of incubation

Table 2: Trial one hatch data

Machine	Day moved	Hatch of fertile	Early mortality[1]	Late mortality[2]	RA[3]
Shake	18 (control)	32.36	14.46	14.79[a]	5.27[a]
	3	47.56	19.29	2.99[b]	0.00[b]
	7	46.65	17.55	4.61[b]	1.16[b]
Mean		42.19[b]	17.10[a]	7.47	2.14
Turn	18 (control)	75.51	3.83	1.96[b]	0.00[b]
	3	55.00	1.58	5.82[b]	0.71[b]
	7	59.34	1.85	3.40[b]	0.43[b]
Mean		63.28[a]	2.42[b]	3.73	0.38
Machine		0.0473	<0.0001	NS	0.0125
Day moved		NS	NS	NS	0.0475
Machine * day moved		NS	NS	0.0320	0.0032

1 oscillation per second for 20 seconds every 30 minutes.
[a-b]Columnar means with a different superscript differ significantly ($P < 0.05$)
[1]Percentage of fertilized eggs with embryos dying between 1 and 3 d of incubation
[2]Percentage of fertilized eggs with embryos dying between 17 and 20 d of incubation
[3]Percentage of fertilized eggs with embryos attaining at least 18 d of incubation and containing residual albumen

Table 3: Trial two hatch data

Machine	Day moved	Hatch of fertile	Late mortality[1]	Pip[2]	RA[3]
Shake	18 (control)	70.43[c]	15.51[a]	10.85[a]	8.88[a]
	3	84.85[b]	3.46[b]	0.87[b]	1.30[b]
	7	80.38[b]	5.67[b]	6.56[ab]	2.20[ab]
Mean		78.55	8.22	6.09	4.13
Turn	18 (control)	92.88[a]	1.92[b]	0.67[b]	0.63[b]
	3	90.84[ab]	5.86[b]	5.91[ab]	4.58[ab]
	7	93.33[a]	1.89[b]	2.99[b]	1.80[b]
Mean		92.35	3.23	3.19	2.33
Machine		<0.0001	0.0119	NS	NS
Day moved		0.0296	NS	NS	NS
Machine * day moved		0.0094	0.0320	0.0017	0.0200

1 oscillation per second for 30 seconds every 10 minutes.
[a-b]Columnar means with a different superscript differ significantly ($P < 0.05$)
[1]Percentage of fertilized eggs with embryos dying between 17 and 20 d of incubation
[2]Percentage of fertilized eggs with embryos that had pipped the eggshell
[3]Percentage of fertilized eggs with embryos attaining at least 18 d of incubation and containing residual albumen

Table 4: Trial three hatch data

Machine	Day moved	Hatch of fertile	Early mortality[1]	Late Mortality[2]	SEE[3]
Shake	18 (control)	24.82[c]	16.83	18.43[a]	6.25[a]
	3	59.21[ab]	18.00	1.92[c]	0.45[b]
	7	47.89[b]	16.42	9.40[b]	3.68[a]
Mean		43.98	17.08[a]	9.92	3.46
Turn	18 (control)	63.87[ab]	1.33	0.56[c]	0.56[b]
	3	59.37[ab]	1.51	14.92[ab]	2.23[ab]
	7	80.64[a]	1.72	0.97[c]	0.55[b]
Mean		67.96	1.52[b]	5.48	1.11
Machine		0.0015	<0.0001	0.0008	0.0264
Day moved		0.0431	NS	NS	NS
Machine * day moved		0.0417	NS	<0.0001	0.0176

1.25 oscillations per second for 20 seconds every 20 minutes.
[a-c]Columnar means with a different superscript differ significantly ($P < 0.05$)
[1]Percentage of fertilized eggs with embryos dying between 1 and 3 d of incubation
[2]Percentage of fertilized eggs with embryos dying between 17 and 20 d of incubation
[3]Percentage of fertilized eggs with embryo's heads in the small end of the egg

Table 5: Trial four embryo weight and hatch data

Machine	Day moved	D 14 Embryo[1]	D19 % Moisture Loss	Hatch of fertile	Late Mortality[2]
Shake	18 (control)	18.15	9.59^{b}	32.97^{c}	21.91^{a}
	3	19.38	10.33^{ab}	71.27^{b}	3.27^{c}
	7	19.35	10.33^{ab}	69.86^{b}	6.73^{bc}
Mean		18.96^{b}	10.08	58.03	10.64
Turn	18 (control)	20.26	10.77^{a}	93.74^{a}	0.45^{c}
	3	19.31	9.69^{b}	58.74^{b}	12.27^{b}
	7	21.45	10.82^{a}	95.71^{a}	1.26^{c}
Mean		20.34^{a}	10.43	82.73	4.66
Machine		0.0037	0.2030	<0.0001	0.0294
Day moved		NS	0.2229	0.0018	NS
Machine * day moved		NS	0.0215	<0.0001	0.0010

1 oscillation per second for 20 seconds every 20 minutes.
[a-c]Columnar means with a different superscript differ significantly ($P < 0.05$)
[1]Embryo without yolk sac at d14 of incubation as a percentage of initial egg weight
[2]Percentage of fertilized eggs with embryos dying between 17 and 20 d of incubation

Table 6: Trial four hatch data

Machine	Day moved	Pipped[1]	RA[2]	SEE[3]
Shake	18 (control)	3.35^{a}	13.92^{a}	11.28^{a}
	3	0.49^{b}	0.45^{c}	0.98^{bc}
	7	1.51^{ab}	1.80^{bc}	2.80^{b}
Mean		1.79	5.39	5.02
Turn	18 (control)	0.00^{b}	0.90^{bc}	0.00^{c}
	3	3.96^{a}	4.55^{b}	2.03^{bc}
	7	0.00^{b}	0.00^{c}	0.63^{bc}
Mean		1.32	1.82	0.89
Machine		NS	NS	0.0019
Day moved		NS	0.0315	NS
Machine * day moved		0.0011	0.0021	0.0015

1 oscillation per second for 20 seconds every 20 minutes.
[a-c]Columnar means with a different superscript differ significantly ($P < 0.05$)
[1]Percentage of fertilized eggs with embryos pipped the eggshell
[2]Percentage of fertilized eggs with embryos attaining at least 18 d of incubation and containing residual albumen
[3]Percentage of fertilized eggs with embryo's heads in the small end of the egg

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Summary

An understanding of embryonic and extra-embryonic component development is helpful to successfully hatch chicks. It is also helpful to appreciate how the various factors of incubation such as temperature, humidity, ventilation, and turning can influence embryo development. There are extreme unfavorable consequences from not turning eggs through a 45° angle through incubation, including, but not limited to, decreased embryonic growth and an increased percentage of extra-embryonic anomalies that lead to mortality.

Researchers have examined turning eggs at a decreased angle, or even not at all, to increase air flow through the incubator, which would remove more metabolic heat later in incubation, and create a better environment for the developing embryos. Not turning eggs is highly detrimental to hatch of fertile and the cause of embryonic malposition, lack of extra-embryonic membrane development, and high mortality. In the present study we turned eggs at a decreased angle of 15° and noted a sharp increase in embryonic mortality, especially d1-3 and d17-20 of incubation. Increasing frequency of turning at a decreased angle, such as 15° three times an hour, will increase the hatchability significantly, but not to a level that would be satisfactory for industry. Again, mortality d1-3 and d17-20 was significantly increased when turning at the reduced angle, even with an increased frequency. Turning 30° was also harmful to hatch of fertile, though not as much as 15°. Turning eggs at slightly less than 45°, (35°-40°) did not significantly impair hatch of fertile. Sub-embryonic fluid as a percentage of initial egg mass was less in the decreased angles at d6 of incubation; however chick weight at hatch was not significantly different. Therefore, even though embryos turned

35° and 40° started out a little less than ideal for successful growth, they were able to compensate during incubation.

Shaking agitation was examined as a possible alternative to turning eggs because it would permit movement of eggs and also allow more even air flow across the eggs. Eggs were displaced 4.5cm at a speed of either 1.0 or 1.25 oscillations per second for various durations and periods of time during different periods of incubation. Shaking eggs continuously through incubation was not adequate for hatchability, but the chicks that did hatch were of equal quality to those that were turned as indicated by their yolk free body mass. We were able to demonstrate that it is critical to turn eggs for at least the first three days of incubation and better for the first seven. Hatch of fertile was increased and there were fewer embryonic anomalies in eggs turned the first three or seven days and then shaken. Also, eggs that were shaken the first three days and then turned demonstrated a better hatch of fertile than those shaken for the entire incubation. It was clear that shaking 1.25 oscillations per second was highly harmful to hatch, compared to 1.0 oscillation per second, which is interesting Randles and Romanoff (1954) demonstrated that shaking eggs at 216 oscillations per minute, (3.6 oscillations per second) was best for their research. Possibly this is because those authors only shook eggs for 2-3 seconds at a time, while we shook them for 10, 20, or 30 seconds at a time.

Overall, it is concluded that shaking agitation is not a suitable alternative for turning for industry use at this time, however, further research is warranted to determine if a proper speed, interval, and duration combination can be identified that would result in successful hatches. Also, while turning 45° is the best choice, turning 40° or even 35° would not be harmful to hatch based on results we obtained.

Future Research

Research on shaking agitation merits further attention, especially at 1.0 oscillation per second for 30 seconds every 10 minutes and also for a shorter duration with a longer interval between shaking. As only one trial was performed with turning treatments 35° and 40°, another trial would be useful to validate our results. One question that has been raised: Is there a genetic component to turning? Does the act of turning through the 45° arc influence the turning on or off of a gene that shaking cannot? If so, when does this occur?