Quantifying Condensation on Shell Eggs and its Effect on *Salmonella* Enteritidis Penetration into Egg Contents

by

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Abstract

Salmonella Enteritidis (SE) prevalence in eggs is a major concern to the egg industry. Some research has shown that egg sweating, which can occur when refrigerated eggs are moved into a warmer ambient temperature with higher humidity from storage to loading docks or delivery trucks, has the potential to increase *Salmonella* penetration into egg contents. Objectives of this project were: 1) to compare three methods of quantifying condensate on sweated eggs, 2) to quantify moisture content on refrigerated shell eggs sweated at two temperatures (21 ^{o}C and 32 ^{o}C) and 3) to assess the effect of egg sweating on SE penetration into shell eggs over a six week period stored at 4 °C. The results of objective 1 showed there was no difference in quantifying egg sweat by either egg weight or weight of moisture absorbed on a paper towel (0.2% vs. 0.19% gain mL condensation/cm^2) ($P > 0.05$). For objective 2, there was a significant difference found in the time it took for an egg to reach a maximum condensation amount (11 min at 32 °C, 60% RH, 17 min at 22 °C, 60% RH), as well as completely dry (25 min at 32 °C, 60% RH, 34 min at 22 °C, 60% RH) between the two temperatures ($P < 0.05$). To evaluate objective 3, a 2x2 factorial of SE inoculation and egg sweating was utilized. To evaluate contamination levels, shell rinse, shell emulsion, and egg contents were enumerated and assessed for prevalence of SE throughout 6 wks of 4 °C storage. Treatments included (SES) nalidixic acid-resistant SE inoculated and sweated, (SENS) NA-resistant SE inoculated and not sweated, (NSES) buffered peptone water (BPW) inoculated and sweated, and (NSENS) BPW inoculated and non-sweated. In week 1, the shell rinse SENS treatment had significantly higher SE counts (0.32 $\log_{10}$

CFU/mL) than the other three treatments, where no SE was detected ($P < 0.05$). After week 1, no SE counts were obtained from the egg shell rinse, shell emulsion or egg contents. The SENS treatment shell rinses had significantly higher SE prevalence than the sweated and inoculated treatment (SES) in wks 1 (100% vs. 34.3%), 2 (57.6% vs. 22.2%), and 3 (38.2% vs. 11.1%) ($P < 0.05$). During weeks 4, 5, and 6, there was no difference in SE prevalence between the SES and SENS treatment. Egg sweating did not increase SE penetration into the shell matrix across treatment or week ($P < 0.05$). The decreasing trend of SE prevalence on the shell rinse obtained over the six week period indicate that refrigeration is a very effective method to mitigate *Salmonella* growth. These results indicate that the normal occurrence of egg sweating is not harmful to egg safety.

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I. INTRODUCTION

Egg safety is of paramount importance to the egg industry, with the presence of *Salmonella* being a particular concern. *Salmonella* can either be introduced into an egg through approximately 10,000 pores in the shell, or vertically laid into the egg contents by the hen (Yamamoto, 1997). Entry could be facilitated by "egg sweating," or the formation of condensation on shell eggs, when they are moved from a cold to a warm environment with a minimum relative humidity and the egg surface temperature is lower than the dew point temperature of the surrounding air (Ernst et al., 1998; Yamamoto, 1997; Zeidler, 1994). The relative humidity (RH) and temperature in the warmer area determines the dew point temperature. A higher relative humidity in the warmer environment encourages condensation formation on the egg surface. Egg sweating sometimes occurs in the egg industry if eggs are set out to increase in temperature before washing, as well as before or after transportation in refrigerated trucks. This varies from plant to plant and is impacted by whether the plant uses off-line, in-line, or mixed production. Eggs sitting on belts from in-line production complexes before they are collected may sweat on a very hot and humid day. Hen houses are physically connected to the plant at in-line processes (Knape et al., 2002). Off-line eggs are transported to the processing facility in plastic or pulp paper flats placed on plastic pallets, metal, or wood carts (Knape et al., 2002).

Previous research to determine if egg sweating promotes *Salmonella* penetration into egg contents is, however, scant and contradictory. When moisture on the surface of the egg, a minimum ambient RH, and a large temperature differential are present (when the egg is warmer than the environment), there is a higher potential for bacterial penetration into the egg contents (Bruce and Drysdale, 1994). The cooling that occurs in the egg when it is taken from a warm

environment and placed in a cooler environment causes the contents to contract, which creates a negative internal pressure, which can then aid in the pulling of bacteria through the egg shell and membranes (Bruce and Drysdale, 1994). This has been confirmed with a mechanical vacuum that simulated a negative pressure, which caused bacterial penetration of a partial egg shell (Haines and Moran, 1940). This situation applies to previously sweated eggs that are placed in refrigerated conditions.

Ernst et al. (1998) concluded that sweating did not increase *S.* Enteritidis numbers in the eggs they tested. They divided shell eggs into intact and cracked groups, and stored them at 7 °C. Half of the eggs were inoculated by immersion with 10^6 CFU/mL *S.* Enteritidis, air dried, and refrigerated. Half of the eggs were uninoculated. Half of each treatment group was removed from refrigeration and allowed to sweat for 3 hrs at 32 °C, 95% RH. The variables of storage time before sweat were also evaluated (0, 8 and 14 days) to determine if additional storage after sweat impacted *Salmonella* penetration. It was concluded that sweating did not increase *S.* Enteritidis numbers in the intact eggs. However, dramatically higher numbers of *S.* Enteritidis were found in both the sweated and non-sweated cracked eggs (63.6% and 77.3%, respectively, compared to 5.7% and 2.8% in the intact sweated and non-sweated intact eggs).

In contrast to Ernst et al., a study by Fromm and Margolf (1958) observed *Salmonella* penetration more frequently in both washed and contaminated eggs allowed to sweat for three and five hrs. This study differed from the previous study in that wet eggs were immediately returned to storage, while the eggs in the study by Ernst et al. were dried before being returned to storage. Another difference between the studies is that Fromm and Margolf utilized immersion washing, which is no longer used to commercially wash eggs. The current FDA egg rule (FDA, 2009) allows previously refrigerated nest run eggs to be left at room temperature for up to 36 hrs,

which could potentially allow enough time for them to form condensation and dry. De Reu et al. (2006) allowed condensation to form on agar-filled eggs for 30 min, while other eggs remained in storage at 20 °C. More frequent penetration of *Salmonella* through the egg shell was observed in the agar-filled eggs upon which condensation was allowed to form, but this effect was not observed using intact shell eggs.

In 2012, FoodNet by the CDC identified 7,842 human illnesses of *Salmonella*. Among these, 1,239 illnesses were serotype *S.* Enteritidis (SE: CDC, 2014) with 29.3% of patients hospitalized. Of 10,319 outbreak-related illnesses caused by a single confirmed etiologic agent in 2012, *Salmonella* resulted in the most outbreak-related hospitalizations (449, 64%) (CDC, 2012). Sixty-eight point two percent of *S.* Enteritidis cases are associated with eggs or egg products (WHO, 2001). While various serotypes have been isolated from egg shells, *S.* Enteritidis has been isolated primarily from egg contents (Saeed, 1998). Models have estimated that *S.* Enteritidis contamination in US-produced shell eggs is 1 in 20,000, or 0.005% (Ebel et al., 2000). *Salmonella* is estimated to be the number one cause of bacterial foodborne illness in the United States (Scallan et al., 2011). A major outbreak in 2010 caused 1,470 reported illnesses, and a recall of almost half a billion eggs (CDC, 2010).

Shell eggs can become contaminated with *Salmonella* as a result of infection of the laying hen's reproductive tract (transovarian route or vertical transmission) or by penetration through the egg shell (horizontal transmission) (Miyamoto et al., 1998). Salmonellosis occurs as an acute gastrointestinal disease that lasts 4-7 days. Symptoms include abdominal pain, frequent diarrhea, vomiting, fever, and chills. Death can also occur in high-risk population groups (Lin, et al., 1997). *Salmonella* growth is related to egg temperature. Many studies have shown that egg storage temperature is one of the most significant factors related to *Salmonella* growth in eggs

(Hammack et al., 1993; Schoeni et al., 1995). It must be noted that keeping eggs in a refrigeration environment for the longest time possible is the best option for egg safety. It was also found that storing *Salmonella* Enteritidis phage type 4 at 4 °C or 8 °C before heating decreased its heat resistance (Humphrey, 1990). An approximate 8% reduction in illness associated with *S.* Enteritidis would occur if all eggs were maintained at an air temperature of 7 °C throughout shell egg processing and distribution (Anon., 2000).

The FDA rule "Prevention of Salmonella Enteritidis in Shell Eggs During Production, Storage, and Transportation" (2009) concerns egg temperature before wash, which allows refrigerated nest run eggs to be tempered at room temperature for up to 36 h immediately prior to washing. It also requires that eggs must be held and transported at or below 7 °C ambient temperature beginning 36 h after time of lay. Post-processing, the Egg Products Inspection Act (USDA, 1998) requires washed shell eggs destined for commercial use to be stored and transported at a temperature no greater than 7.2 °C. Eggs can sweat before being placed in cold trucks or after being taken out if the correct conditions are present. Egg cooling can be time consuming: from post-processing to final cooling, eggs take an average of 2.5 days to cool from an average of 25 °C to the ¾ cooling point of 7 °C (Koelkebeck et al., 2008). The ¾ cooling time is the time required to remove three-fourths (75%) of the temperature difference between the starting egg temperature and the temperature of the surrounding air (Koelkebeck et al., 2008). The USDA Risk Assessment data suggests that cooling eggs to 7 °C or below within 12 hr of lay would reduce foodborne illness in eggs by 78% (FSIS, 2005). Czarick and Savage (1992) reported that eggs cased in cardboard cases required almost one week to cool from 27 °C to 7 °C.

The gene *yafD* found in *S. enterica* serovar Enteritidis can contribute resistance to the egg albumen by repairing DNA damage caused by the egg albumen (Lu et al., 2003). Egg albumen contains ovotransferrin, which chelates iron, making it unavailable for bacterial activity (Baron et al., 1997). It also contains lysozyme, which has been shown to form pores in gram negative bacteria (Pellegrini et al., 2000). After lay, the egg shell can become contaminated by all contact surfaces. The extent of contamination is directly related to the cleanliness of these surfaces (Board and Tranter, 1995). The egg-packing plant may house unclean surfaces where contamination can occur. Sterilized eggs that had passed through five farm-packing plants showed a contamination rate of 0.3%, but this level of contamination could be due to laboratory error (Davies and Breslin, 2003). The combination of refrigeration and low relative humidity has the potential to enhance *Salmonella* survival. According to Messens et al. (2006), *S.* Enteritidis survived longer at 10 °C compared to 15 °C and 23 °C when the % RH was lowered from 97% to 75%. *Salmonella* can probably survive longer in these conditions because the disadvantageous conditions may induce slower metabolism in the bacteria (Radkowski, 2002). The presence of moisture on eggs in storage may also enhance *Salmonella* survival (Rizk et al., 1966).

There are inconclusive results as to whether egg sweating (the formation of condensation on the egg shell surface) encourages bacterial penetration. Egg producers have the option to leave off-line eggs that have been previously refrigerated at room temperature for up to 36 h before wash. During this time, condensation and possible microorganism growth could occur. The objectives of this project were to quantify egg condensation in two environments, and correlate *Salmonella* penetration into eggs as a result of egg sweating. The FDA Egg Rule allows eggs that have been previously refrigerated to be left out at room temperature before wash for up to 36 h. If the egg industry continues to allow eggs to sweat under current conditions, unwanted

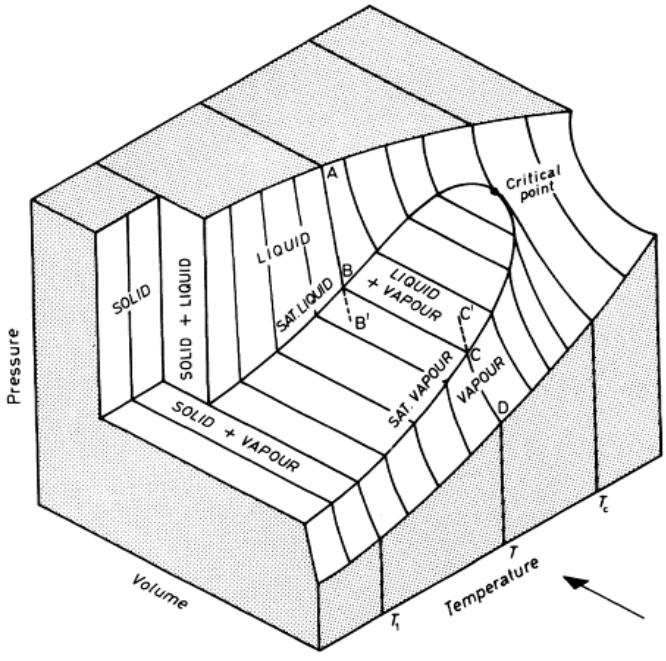
Salmonella contamination may occur. The current study will expose eggs to maximum condensation that will mimic industry conditions. Eggs will be inoculated with the drop method, which represents an industry-like scenario where *Salmonella* living in a spot of feces on an egg trans locates into the egg contents. Egg surface and contents will be tested for *Salmonella* weekly for six weeks. This research will provide a more definitive answer as to whether the normal occurrence of egg sweating is harmful to egg safety in the short and long term.

II. LITERATURE REVIEW

THE PROCESSES OF CONDENSATION AND EVAPORATION

Condensation is defined as the removal of heat from a system in such a way that vapor is converted into liquid (Collier, 1994). The stable equilibrium phases are liquid, liquid and vapor, and vapor alone. At equilibrium, the number of molecules hitting and being absorbed by the interface from the vapor phase is exactly equal to the number of molecules being emitted through the interface from the liquid phase. The state of equilibrium is impacted by pressure, volume, and temperature. Referring to Figure 1 (Collier, 1994), liquid exists along line AB, and vapor exists along the line CD. Liquid and vapor exist on the line BC. The saturation curve is at the locus point between points B and C (Collier, 1994).

Figure 1. Pressure-volume-temperature surface for a pure substance (Collier, 1994)

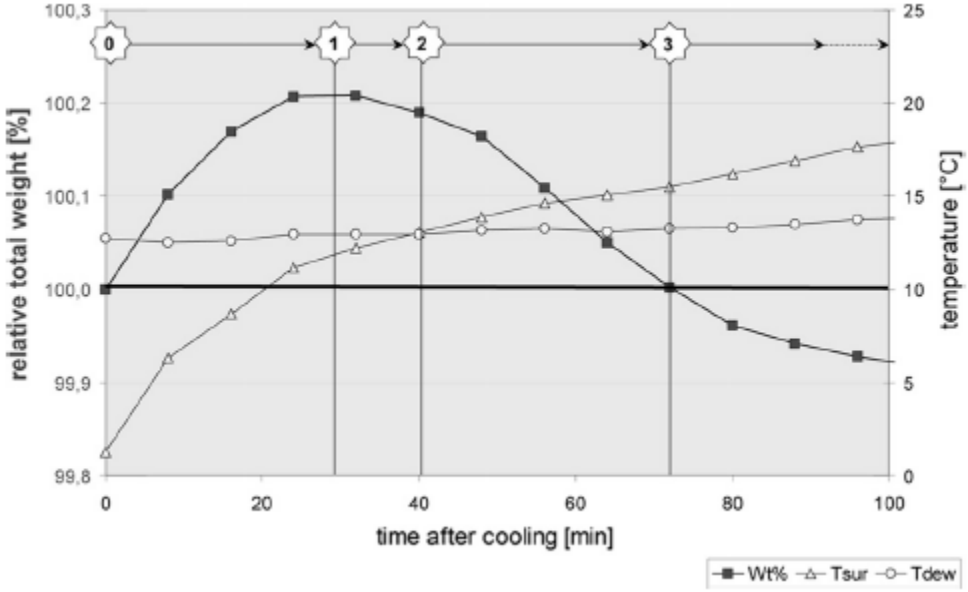


Condensation and evaporation are dynamic processes. They involve a flux of vapor to and from a surface. In the case of eggs, the flow is to or from the surface of the egg shell. It is assumed at the surface of the egg there is a fully saturated boundary layer of air that is the same

temperature as the egg surface. This boundary layer interacts with the surrounding air, which causes either evaporation or condensation. Evaporation and condensation are driven by a vapor gradient. A vapor gradient is defined by the moisture flux across the gradient and the level of resistance to the diffusion of vapor across the boundary layer (De Freitas, 2003). The vapor gradient is also controlled by the rate of air movement and the roughness of the surface, known as the combined convection moisture transfer coefficient (Monteith, 1957).

Depending on the environmental conditions (ambient temperature, surface temperature, and RH), mass flow density from the surface of an egg (or in the next example, fresh plums) can be determined. When mass flow is directed to the surface of the plum, condensation takes place. The air within the boundary layer is dried off; therefore, the air humidity decreases close to the plum surface, demonstrated in Figure 2 (Gottschalk et al., 2007). Due to the condensation process, the plum surface temperature increases until equilibrium is reached at a final surface temperature equal to the adiabatic saturation temperature. When this point is reached, the condition reverses and evaporation takes place to dry off the surface. This process takes place until the surface is totally dried off from the condensed water (Gottschalk et al., 2007). This exact situation occurs when cool eggs are allowed to temper before wash.

Figure 2. Simulation example of a course of humidity x in air apart from a plum surface in units of R0. Surface temperature 5.1 °C, ambient temperature 19.0 °C, ambient air RH 66% (Gottschalk et al., 2007)

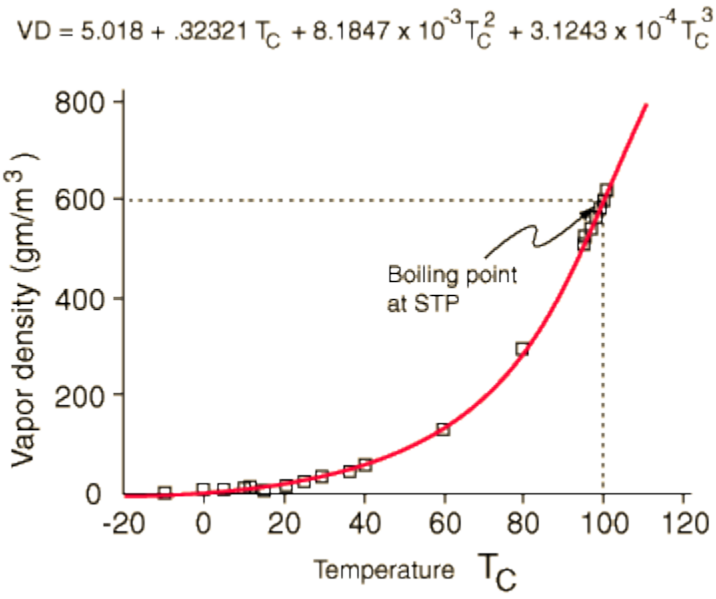


During the condensation of water vapor, heat is released, causing an increase in temperature at the point of condensation. If the measured surface temperature is equal or lower to the determined dew point, condensation forms (Linke et al., 2013). Condensation occurs when the dew point temperature of the room air is higher than the temperature of the egg surface. When the amount of condensation over a given period exceeds the evaporation of condensate over that same period, condensation is observed to have occurred (De Freitas, 2003).

Relative humidity (RH) is a ratio of how much water vapor is in the air to the amount of water vapor the air can hold at a certain temperature. At 100% relative humidity, the air is considered saturated. It is possible to create a good fit of the saturated vapor density curve at various temperatures (Figure 3) (Nave, 2012). Warmer air can hold more moisture than colder air. For example, the saturated vapor density of 22 °C air is 19.4 gm/m^3 while the density of 32 °C air is 33.97 gm/m^3 (Nave, 2012). The specific humidity is the amount of moisture in the air

per unit mass of air. It is proportional to the enthalpy (total energy content) of the air mixture. Specific humidity changes when moisture is added or removed. It does not change when the temperature changes, unless the air temperature is below the dew point. The dew point is the temperature at which moisture begins to condense out of the air (Elovitz, 1999).

Figure 3. Empirical fit of the saturated vapor density of water (Nave, 2012)



The shape, dimensions, and surface structure of the egg can have an impact on the intensity and transient response of condensation processes. Environmental factors including air temperature, humidity, and variable flow conditions around the eggs such as packaging impact both the amount and retention time of the condensation formed (Linke et al., 2013). The material factors also include egg surface area, egg surface roughness, egg surface composition, and presence of a cuticle, presence of oil on the egg, film packaging, and the type of egg carton. How surface condensation forms and the rate of formation is dependent on the surface properties of the egg. Water may form at different rates on a hydrophobic and hydrophilic surface (e.g., oiled and non-oiled eggs). On hydrophilic surfaces, a thin liquid film forms on the high surface energy substrate and acts as a conduction barrier for heat transfer. On hydrophobic surfaces,

condensation forms in droplets (Nenad et al., 2013). The temperature at which the air and water vapor reach 100% relative humidity is known as the dew point. The colder a surface, the higher the relative humidity adjacent to that surface. The coldest surfaces in the room will be where condensation will likely occur first (Lstiburek and Carmody, 1994). This is true in the case of refrigerated eggs set out at a warm temperature.

When moving eggs or in the next example, a produce item, from a cold to warmer temperature, the minimum relative humidity at which condensation will form can be determined using a psychrometric chart (Figure 4) (Ciobanu, 1976). The minimum surface temperature of warmed produce to avoid condensation can also be determined. Table 1 lists the predicted values of the temperature at which condensation will form on cold eggs when being transferred to ambient conditions (Zeidler et al., 1994)

Figure 4. Conditions of condensation on the surface of cold produce (Ciobanu, 1976)

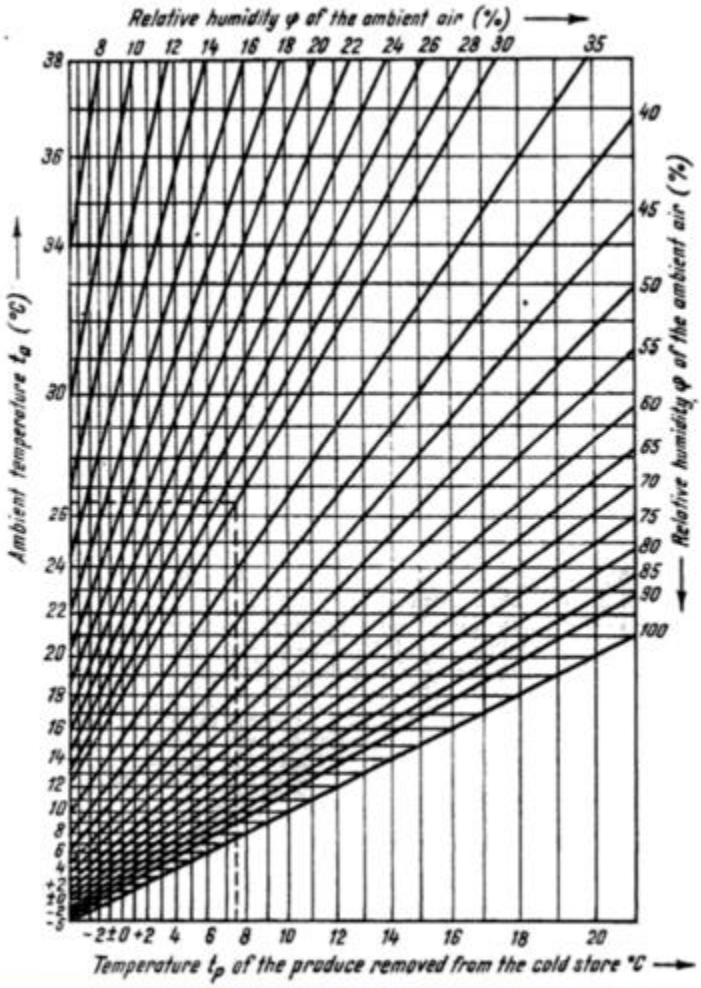


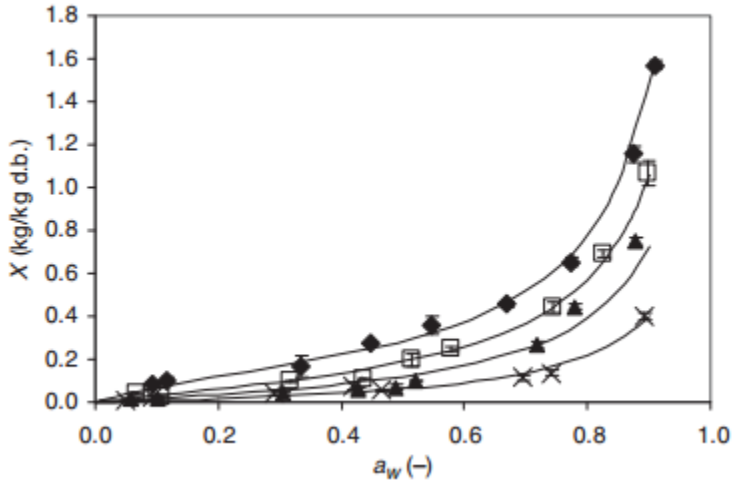
Table 1. Ambient conditions when moisture condenses on cold eggs (Zeidler et al., 1994)

Outside Temperature, °F	Outside Relative Humidity, % — Egg Temperature 45°F	Outside Relative Humidity, % — Egg Temperature 55°F
55	70	—
60	60	85
65	50	70
70	40	60
75	35	50
80	30	43
85	25	36
90	21	31

Evaporation, also known as vapor formation, occurs when a liquid temperature is increased fractionally above the corresponding temperature in which saturation occurs (Collier, 1994). In the case of eggs, the condensation formed on the surface of sweated eggs eventually evaporates when left out at a constant temperature for an extended time. The surface vapor flux for food drying processes can be expressed at the surface of the egg. The boundary-layer vapor concentration is determined by factors such as the chemical composition of the egg surface, and the rate of water supply from the inside of the egg to the surface (Chen, 2008). If the egg surface is at equilibrium conditions, the vapor concentration is determined by the water sorption isotherm. A water sorption isotherm compares the equilibrium relationship between water activity and percent water content on a dry basis at constant temperature and pressure for a particular food item. Water activity is defined as the partial pressure of water in a food divided by the vapor pressure of pure water at the same temperature. It describes the degree of binding of water and its availability to participate in physical, chemical, and microbiological reactions (Scott, 1957). This relationship is essential for the design and optimization of food drying and storage. The importance of the moisture sorption isotherm is that it shows when the drying process ceases for a particular food item. Figure 5 shows a moisture desorption isotherm for fresh eggplant at four temperatures (Moreira, 2010). The temperatures of most interest are 20 °C and 35 °C, because these are the temperatures at which eggs most likely will form condensation and then dry. As the moisture content of the eggplant decreased at a given water activity, temperature increased. This is because at higher temperatures, the activation of the water molecules shifts to higher energy levels causing the links to become less stable and break away from the water-binding sites of food; consequently, the equilibrium moisture content decreases (Palipane and Driscoll, 1992). Water sorption isotherms also show how hygroscopic, or

readiness to form condensation on the surface, the material is. Materials containing bound water are considered hygroscopic (MacCabe, 1994). Bound water exerts less vapor pressure than pure liquid water at the same temperature (Aguilera, 1999).

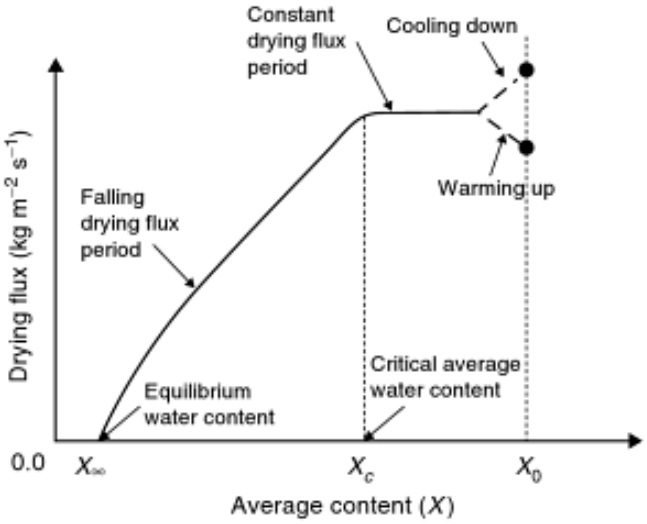
Figure 5. Experimental data of equilibrium moisture contents. Lines correspond to the Halsey model. (◊) 20 °C, (□) 35 °C, (▲) 50 °C and (x) 65 °C (Moreira, 2010)



Condensation and evaporation are impacted by the relative humidity of the air, which can be measured by a psychrometer. Psychrometers consist of two thermometers. One bulb is covered by a wick moistened with distilled water, and the second bulb is bare. In a fan-driven psychrometer, the fan moves the air around, promoting evaporation of water from the wick. As the water evaporates, the temperature of the wet bulb falls, eventually reaching the wet bulb temperature. The wet bulb temperature is the temperature at which the air is fully saturated with water. The dry bulb temperature is the temperature of the air shielded from moisture (Turns, 2006). As shown in Figure 6, drying consists of three stages: a warming up and cooling down period, a constant rate drying period, and a falling rate drying period (Chen, 2008). During the warming up and cooling down period, the food item adjusts to the new hotter temperature to which it is exposed. During constant rate drying, the drying rate is controlled by how fast water

molecules are removed from the droplet surface. The moisture content at the food surface is constant, and the rate of moisture removal from the surface is constant. The water droplet temperature is maintained at the wet-bulb temperature of the air (Chen, 2008). During falling rate drying, the water droplet eventually reaches the same temperature as the drying temperature air, since evaporative cooling is insufficient to maintain the surface wet-bulb temperature (Heldman and Hartel, 1997). The flux from the drying curve is described as the rate per unit surface area of the food (May and Perre, 2002). Evaporation of condensation from the surface of an egg is impacted by the egg surface structure, surface area, the temperature of the environment and food, and relative humidity.

Figure 6. Drying rate characteristics (Chen, 2008)



METHODS OF MEASURING MOISTURE

There is no standard method to measure condensation. There is also no research on methods of measuring moisture on egg shells. Therefore, methods used on other items were explored. There are some instruments available to measure dew formation such as lysimeters and wood moisture meters. Lysimeters are used to measure dew on soil and grass (De Freitas, 2003).

However, the drainage-like structure of the apparatus does not fit the requirements needed to collect condensate on a shell egg surface. A piece of equipment known as a pinless moisture meter, normally used to measure wood surface moisture, was tested to check its effectiveness in quantifying moisture on the egg shell surface. This meter works using electromagnetic wave technology, which measures the density in a 3D field underneath a measuring pad. Pinless moisture meters do not damage the egg shell surface, which gives it a significant advantage over pinned moisture meters (Wood and Wood Products, 2013). A pinless moisture meter is a "relative" measurement device. The moisture meter works as one of the plates of a capacitor, and the egg shell, or other item, works as an insulator. Any rise in reading given from a known dry product indicates moisture. The meter measures the wettest portion of the material from the surface to its lowest penetration point, which is 20 mm into the material (Byk, 2014). All moisture meters are designed to measure the "bound water" which are water molecules bound to the cell wall of wood or food. They cannot measure "free water" which is liquid water accumulating in the wood or food item and is not bound to anything.

Richards used absorbent paper on grass by first pressing lightly to avoid run-off from the grass to the ground, and then harder to absorb the rest of the dew (Richards, 1999). The absorbent paper was then sealed in a plastic bag of a known weight, and the whole sample and container weighed, with the weight compared with the weight of dry paper. A problem with this approach is that not all condensation water is absorbed, making the measured amount an underestimate of the true amount, which, according to Monteith, can amount to as much as 50–100%. (Monteith, 1957) This potentially could be a similar problem when measuring condensation on eggs.

SHELL EGG SWEATING

Water in foods plays a key role in determining the chemical and physical properties of a food, as well as its shape and structure. It is also a major contribution to chemical reactions and microorganism activity. Water is a tight hydrogen-bound structure that can bend to accommodate different types of molecules or surfaces in its interior (Rockland, 1987).

Temperature changes cause surface condensation problems on foods. Condensation causes localized increases in water activity, which can lead to microorganism growth on the surfaces of foods. Commercial egg processing systems create environments where temperature is not at a steady state. Immediately after lay, an egg takes up to ninety min to equilibrate with room temperature (22 °C) (Hillerman, 1955). Eggs received from an off-line (off-site) facility have an average internal temperature of 17-20 °C. Eggs received from the premise (in-line) have an average internal temperature of 31-36 °C (Curtis, 2005).

An extensive study by Koelkebeck et al. (2008) recorded surface egg temperatures and internal egg temperatures at the accumulator, post-wash, post-candling, and at the packer head in the summer and winter seasons. Average egg surface temperatures of in-line eggs at the accumulator were 24.2 and 17.8 °C in the summer and winter, respectively. Average egg surface temperatures of in-line eggs at the packer head were 26.6 and 21.6 °C in the summer and winter, respectively. In-line eggs at the accumulator in the summer and winter combined had an average surface temperature of 19.6 °C, while off-line eggs had an average surface temperature of 12 °C. At the packer head, in-line and off-line eggs had an average surface temperature of 23.2 and 19 °C, respectively. This result was expected, because off-line eggs come from a pre-shell processing cooler, where egg surface temperatures were 13.3 and 11.1 °C in the summer and winter, respectively. Most processors wash eggs in water ranging from 46.1 to 51.7 °C

(Anderson et al., l992). This wash water temperature would cause thermal cracking in previously 7 °C eggs, which would increase the probability of remaining bacteria on the eggs post-processing penetrating into the egg contents during the cooling process. Therefore, eggs should be at a minimum temperature of 15.5 °C before wash to prevent this (Zeidler et al., 1994). Eggs can sweat during the time period required to increase the egg temperature from 7 to 15.5 °C. Anderson (1993) showed that after wash, internal egg temperatures continued to rise, resulting in an 8 to 12 °F internal temperature increase above their starting temperature. After washing and packing in in-line systems, eggs can reach 24 to 29.4 °C and in rare cases, 37.8 °C.

After processing, shell eggs required at least five days to reach an internal 7.2 °C when stored at 7.2 °C (Chen et al., 2002; Jones et al., 2002a). Anderson et al. (1992) showed that post-processing, before packing internal egg temperatures can continue to rise for six hours after being placed in a cooler, and can be 6.1-7.8 °C higher than initial internal egg temperatures. Egg cooling can be time consuming: from post-processing to final cooling, eggs take an average of 2.5 days to cool from an average of 25 °C to the ¾ cooling point of 7 °C (Koelkebeck et al., 2008). The ¾ cooling time is the time required to remove three-fourths (75%) of the temperature difference between the starting egg temperature and the temperature of the surrounding air (Koelkebeck et al., 2008). After processing, eggs are placed in cartons or flats, placed into cases, then palletized. Eggs packed in cases cool at a seven times slower rate than uncased eggs (Feddes et al., 1993).

Eggs can form condensation when removed from refrigerated storage before wash, when being placed on transportation vehicles headed towards retail establishments, or when taken off trucks to be placed on grocery store shelves. They can also form condensation while on belts in in-line facilities on a very hot and humid day. For example, in food warehouses in the winter

season, heating is turned on and off to save energy. The same situation exists in a consumer's kitchen, where air conditioning and heating is similarly turned on and off at various points. If these temperature cycles continue for a period of time, this could cause an issue with the repetitive creation of high water activity regions on food surfaces (Rockland, 1987).

In commercial egg production, the main factors causing condensation on eggs are the transfer from a cold environment to a warm environment, in addition to a minimum relative humidity level. This can occur in certain conditions when refrigerated (7 °C, 75-80% RH) eggs are set out at room temperature for up to 36 h to increase in temperature before wash (Zeidler et al., 1994). The conditions (RH and temperature) required for eggs to sweat can be determined using a psychrometric chart. Sweating could occur when cold eggs are transferred in a warmer ambient temperature with a minimum RH to a cold truck for transportation to stores. The environment in a commercial egg transport vehicle could also be variable within the truck (Damron et al., 1994), creating additional opportunities for eggs to sweat. A study by Anderson et al. (2008) examined internal egg temperatures in the pre-delivery short-term storage and delivery phases. In the pre-delivery short term storage phase, average internal egg temperatures were 19.6 and 18 °C when eggs were stored for more than 12 h or less than 12 h, respectively. The total ambient temperature change was -4.8 °C for long term (>12 h) short term storage and -5.7 °C for short term (<12 h) short term storage. In the delivery phase, the total ambient temperature change from the beginning to end of the trip for long term (>10 h) and short term (<10 h) deliveries were -5.5 and -0.4 °C, respectively. The average internal egg temperature at the end of long and short deliveries were 9.7 and 17.7 °C, respectively.

Egg condensation can occur during unloading of the product to the store if the ambient outside temperature and relative humidity is high. Table 2 shows temperature and relative

humidity values and the corresponding likelihood for the eggs to form condensation in a new environment (Bell, 2002). Note that these values were determined before the mandated USDA egg holding temperature of 7.2 °C post-processing went into effect in 1998.

Table 2. Effect of humidity and temperature on moisture condensation on egg shells (Bell, 2002)

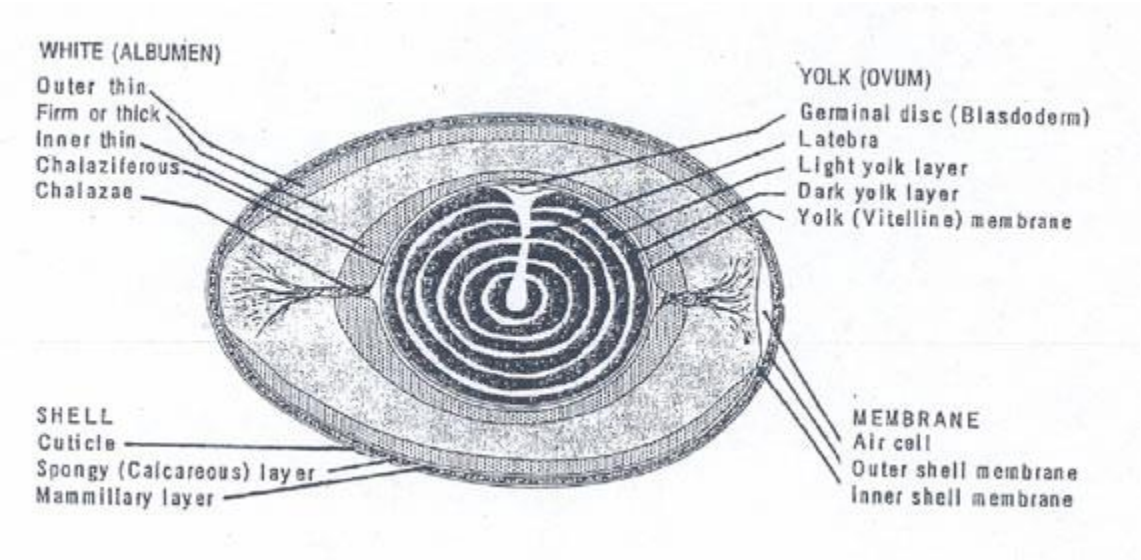
Temperature of New Room		Egg Room Temperature		
		55°F (13°C)	60°F (16°C)	65°F (18°C)
		Eggs Will Sweat if Relative Humidity in Egg-traying Room Is Higher Than		
°F	°C	%	%	%
60	16	82	—	—
65	18	70	85	—
70	21	58	71	83
75	24	50	60	71
80	27	42	51	60
85	29	36	44	51
90	32	30	37	43
95	35	26	32	38
100	38	22	28	32

During the hot summer months in the southeast United States, relative humidity can reach up to 100%. In addition, many indoor rooms in processing plants in the southwest US are cooled by evaporative cooling, which adds moisture to the air. Recommendations to avoid shell egg sweating include decreasing the humidity level in the warmer room where the eggs are being moved, creating good air flow with circulating fans to promote the evaporation of condensation, and allowing more time for cold eggs to temper before moving the eggs to a warmer environment (Bell, 2002). Additionally, a pallet of eggs can be covered until it warms up to avoid heavy condensation (Ciobanu, 1976). Methods to decrease humidity in a room include controlling the source of the moisture, dehumidification, and air change, or the exchange of interior air with exterior dry air (Lstiburek and Carmody, 1994).

FORMATION AND STRUCTURE OF THE HEN'S EGG

A hen egg is composed of a shell, albumen, and yolk. The yolk is surrounded by the albumen layer, which is surrounded by a hard egg shell (Figure 7, USDA, 2000). The distribution of these three parts varies depending on the type of hen and their age. According to Zeidler (2002), weights of eggs are divided into 6 size categories. Minimum weight requirements in the United States for these categories are: jumbo (68.6 g), extra large (61.5 g), large (54.4 g), medium (47.3 g), small (40.3 g), and peewee (no minimum requirement). Egg weight increases with the age of the hen (Van den Brand et al., 2004). The weight of the shell, albumen, and yolk represents 9-11%, 60-63%, and 28-29% of the egg weight respectively (Yamamoto, 1997).

Figure 7. Drawing of a midsagittal section of a hen egg (USDA, 2000)



An egg shell is composed of a thin layer of cuticle, a calcium carbonate layer, and two shell membranes. The cuticle is a hydrophobic thin stratum of glycoprotein spheres that extends a short distance into the egg pores, creating a first line of defense against microbial invasion (Romanoff and Romanoff, 1949). Figure 8 shows a tangential section of an egg shell (Igic et al., 2011). Figure 9 shows a microstructure view of the egg shell (Yamamoto, 1997). Pore canals are

funnel-shaped small holes on the surface of the shell for gas exchange. These canals are located scattered through the palisade layer of the egg shell, opening to the exterior. Pore canals range in diameter from 10-30 µm. An egg has approximately 10,000 pore canals on the shell surface. These canals allow air and moisture to pass through, but not liquid water (Yamamoto, 1997). The greatest concentration occurs at the equator of the egg (Board and Fuller, 1994). The cuticle is the most external layer of the egg. It is about 10 µm thick and covers the pore canals. It is the outermost physical defense of the egg against microbial invasion and moisture. It acts as a covering that closes many of the pores of the shell, which decreases shell permeability (Board et al., 1979). It also permits gas exchange in the egg. To assess the effect of common commercial egg washing detergents on the egg shell surface, Wang and Slavik (1998) washed eggs using a quaternary ammonium compound, sodium carbonate, and sodium hypochlorite. They found that the quaternary ammonium compound and sodium hypochlorite did not cause excessive damage to eggshell surface. Sodium carbonate removes large parts of the eggshell surface layer and most of the cuticle layer. The cuticle can also be damaged with contact with cage floors, abrasion from water brushes, and exposure to large amounts of water (Sauter et al., 1978; Wang and Slavik, 1998; Favier et al., 2000).

Figure 8. Tangential section of egg shell (Igic et al., 2011)

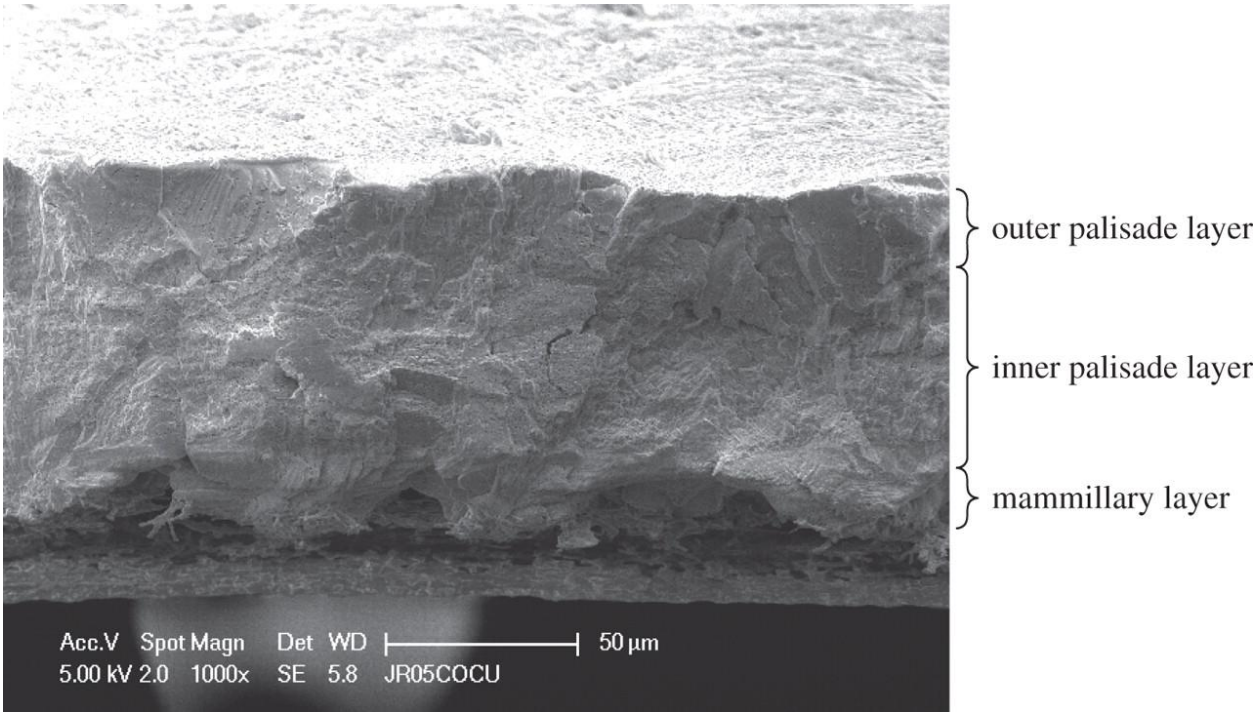
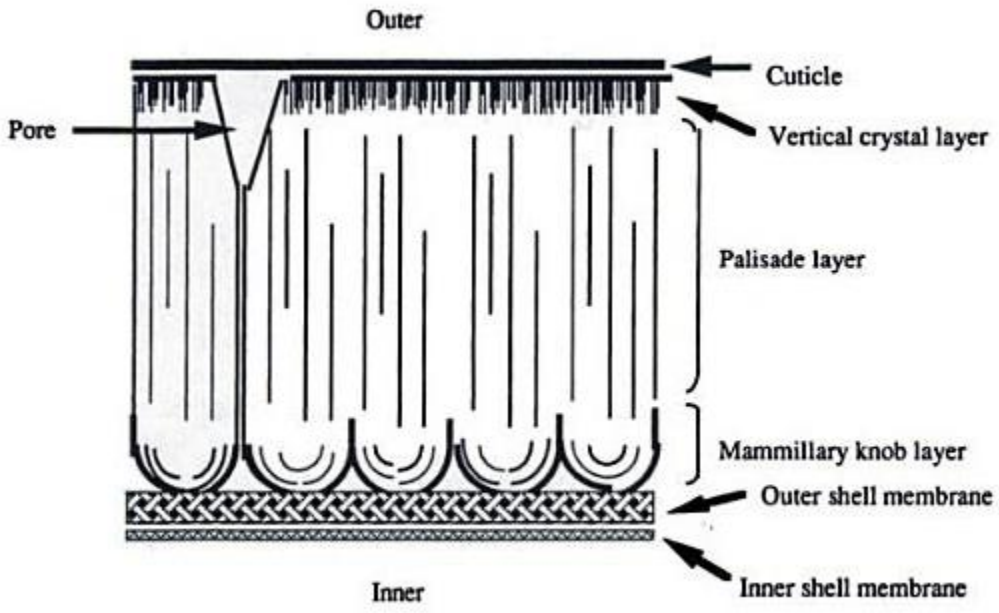


Figure 9. Microstructure view of the egg shell (Yamamoto, 1997)



As shown in Figure 9**,** the egg shell consists of a vertical crystal layer, a palisade layer, and a mammillary knob layer, with average thicknesses of 5 µm, 200 µm, and 110 µm,

respectively (Parson, 1982). It consists of 95% inorganic substances, 3.3% protein, and 1.6% moisture. Calcium carbonate composes most of these inorganic substances. The vertical crystal layer consists of short thin crystals running in the vertical direction of the shell. The palisade layer is dense and hard. Its crystalline structure is formed by calcification of calcium carbonate containing a small amount of magnesium, which forms a spongy matrix with the addition of collagen. Each mammillary knob is in contact with the outer shell membrane, which when distributed on the membrane hardens the shell (Hincke et al., 2012).

The shell membrane consists of an inner and outer membrane. The structure resembles randomly knitted nets. This structure obstructs invading microorganisms by catching them in the network. The outer membrane is about 50 µm thick, and the inner membrane is 15 µm thick (Lifshitz and Baker, 1964). The fibers are 0.8-1 µm thick, and each is composed of an elastin-like protein surrounded by a mucopolysaccharide mantle (Yamamoto, 1997). The outer shell membrane is more porous than the inner shell membrane. Figures 10 and 11, captured by the author, show inner shell membrane. Figure 12, also captured by the author, shows the top surface of the outer and inner shell membranes. The bumps represent calcium deposits on the outer shell membrane. Both membranes consist of 70% organic substances, 10% inorganic substances, and 20% moisture. The most common organic constituent is protein, followed by a small amount of carbohydrates and lipids. The shell membrane is composed of a thin insoluble fibrous layer of protein with many mesh-works. Wong et al. (1984) determined that collagen-like proteins are present in the shell membrane. The shell membrane is highly likely to contain collagen, elastin, or keratin-like proteins (Yamamoto, 1997).

Figure 10. The inner membrane of an egg

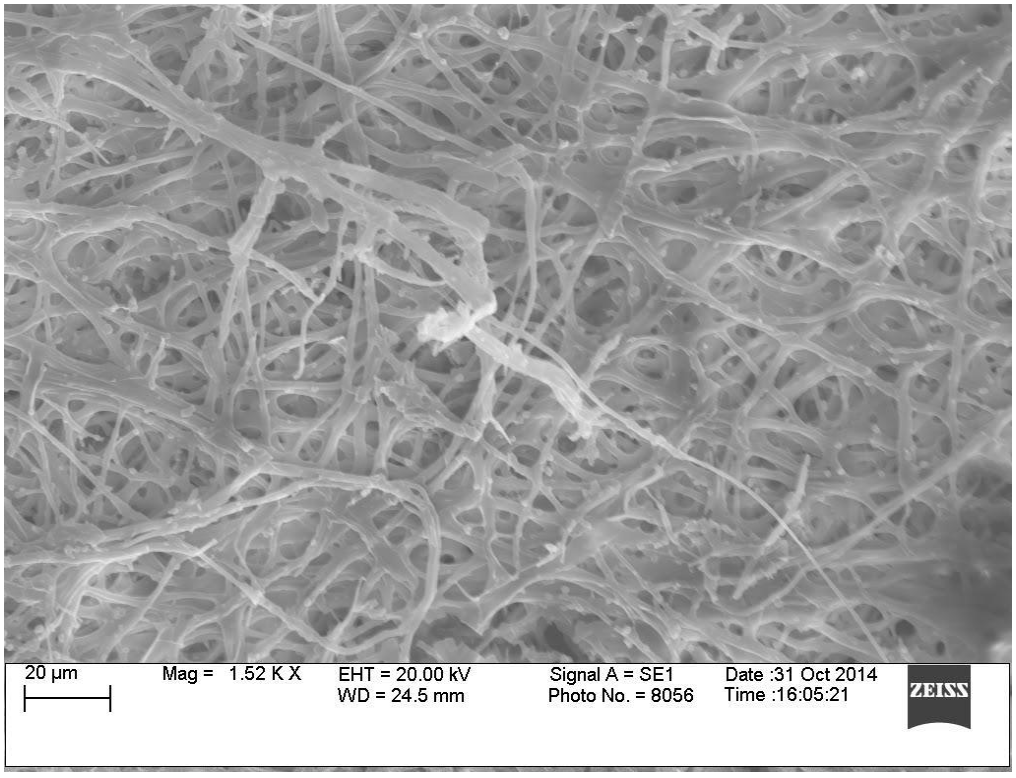


Figure 11. The inner shell membrane of a shell egg

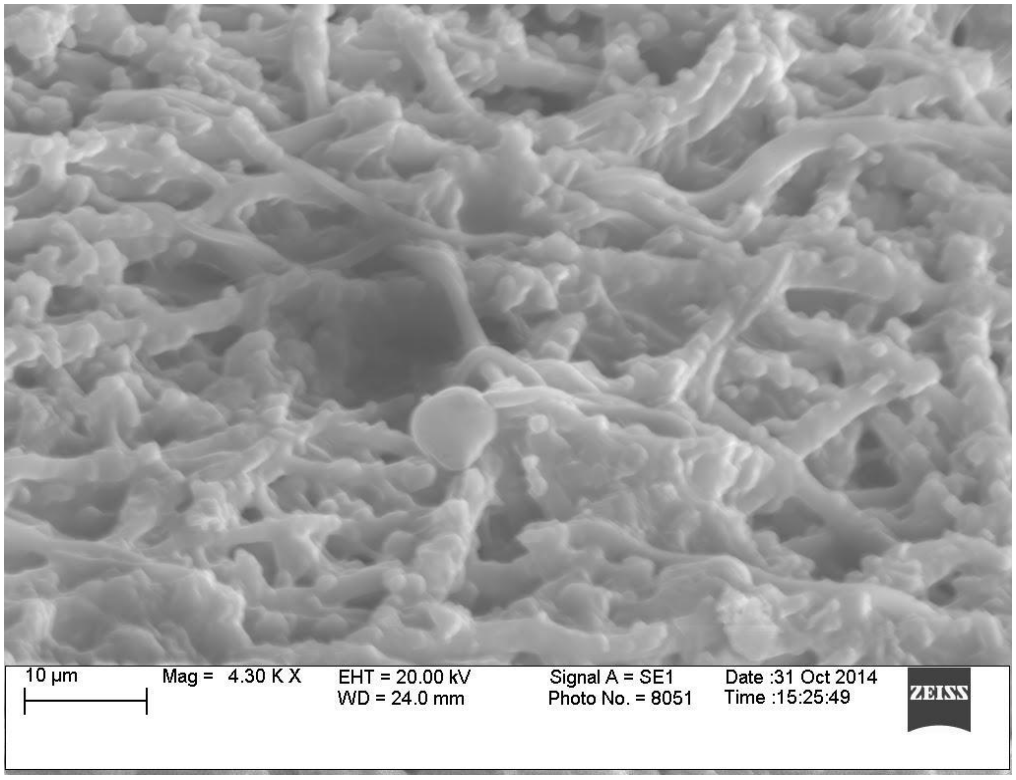
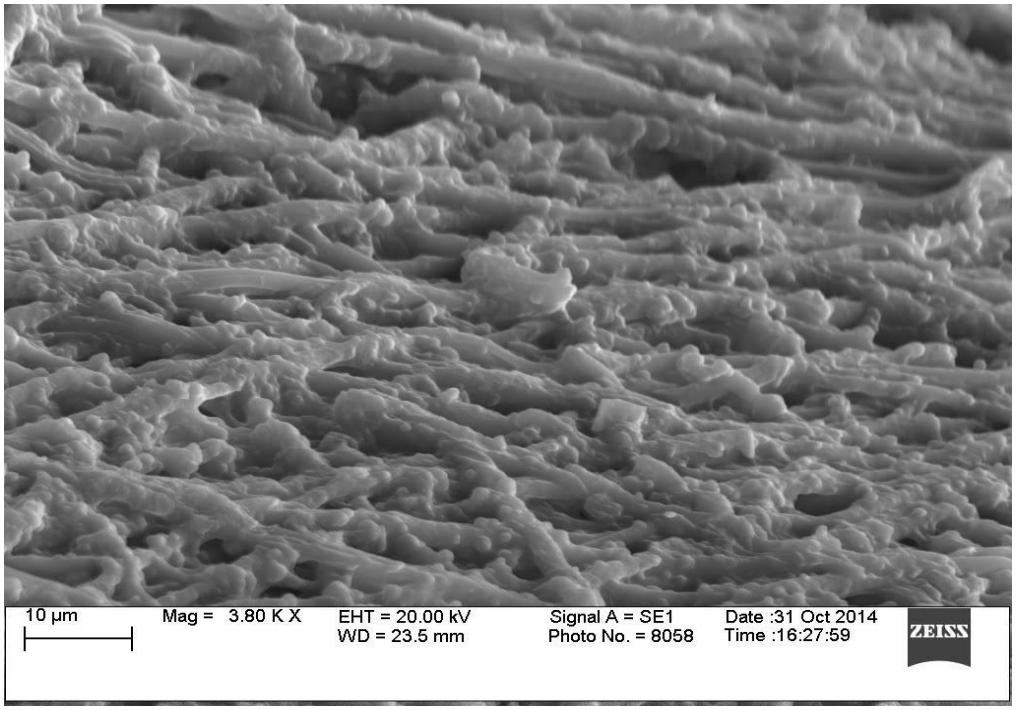


Figure 12. Inner and outer shell membranes of an egg



The albumen portion of the egg consists of thick and thin albumen and a chalaziferous layer. The thick albumen has a higher viscosity than the thin albumen. This is due to a high content of ovomucin in the thick albumen. In a fresh egg, the thick albumen is in contact with the shell membrane on one side, and surrounds the inner thin albumen and chalaziferous layer. The chalaziferous layer is fibrous and surrounds the entire egg yolk. This layer twists at both sides of the yolk membrane, forming a chalazae cord that stretches into the thick albumen, suspending the yolk in the center of the egg (Romanoff and Romanoff, 1949).

The egg yolk is surrounded by the vitelline membrane. The egg yolk consists of 2% white yolk and 98% yellow yolk (Okubo et al., 1997). The vitelline membrane is composed of an inner layer, a continuous membrane, and outer layers (1.0-3.5, 0.05-0.1, and 3-8.5 µm, respectively). The inner and outer layers are composed of a fine three-dimensional meshwork that is composed with fibers. The continuous membrane is a layered sheet-like structure consisting of 7 nm granules. The vitelline membrane is approximately 87% protein, 3% lipids, 10% carbohydrates,

and some DNA and RNA. Yellow yolk is a lipoprotein emulsion consisting of deep and light yellow yolk. The two yellow yolks appear in consecutive circular layers (Romanoff and Romanoff, 1949).

The ovary and oviducts of the hen originate from the left gonad. The hen is at peak production at 30 weeks of age (Campbell et al., 2010). Twelve thousand ova exist in a mature ovary. An ovum becomes a follicle when it is covered with a granular layer. Most of the follicles eventually degenerate, leaving about 2,000 that will accumulate white yolk to grow to 6 mm in diameter. These remaining follicles are called white follicles (Yamamoto, 1997).

Seven to twelve days prior to ovulation, the white follicle rapidly accumulates yellow yolk. After an average of 9 days the follicle stops accumulating yellow yolk. The follicle is then called a yellow follicle. The yellow yolk stops accumulating a day before the mature follicle of 16 to 18 g is ovulated in 24-27 h intervals from the ovary to the oviduct. An ovary of an egg-laying hen contains nine yellow follicles in a hierarchy. The growth of follicles and ovulation is regulated by follicle stimulating hormone (FSH) and luteinizing hormone (LH). The oviduct is a long (40-80 cm) tubular organ that extends from the ovary to the cloaca. It ensures that the egg is transported smoothly, and also excretes extracellular matrix components to surround the egg albumen. The five portions of the oviduct are the infundibulum, magnum, isthmus, shell gland, and vagina (Yamamoto, 1997).

The infundibulum is 11 cm long and opens its ampula to receive the follicles. The follicle remains there for 15 to 30 min where it can encounter the male chicken's sperm for fertilization. The outer vitelline membrane layer and the albumen chalazal layer are probably generated here (Burley, 1989). The magnum of the oviduct is approximately 34 cm long. The follicle is held there for 174 min while the egg albumen is secreted to cover the egg yolk. The isthmus is 11 cm

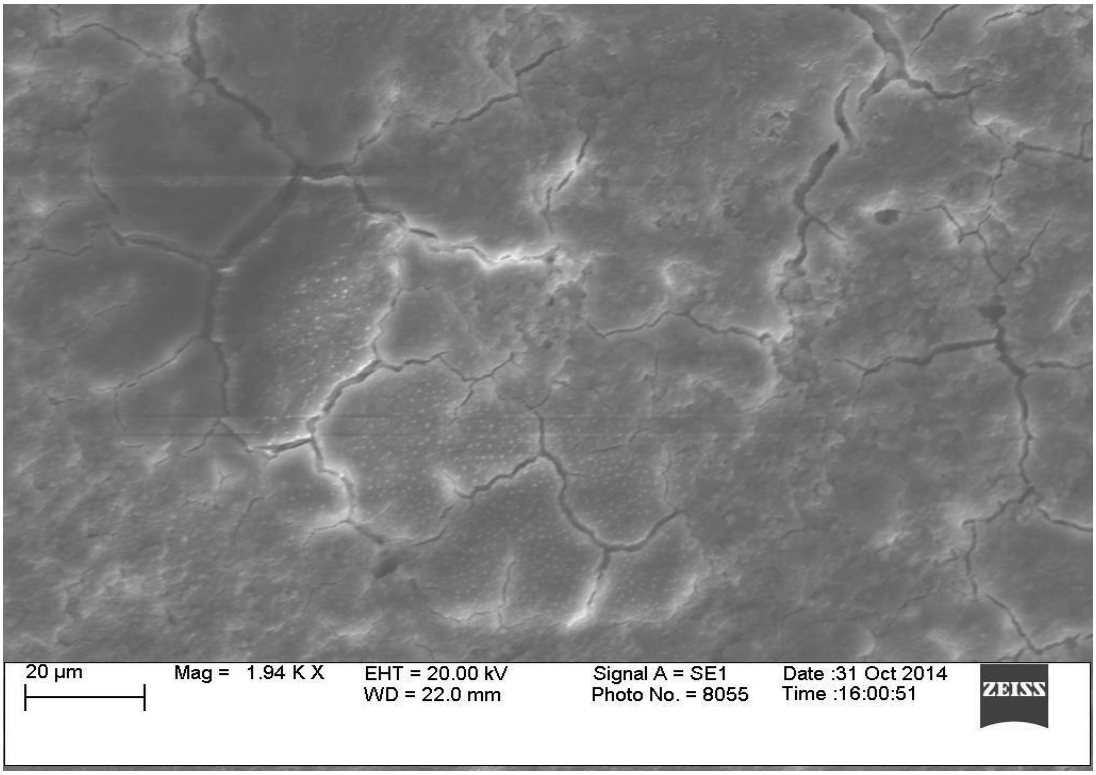
long, where the shell membranes are formed, wrapping the egg albumen from the outer side. This process takes 74 min (Yamamoto, 1997).

The next portion of the oviduct, the shell gland, also known as the uterus, is 10 cm long. The egg is held there for 21 h. A fluid consisting of sodium bicarbonate, sodium chloride, potassium chloride, and calcium chloride is secreted by several glands onto the inner surface of the uterus (Nys et al., 2004). It is still unknown how calcium reaches the egg shell. The egg shell structure is formed by assembling a crystalline calcium structure on the egg shell membrane. The next portion, the vagina, is 9 cm long. During oviposition, the edge of the vagina everts through the cloaca, the egg makes a 180 degree horizontal rotation and is laid large end first (Mayes and Takeballi, 1983). The egg passes through this muscled portion in 5 min (Bell, 2002).

MICROBIAL DEFENSES OF THE EGG

The egg consists of physical and chemical barriers against microbial invasion. The outermost physical defense of the egg against invaders is the cuticle. The cuticle is a major barrier to water. It covers the shell, and acts as a covering that closes many of the pores of the shell, which decreases shell permeability (Board et al., 1979). The function of the cuticle in preventing spoilage of eggs by microorganisms has an effectiveness of 96 h after lay (Vadehra et al., 1970b). Figure 13 shows a scanning electron microscopic (SEM) image of the cuticle taken by the author. The cuticle, also known as shell accessory material (Sparks, 1994), can enhance the egg's defense against bacterial invaders in two ways. First, it increases the shell strength, thereby reducing the chance of shell cracks (Van Immerseel et al., 2011). It also poses a physical and sometimes chemical barrier to microorganisms before they reach the pore canal. The cuticle is the egg's main barrier to water uptake, and therefore bacterial penetration (Board and Halls, 1973). Drysdale (1985) showed that the cuticle deposition deteriorates with flock age.

Figure 13. Outer surface of the egg cuticle. x1.94K



Research by Sparks and Board (1985) showed that 16% of eggs after oviposition (30 seconds or less) with mature cuticles challenged with feces were penetrated, compared to 100% of eggs with an immature cuticle. The cuticle can become damaged and provide an entry method for spoilage and pathogenic bacteria to enter the egg (Board, 1966; Wang and Slavik, 1998). Methods of damage include abrasion or chemical treatment. Washing eggs with alkaline sodium hydroxide can alter the egg surface, increasing bacterial penetration (Wang and Slavik, 1998). Hypothetically, a weakened cuticle could increase the chance of egg sweating causing bacterial penetration into the egg contents. The cuticle can also be damaged by *Pseudomonas* or *Enterobacteriaceae* during storage in a humid atmosphere (Bruce and Johnson, 1978; Board et al., 1979).

Pore openings impact the microbial integrity of the shell. Fromm and Monroe (1960) correlated bacterial penetration with porosity. Reinke and Baker (1966) refuted these findings by

correlating percent water loss and carbon dioxide penetration into the egg shell with egg shell porosity. A linear relationship was found when cumulated carbon dioxide values were plotted against percent weight loss for both the top and bottom shell sections. Nascimento (1993) showed that there is a positive correlation between aberrant crystal form shell defects such as aragonite, cubic calcite, and rounded "B" type bodies and bacterial penetration. These structures are characteristic of young birds and birds under stress. The data also suggested that pores played a minor role in bacterial penetration. The egg shell provides mechanical protection against invaders (Board and Tranter, 1995). Any form of damage to the egg shell increases the risk of bacterial penetration and hypothetically, bacterial penetration due to egg sweating. In the conventional caging system, the force of the egg dropping onto the wires from a certain height, an egg colliding with another, or the egg interacting with the collection machinery can damage the egg shell. Packaging and handling during transportation can also impact egg shell integrity. The egg shell strength of intact eggs is not correlated to *S.* Enteritidis penetration into egg contents (Jones and Musgrove, 2005).

The inner and outer shell membranes act as a filter to bacterial penetration. The membranes are harder to transverse by bacteria than the shell. The inner shell membrane is reported to be a better barrier than the outer shell membrane to bacterial translocation (Vahedra and Baker, 1972). Lifshitz et al. (1964) reported that in order from most to least, the inner shell membrane, then the shell, then the outer shell membrane were important in preventing bacterial penetration. This might explain why *Salmonella* penetration through the egg shell into the shell membranes has not been shown to increase with the occurrence of egg sweating (De Reu et al., 2006; Ernst et al., 1998). Even if the shell is penetrated, the shell membranes might be more important for preventing microbial penetration into the egg contents. However when large

bacterial inocula are used, membrane integrity is quickly overcome, especially when eggs are at 37 °C (Board and Fuller, 1994).

The enzymes mucinase and polysaccharidase are hypothesized to be involved with the bacterial penetration of the shell membranes. Zones of hydrolysis have been found surrounding bacteria in the shell membrane (Brown et al., 1965; Candlish, 1972). Evidence to refute this has also been found (Wedral, 1971). Board (1966) reported lysozyme in the shell membranes, hypothesizing that these have bactericidal activity.

The vitelline membrane plays a role in microbial defense by separating the nutrient-rich yolk from the rest of the egg. A study by Gast et al. (2010**)** assessed the ability of small numbers of *S.* Enteritidis to penetrate the vitelline membrane and multiply inside the yolks of eggs from six genetically different commercial lines of hens during 24 h storage at 30 °C. Eggs were also tested at 4 different ages (33, 39, 44, and 51 weeks) by inoculating 100 CFU/mL of *S.* Enteritidis onto the outside of vitelline membranes of intact yolks in plastic centrifuge tubes, and then adding back the albumen into each tube before incubation. The frequency of *S.* Enteritidis penetration into the yolk contents ranged from 30-58%, and the mean concentration of *S.* Enteritidis in the yolk contents was between 0.8 and 2 $\log_{10}$ CFU/mL. Hen age did not have a significant impact on *S.* Enteritidis penetration.

The likelihood of bacterial penetration into the yolk increases with extended storage at warm temperatures (Guan et al., 2006), possibly due to declining albumen viscosity and vitelline membrane integrity (Chen et al., 2005). The vitelline membrane integrity declines with egg age because osmotic movement of water across the membrane leads to a flattened and enlarged yolk, as well as a stretched and consequently weakened membrane (Romanoff and Romanoff, 1949). The reduced quality of the albumen and yolk are a function of temperature, reduced carbon

dioxide, increased pH, egg age, and the loss of moisture (Chen et al., 2005; Romanoff and Romanoff, 1949; Samli et al., 2005). Refrigeration of eggs has been shown to sustain yolk membrane integrity against physical rupture (Chen et al., 2005**)**.

During contamination, translocation across the shell into the egg contents is either active or passive. Micro-fungi actively translocate and bacteria passively translocate (Board and Fuller, 1994). The albumen contains various antimicrobial peptides and proteins (AMPPs). These function in three ways. The first is chelation of compounds needed for bacterial growth. Iron is chelated by ovotransferrin and biotin by avidin (Tranter and Board, 1982). The second is inactivation of proteases involved in invasive or metabolic processes by antiproteases. Cystatin and ovoinhibitor work this way (Bourin et al., 2011). The third is the direct binding to microorganisms, leading in some cases to the destruction of their cell walls. For example, lysozyme hydrolyses beta 1-4 glycosidic bonds in peptidoglycans (Tranter and Board, 1982). AMPPs have been identified in all egg compartments, including the shell, shell membranes, egg white, vitelline membrane, and yolk (Bedrani, 2013). The two most common AMPPs found in the albumen by weight are ovotransferrin (12%) and lysozyme (3.4%) (Stadelman and Cotterill, 1995). Other proteins found in the albumen are ovomucoid, which is a trypsin inhibitor, ovomucin, ovomacroglobulin, ovoglycoprotein, and ovoflavoprotein, which chelates riboflavin (Tranter and Board, 1982). The presence of these antimicrobial components might explain why egg sweating has not been shown to increase *Salmonella* penetration into the egg contents (a 9-11% rate of *S.* Enteritidis penetration for eggs with condensate and control eggs, respectively (De Reu et al., 2006; Ernst et al., 1998).

MICROBIAL PENETRATION AND EGGS

There are two ways in which eggs can become microbially infected, by the transovarian

and trans-shell routes. In the transovarian route, the microorganism infects the hen's reproductive tract. The organism can infect the oviduct or ovary, eventually contaminating the contents of the egg before it is laid (Board and Fuller, 1994). *Salmonella* Enteritidis can colonize the ovary of the hen with high frequency (Gantois, 2008). In some cases, due to the many defenses obstacles that the egg poses, microorganisms may penetrate only the egg shell (Board, 1966). Different microorganisms have varying abilities to penetrate and grow in the egg contents (Board, 1969). Bacterial penetration of the shell may occur without any damage to the egg or any further growth of the microorganism.

Trans-shell transmission was demonstrated by Haines and Moran (1940) by immersing warm eggs in a cool bacterial solution so that the contraction of the egg contents drew the bacteria through the pores. Another method is to incorporate dyes or black carbon into the bacterial suspension, and then inspect the shell membrane for color (Alls et al., 1964; Board and Halls, 1973). Board and Board (1967) used a method in which the sharp end of the egg to be examined was sterilized and a 1.5 cm hole created in the shell. The shell contents were then aseptically removed, and remaining albumen adhering to the membranes was removed with sterile Ringer's solution. The egg was then filled with a molten agar solution containing 0.1% 2,3,5-triphenyltetrazolium chloride, and the hole was sealed with molten wax. After 24 h of incubation at 37 °C, the agar block was removed. Where bacterial penetration of the shell occurred, organisms grew and reduced the tetrazolium compound to formazen, which is a red color. A disadvantage of this method is that not all bacteria reduce tetrazolium, therefore not all bacteria will be detected.

The temperature differential between a warm egg and the environment is a factor that affects the trans-shell transmission of microorganisms into the egg. As the egg cools, a negative

pressure is created down the egg pores which may cause contaminated material on the egg shell to be drawn through the pores into the egg contents (Haines and Moran, 1940). Contamination that is already present on the egg shell is a prerequisite for penetration of microorganisms into the egg contents. The level of contaminations on eggs is related to the environment in which they are laid. For example, a study by Jones et al. (2011) examined shell contamination levels on conventional cage produced eggs (CC), free range produced eggs from nest boxes (FRNB), the grass (FRG), and the floor (FRF). Shell emulsion aerobic contamination was higher in the winter from the conventional eggs (3.3 $\log_{10}$ CFU/mL) than the free range eggs (FRNB and FRF, 2.19 $\log_{10}$ CFU/mL vs. 3.60 $\log_{10}$ CFU/mL, respectively). During the spring, FRNB and FRF shell emulsions had significantly ($P < 0.05$) lower aerobic levels than CC shell emulsions (2.79 and 3.06 $\log_{10}$ CFU/mL vs. 3.87 $\log_{10}$ CFU/mL). Shell emulsion coliform levels in the summer for FRNB and FRF were 2.61 and 2.00 $\log_{10}$ CFU/mL. The CC shell emulsion coliform levels ranged from 0.02-0.42 $\log_{10}$ CFU/mL throughout the duration of the study. The FRF shell emulsion yeast and mold levels were significantly greater than FRNB and CC levels throughout the entire study ($P < 0.05$). Hannah et al. (2011) evaluated contamination levels on unwashed and washed shell eggs from caged and cage-free housing systems. Hens were housed on all wire slats or all floor shavings. Rinsates from the unwashed eggs from the shavings pen had significantly higher aerobic plate counts (3.8 $\log_{10}$ CFU/mL) than eggs produced on slats (3.2 $\log_{10}$ CFU/mL), while the eggs produced in cages had similar levels to the eggs produced on slats (3.1 $\log_{10}$ CFU/mL; $P < 0.05$). The level of contamination on the egg shell is not necessarily related to whether these organisms will penetrate the shell (Board and Fuller, 1994). Eggs laid on the floor or in dirty nests are more likely to be contaminated (Smeltzer et al., 1979). An explanation for this is that the soiling material may contain unknown substances that reduce the

surface tension of moisture present (Board and Halls, 1973).

In contrast, the USDA-FSIS *Salmonella* Enteritidis risk assessment in shell eggs publication does not agree with the theory that eggs contain no viable organisms before oviposition (USDA, 2005). The risk assessment model is based on the assumption that only an infected hen can lay an internally contaminated egg. An algorithm was used to estimate the fraction of eggs with no *S.* Enteritidis contamination. It uses the fact that the number of United States flocks estimated to be infected is 20%. Given that a flock is infected, the fraction of hens within the flock that are infected varies from flock to flock. The variation in the number of infected laying hens is represented by a Weibull distribution. The likelihood that an egg is *S.* Enteritidis contaminated at lay has an estimated value of about 0.00028 or approximately 1 in every 3,600 eggs. The initial number of contaminating bacteria when the albumen or shell is contaminated is a random value from the normal distribution for about 80% of contaminated eggs. When the yolk or vitelline membrane is contaminated, the initial number of bacteria is estimated to be a random number from the Poisson distribution for 19% of eggs (USDA, 2005).

Common contaminants that penetrate through hen egg contents are *Enterobacteriaceae*, *Staphylococcus* spp., *Micrococcus* spp., and *Streptococcus* spp. (Bruce and Johnson, 1978; Musgrove et al., 2004). Gram-negative bacteria can better withstand the antimicrobials present in the albumen (Board, 1966; Jones et al., 2004); therefore, bacteria that penetrate into the egg contents are commonly Gram-negative organisms such as Alcaligenes, Achromobacter, *Pseudomonas fluorescens, Salmonella,* and Eschericia (Hutchinson et al., 2003). Common contaminants on the egg shell tend to be Gram-positive cocci and bacillus such as Micrococcus and Arthrobacter (Hutchinson et al., 2003). In a study by Musgrove et al. (2008), eggs were sampled from three US commercial shell egg-processing plants on three separate visits. On each

plant visit, 12 eggs were collected from 12 sites along the processing line: the accumulator, prewash rinse, first washer, second washer, sanitizer rinse, dryer, oiler, check detection/scales, egg grader/packer head lanes, rewash belt entrance, and rewash belt exit. Organisms found during all nine visits included *Escherichia coli* and *Enterobacter* spp. Other genera isolated from at least one of the three plants included *Cedecea, Citrobacter, Erwinia, Hafnia, Klebsiella, Kluyvera, Leclercia, Morganella, Proteus, Providencia, Rahnella, Salmonella,* and *Serratia*. Non-Enterobacteriaceae identified included *Aeromonas, Chryseomonas, Listonella, Pseudomonas, Sphingobacterium, Vibrio,* and *Xanthomonas.* In addition, Jones et al. (2012) detected *Salmonella* and *Listeria* from shell emulsion pools from free range floor and conventional cages, as well as *Campylobacter* in shell emulsion pools from conventional cages and free-range nest boxes.

The growth of bacteria in eggs has been studied by inoculating the air sac in between the shell membranes. Board (1964) showed that once gram-negative bacteria penetrate the egg shell, there is a localized and restricted phase of growth in the membrane area where penetration occurred. The degree of growth at this stage is dependent on the presence of iron, which counteracts the mechanisms of conalbumin, a chelating agent in the albumen. The second and more proliferative phase of growth occurs when the yolk makes contact with the inner shell membrane.

The state of the cuticle is a major factor in the ability of bacteria to penetrate the egg shell. Sparks and Board (1985) showed that bacteria penetrated more frequently on "wet" portions of the cuticle as compared to dry portions. The cuticle is wet when the egg is freshly laid and appears dry after three min. Using scanning electron microscopy, the wet cuticle appears frothy, granular, and open. The dry cuticle has a more spherical structure. The ability of bacteria

to penetrate into the egg contents is determined by the number of open pores not covered by cuticle. The pores are largest and most often found on the blunt end of the egg (Mayes and Takeballi, 1983). Since the cuticle is the first line of defense against bacterial penetration (Board and Halls, 1973), the presence of a cuticle might impact trans-shell penetration of moisture and bacteria into eggs that have been sweated. De Reu et al. (2006) found greater bacterial penetration of the egg shells of agar-filled eggs with condensate than with a control group. They hypothesize that these results indicate that the major cuticle deposition was less effective as a barrier to bacteria, possibly because of the presence of condensate. Drysdale (1985) found significantly higher bacterial penetration in eggs which had a poor cuticle (40%) compared to eggs with a medium or good quality cuticle (26%). Alls et al. (1964) showed that cuticle removal increased bacterial contamination from 20% to 60%. It was found that *Pseudomonas* Aeruginosa penetrated the egg most frequently at the poles of the egg, with the blunt end of the egg being most prone to penetration. It has also been shown that cuticle deposition is not always complete at the poles (Board and Halls, 1973; Drysdale, 1985).

Shell integrity impacts bacterial penetration into egg contents. Mechanical protection is the shell's most important contribution to microbial safety (Board and Tranter, 1995). Heavy contamination has been found in cracked eggs, especially if eggs are wet **(**Brown et al., 1966). The presence of moisture on a cracked eggs sweeps bacteria onto the shell membranes (Board and Fuller, 1994). Ernst et al. (1998) found a significant increase in *S.* Enteritidis sweated egg content contamination due to cracked eggs (hair-cracks). Eggs were inoculated with 10^6 CFU *S.* Enteritidis/egg shell. 2.8% of intact eggs were contaminated versus 77% of cracked eggs. Between 8 and 10% of the eggs laid for the table industry suffer damage to the shell during routine handling (Hamilton et al., 1979). It was found that there is no relationship between shell

thickness and the likelihood of *S.* Enteritidis penetrating the eggshell (Kraft et al., 1959; Messens et al., 2005; Williams et al., 1968). Hincke et al. (2000) found lysozyme and the shell gland specific protein ovocalyxin, both present in the shell, are also important for the bacterial defense.

The purpose of the shell membranes is to protect the albumen from microbial infection (Bean and MacLaury, 1959; Williams and Whittemore, 1967). Penetration studies to assess the importance of the shell and outer and inner shell membranes in preventing bacterial penetration found that the inner membrane was the most important (Lifshitz et al., 1964). The effect of the cuticle was not accounted for in this study. When whole eggs were challenged with large numbers of bacteria, bacteria were found on the inner surface of the inner membrane within minutes (Bean and MacLaury, 1959). Challenging the eggs consisted of warming them to 37.8 °C and submerging them in a water-broth mixture for 15 min. A tracer study indicated that the inner membrane could provide physical resistance for 15 to 20 h (Walden et al., 1956). The membranes provide a micron filter action that makes it difficult for bacteria to penetrate (Anderson et al., 2004). Scanning electron microscopy has shown that the membranes become more permeable to bacteria after penetration, suggesting that enzymatic activity is involved during bacterial penetration (Brown et al., 1965). Board (1965**)** has shown that that contaminants occurring in rotten eggs can multiply in a buffered solution of mineral salts and intact shell membrane. Also, Lifshitz et al. (1965) have demonstrated that the egg cuticle, shell, and shell membranes contain sufficient nutrients for bacteria including *Salmonella* Paratyphi and *Pseudomonas fluorescens* to grow while penetrating the egg shell. Therefore, if eggs sweat and subsequently cool down, the negative pressure may cause moisture and bacteria to penetrate the egg shell, which in addition to the cuticle and shell membranes, can sustain bacterial growth. In fresh eggs, the chalazae, the albuminous sac, and the viscosity of the albumen ensures that

bacteria that have penetrated through the membranes will stay localized and away from the egg yolk (Board and Fuller, 1994). Jones et al. (1995) showed that despite having a *Salmonella* incidence of 7.8% on egg shells, no *Salmonella* was found in the egg contents of the same sampling, indicating the strong antimicrobial properties of the albumen. This explains why modern egg sweating studies have not observed significant *S.* Enteritidis penetration into the egg contents (a 9-11% rate of *S.* Enteritidis penetration in eggs allowed to sweat and control eggs, respectively) (De Reu et al., 2006; Ernst et al., 1998).

Freshly laid eggs usually contain no more than a few hundred *Salmonella* cells (Chen et al., 2002). An egg can be contaminated with any microorganism through contact with nesting material, dust, soil, feedstuff, inspects, blood, shipping and storage containers, human beings, and other animals (Board and Tranter, 1995). Feed can already be contaminated when it arrives at the farm (Davies and Hinton, 2000**)**. The likelihood of trans-shell penetration increases with the amount of time the eggs are in contact with the contaminated materials (FDA, 2009). Messens et al. (2005) found a high correlation between shell contamination with *S.* Enteritidis and its penetration of the egg shell. Gast and Beard (1990) reported that there is a relationship between feces positivity and egg shell contamination in hens artificially infected with *S.* Enteritidis. Hen strain and hen age affect the frequency of *S.* Enteritidis penetration into egg contents after inoculation on the surface of egg shells (Jones et al., 2002; 2004). There is also some evidence that suggests that egg shell translucency is related to *Salmonella* and *Escherichia coli* penetration into egg contents (Chousalkar, 2010).

Experts now believe that eggs can become contaminated with *S.* Enteritidis by the transovarian route. Eggs from naturally *S.* Enteritidis infected hens had no relationship between shell contamination and the presence of *S.* Enteritidis in the egg contents (Mawer et al., 1989;

Humphrey et al., 1989b). Studies with artificially *S.* Enteritidis infected hens have also shown no relationship between fecal carriage of *S.* Enteritidis and presence in egg contents (Gast and Beard, 1990a; Humphrey et al., 1991a). In the absence of intestinal colonization of *S.* Enteritidis in hens, *S.* Enteritidis has still been isolated from the reproductive tissues of naturally infected hens (Lister, 1988; Bygrave and Gallagher, 1989) and artificially infected hens (Timoney et al., 1989).

Ultimately, the deposition of *S.* Enteritidis into egg contents is a result of the colonization of reproductive tissues of systemically infected hens (Gantois et al., 2009, Gast et al., 2011a). Opportunities for hens to be exposed to *S.* Enteritidis are created by the persistence of the pathogen in poultry facilities (Davies and Breslin, 2003). Fecal shedding of bacteria by infected hens can be a frequent cause of environmental contamination with *S.* Enteritidis (Trampel et al., 2014). *S.* Enteritidis can spread throughout a poultry house through dust and feces (Garber et al., 2003; Kinde et al., 2005). Rodent and insect infestations can also increase a poultry house *S.* Enteritidis problem (Carrique-Mas et al., 2009b). Other risk factors for *S.* Enteritidis include larger flock sizes, older flock age, and housing in older facilities (Huneau-Salaün et al., 2009; Van Hoorebeke et al., 2010a; Pitesky et al., 2013). Poultry housing facilities are varied and no definite conclusion about their implications for food safety have been made in scientific literature (Holt et al., 2011). *S.* Enteritidis has been detected in both cage-based and cage-free housing systems in different studies, but no advantage has been shown for either system regarding the persistence of *S.* Enteritidis in either infected hens or the environment (Holt et al., 2011).

Only a small number of hens in an infected block shed *S.* Enteritidis at given time and an infected hen may lay many uncontaminated eggs (Humphrey, 1994). According to the U.S.

Department of Agriculture's Food Safety and Inspection Service (FSIS) *S.* Enteritidis risk assessment, it is estimated that of 47 billion table eggs consumed annually, 2.3 million are *S.* Enteritidis positive (USDA, 1998). The CDC established an epidemiological and laboratory association between eggs and *Salmonella* outbreaks in the 1980s.

The model created by the 1998 *Salmonella* Enteritidis risk assessment assessed the relationship between egg holding time, holding temperature, yolk membrane breakdown, and *S.* Enteritidis risk (USDA, 1998). The vitelline membrane separates the nutrient-rich yolk from bacteria that may be in the albumen. If the vitelline membrane breaks down, this results in rapid growth of *S.* Enteritidis. The model showed that at temperatures of 21.1 to 32.2 ℃, there was much less yolk membrane breakdown in eggs held no longer than 36 h compared to no longer than 72 h. For example, eggs held at 21.1 °C will experience a 16% yolk membrane breakdown after 36 h, and a 25% breakdown after 72 h (USDA, 1998).

The 2005 FSIS egg refrigeration risk assessment found that limiting eggs to 12 h at ambient temperature before refrigeration, the shortest timeframe between laying and refrigeration that was evaluated, provided the greatest public health benefit among the time frames studied (USDA, 2005). In terms of storage conditions, Martelli and Davies (2012) suggested that egg storage temperature conditions should not exceed 20 °C, because *Salmonella* species in the egg albumen can grow at temperatures higher than 20 °C, but they cannot grow at temperatures below 10 °C.

The effectiveness of refrigeration for preventing the multiplication of small populations of *Salmonella* is dependent on the initial level and location of contamination, the potential for movement of bacteria or nutrients within eggs during storage, and the rate at which growth-restricting temperatures are reached. Infected laying hens typically deposit *Salmonella* in the

albumen or on the surface of the vitelline membrane more often than inside the nutrient-rich yolk contents (Gast et al., 2003). In vitro egg contamination models have demonstrated that migration across the vitelline membrane into the yolk contents can occur within 24 h of storage at temperatures above 20 °C (Braun and Fehlhaber, 1995). Various *Salmonella* strains are capable of penetrating the yolk membrane and growing during 36 h of unrefrigerated storage (Gast et al., 2007).

S. Enteritidis can grow in the contents of naturally contaminated eggs at room temperature (20 °C) and it does not lead to changes in the color, smell or consistency of the egg contents. (Humphrey and Whitehead**,** 1993). Cogan (2001) reported *S*. Enteritidis growth after 8 days at 20 °C in 7% of whole eggs inoculated in the albumen near the shell with as few as 2 CFU. At inoculum levels of 25 CFU/egg when eggs were subsequently stored at 20 °C or 250 CFU/egg when eggs were stored at 30 °C, high levels of growth of *Salmonella* in the egg occurred significantly more frequently than when the inoculum dose was smaller (Cogan et al., 2001).

Chen et al. (2005) compared the storage of table eggs at 4 °C, 10 °C, and 22 °C. The whole eggs were inoculated with 10^2, 10^4, and 10^6 *S*. Enteritidis cells. At 22 °C, for all concentrations of inoculum, *S*. Enteritidis was able to grow, while at 4 °C and 10 °C, its growth was inhibited, regardless of the initial inoculum concentrations used. The authors believed that storage at 4 °C and 10 °C postponed the aging process of the eggs, preserving the antimicrobial agents of the albumen, maintaining the integrity of the vitelline membrane, thus preventing *S.* Enteritidis penetration into the egg contents. Catalano and Knabel (1994) found that slowly chilled eggs to 7 °C were more prone to penetration by *S.* Enteritidis than rapidly chilled eggs.

A study by Gast and Holt (2000) assessed the ability of *S.* Enteritidis to multiply in eggs

following oviposition prior to being placed in refrigeration conditions. It was found that *S.* Enteritidis multiplied less frequently with lower inoculum doses (15 cells), shorter storage times (1 day), and lower temperatures (10 to 17.5 °C). At warmer incubation temperatures (25 °C) and a higher inoculum dosage (150 cells), *S.* Enteritidis rapidly multiplied, especially with longer storage times of 2 to 3 days. Braun and Fehlhaber (1995) inoculated egg albumen with different doses of *S.* Enteritidis and assessed the impact of temperature and storage. *S.* Enteritidis was able to migrate from the albumen into the egg yolk during storage at 20 and 30 °C after one day. The first *S.* Enteritidis positive egg stored at 7 °C was found after 14 days of storage.

The egg yolk is a good source of high quality nutrients, therefore fast growth of *Salmonella* is expected to occur in this site when temperature will allow it. Experimentally infected laying hens often deposit *S.* Enteritidis on the vitelline membrane (Gast et al., 2007). The fast growth of *S.* Enteritidis occurs after a certain delay, during this period the integrity of the vitelline membrane is lost, resulting in a leakage of nutrients into the albumen. This enhances further migration and multiplication of *S.* Enteritidis in the yolk (Humphrey and Whitehead, 1993). The initial growth phase potentially involves the use of iron reserves. This appears to be sufficient to support four generations of the bacteria, but once these reserves are depleted, *Salmonella* cells enter a lag phase (Gantois et al., 2009).

SALMONELLA

The *Salmonella* genus is a part of the *Enterobacteriaceae* family, which contains gram negative rod-shaped, non-spore forming bacteria (Bhunia, 2007). *Salmonella* species were discovered more than a century ago (Bell and Kyriakides, 2002). *Salmonella* organisms are ubiquitous, and are often found in the digestive tracts of mammals, birds, and reptiles. They are facultative anaerobes, small 0.7-1.5 x 2-5 µm rods that are usually motile with peritrichous

flagella (Bell and Kyriakides, 2002**)**. Of the different serovars of *Salmonella enterica*, *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium account for the most non-typhoidal *Salmonella* infections in both developed and developing countries (CDC, 2014).

CDC surveillance data has shown that 600 different *Salmonella* serotypes have caused salmonellosis in the United States (CDC, 2005). Since 1995, *Salmonella enterica* serotype Enteritidis has been the second most frequently reported cause of *Salmonella* infection (CDC, 2005). There are over 2,400 confirmed serotypes of *Salmonella* that are of concern to the food industry (Bell and Kyriakides, 2002). The species of most concern to food safety is *Salmonella enterica* subspecies *enterica*. Over 99% of *Salmonella* isolated from humans belong to this subspecies (Old**,** 1992**).** *Salmonella* are widespread in the natural environment and may survive for long periods of time in the soil, water, or dried animal feces. *Salmonella* can be present in or on any raw food material such as seafood, poultry, and produce (Bell and Kyriakides, 2002**).**

Salmonella species cause illness by infection. Outbreaks of Salmonellosis have occurred with an infective dose of 10-100 cells (Bell and Kyriakides, 2002). The organism grows and multiplies in the host's body and becomes established in or on the cells or tissue of the host. *Salmonella* multiply in the small intestine, then colonize and invade the intestinal tissues, producing an enterotoxin. This causes an inflammatory reaction and diarrhea. Human illness usually results from ingesting contaminated food or drink. However, *Salmonella* can also be transmitted through the fecal-oral route or by the animal-to-man route (CDC, 1996; 1999). It results in mild to severe gastroenteritis, which could entail a sudden onset of possibly diarrhea, abdominal cramps, fever, plus nausea, vomiting, and headaches. Symptoms begin 6-72 h after consuming the contaminated food or drink, and the infection can last 4-7 days. Most people

recover without antibiotic treatment, but in some cases, hospitalization is required. Most people who are infected with *Salmonella* recover completely, but it may be a few months before bowel habits return to normal (CDC, 2015).

If *Salmonella* spreads to the blood, urine, bones, joints, or the brain, the severity and danger of the infection increases. According to the CDC (2013), it occurs in 8% of people infected with *Salmonella* and can appear as bacteremia (blood infection), meningitis, osteomyelitis (bone infection), or septic arthritis (joint infection). This most often occurs in the young or elderly who have a weakened immune system. The CDC estimates that 1.2 million illnesses due to *Salmonella* and 450 deaths occur annually (Scallan, 2011). Compared to 2010-2012, 2013 showed a 9% decrease in the incidence of *Salmonella* infection (CDC, 2014).

Salmonellosis is a reportable disease, which means physicians and health laboratories are required to report cases of the illness to local health departments according to the procedures established in each state. These cases are reported to the state health departments and the *Salmonella* isolates are sent to the state public health laboratories for serotyping. All cases and all serotyped isolates are reported to the CDC. A case is only reported to the CDC if it is a confirmed isolate of *Salmonella*. However, the number of reported illnesses is likely much lower than the actual number of illnesses because (1) ill people do not always seek care by physicians, especially if symptoms are not severe; (2) medical professionals may not determine the cause of the illness and only treat the symptoms; and (3) medical professionals do not always report *Salmonella* cases to public health officials (FDA, 2009). The CDC estimates that there are 38 cases of salmonellosis for every reported culture confirmed case (Voetsch et al., 2004).

EGG REGULATIONS AND THE CONTROL OF SALMONELLA

In 2012, FoodNet by the CDC identified 7,842 human illnesses of *Salmonella*. Among these, 1,239 illnesses were serotype *S.* Enteritidis (CDC, 2014) with 29.3% of patients hospitalized. Of 10,319 outbreak-related illnesses caused by a single confirmed etiologic agent in 2012, *Salmonella* resulted in the most outbreak-related hospitalizations (449, 64%) (CDC, 2012). Sixty-eight percent of *S.* Enteritidis cases are associated with eggs or egg products (WHO, 2001). While various serotypes have been isolated from egg shells, *S.* Enteritidis has been isolated primarily from egg contents (Saeed, 1998). Current models have estimated that *S.* Enteritidis contamination in US-produced shell eggs is 1 in 20,000, or 0.005% (Ebel et al., 2000). *Salmonella* is estimated to be the number one cause of bacterial foodborne illness in the United States (Scallan et al., 2011). A major outbreak in 2010 caused 1,470 reported illnesses and a recall of almost half a billion eggs (CDC, 2010).

The temperature at which eggs are stored is very important. Refrigeration has been shown to inhibit the growth of *Salmonella* in the yolk and albumen **(**Board et al., 1989). Contaminated eggs that are stored at room temperature provide conditions for microorganisms to grow rapidly. The FDA's final rule on egg safety, titled "Prevention of *Salmonella* Enteritidis in Shell Eggs during Production, Storage, and Transportation" (Egg Rule) states that eggs must be held and transported at or below 7 °C ambient temperature beginning 36 h after time of lay (FDA, 2009). The United States Department of Agriculture, Food Safety Inspection Service, risk assessment data suggests that cooling eggs to 7 °C or below within 12 h of lay would reduce food-borne illness in eggs by 78% (FSIS, 2005).

The Egg Rule raised questions about the holding of eggs to be processed into products or table eggs at refrigeration temperatures. Specifically, when the refrigerated eggs are set out at ambient temperature with a minimum RH level before wash, they will sweat, which could permit

an increase in penetration of surface bacteria into the shell contents. The other concern is that immediately subjecting cold eggs to hot wash water will lead to an increase in thermal checks or cracks, which deteriorate egg quality and promote microbial penetration into the egg contents. The FDA responded to these questions by stating that the refrigeration requirement of 7 °C is consistent with the rule on the refrigeration of shell eggs at retail (FDA, 2000). Both laws are based on the findings that refrigeration significantly reduces the rate of *S.* Enteritidis multiplication in eggs (Kim et al., 1989; Humphrey, 1990). However, the FDA agrees that there can be quality and safety problems such as thermal checks (hairline cracks in the shell) associated with refrigerating eggs immediately prior to processing into either table eggs or egg products. Therefore, the FDA modified the rule to allow an equilibration step (a step during which the eggs reach room temperature) before eggs are processed. Specifically, under §118.4(e) of the final rule, shell eggs that have been refrigerated may be held at room temperature for no more than 36 h just prior to processing to temper them, which will reduce the risk of hairline cracks in the shell that could contribute to bacteria entering the egg. The FDA states in the law that it believes the benefits of refrigeration accompanied by equilibration outweigh any possible risk associated with sweating of the eggs (FDA, 2009).

There are several laws that impact the various instances where eggs could potentially form condensation due to the change in temperature they are exposed to. For example, eggs destined for retail transported on trucks are required to be refrigerated at 7 °C according to the FDA Food Labeling, Safe Handling Statements, Labeling of Shell Eggs; Refrigeration of Shell Eggs Held for Retail Distribution (FDA, 2000). The FDA's final rule on egg safety requires refrigeration during all egg storage and transportation beginning at 36 h after time of lay (FDA, 2009). When previously refrigerated eggs are put on a refrigerated truck during production for

transport, there could be brief periods of time where the eggs could form condensation if exposed to ambient temperatures with high relative humidity.

The Egg Rule also requires shell egg producers to implement various other measures to prevent *S.* Enteritidis from contaminating eggs on the farm. These include a written egg safety plan, proper biosecurity practices, cleaning and disinfection of a poultry house if an environmental test or an egg test is positive for *S.* Enteritidis at any point during the life of a flock, and a successful pest monitoring program. Poultry houses and eggs must be inspected for *S.* Enteritidis. A *S.* Enteritidis prevention plan must also be created and documented by an egg facility (FDA, 2009).

The Egg Rule is the first federal rule that addresses the introduction of *S.* Enteritidis into the egg during production (FDA, 2009). All previous regulations have addressed transportation and storage (USDA, 1998) and labeling and refrigeration at retail locations (FDA, 2000). At the grocery store level, the FDA issued a rule designed to help prevent the growth of *S.* Enteritidis in eggs by requiring refrigeration of shell eggs at retail and by requiring shell egg labeling (FDA, 2000).

The Egg Product Inspection Act (USDA, 1975), defines ambient temperature as the air temperature maintained in an egg storage facility or transport vehicle. Amendments made to the Egg Products Inspection Act in 1998 require that shell eggs destined for the consumer must be stored and transported at an ambient temperature of no greater than 7 °C. The amendment also requires a label indicating that refrigeration is required, e.g., ‘‘Keep Refrigerated,’ (USDA, 1998). When previously refrigerated eggs are put on a refrigerated truck for transport to retail stores, there could be brief periods of time where the eggs will form condensation. This could also occur after the eggs are unloaded from the retail trucks into grocery stores if the temperature

and RH conditions are appropriate. In addition, the FDA rule on the refrigeration of shell eggs for retail distribution has additional measures in place to prevent *Salmonella* growth (FDA, 2000). It requires that all shell eggs held for retail distribution are to be refrigerated at no greater than 7 °C.

MOISTURE AND SALMONELLA PENETRATION OF SHELL EGGS

Water in liquid or vapor state is necessary for microbial penetration through the pores of the egg shell (Board et al., 1979). The flooding of the egg pores with contaminated water is considered the first stage of microbial contamination (Board and Fuller, 1974). When eggs are transferred from a cold environment to room temperature or higher temperatures with a minimum RH, they may "sweat" due to condensation formed on the egg from the moisture in the air. It often happens when refrigerated eggs are brought to room temperature (21-24 °C). This occurrence is common in the egg industry, when refrigerated eggs are set out at room temperature with a minimum RH level for anywhere from 4 to 36 h to increase their temperature before wash. However, during this warming period, the contents of the eggs are in an expansion mode which creates a positive pressure in the egg which does not allow for movement from the surface to the contents. When eggs are allowed to condense moisture on the surface and are then moved from the warm to the cold environment, moisture on the surface sometimes does not evaporate. This change in temperature causes the egg contents to contract, creating a negative pressure that allows for microbial translocation in to the egg contents. This situation caused by egg sweating is widely believed to be a cause of bacterial penetration (Fromm and Margolf, 1958).

The presence of moisture on the surface of eggs in addition to a temperature change may provide an opportunity for the egg contents to contract and for moisture to be pulled along with

bacteria into the shell pores and into the egg contents (Haines and Moran, 1940; MacLaury and Moran, 1959; Williams et al., 1968; Vadhera et al., 1970**;** Board and Halls, 1973). In addition to liquid water being a factor in trans-shell transmission, Graves and MacLaury (1962) showed that there is a positive correlation between the water vapor present in the atmosphere at the time of lay and the incidence of contamination in eggs. According to Braun et al. (1999), the level of *S.* Enteritidis penetration into the egg contents increases with temperature and relative humidity. Bacteria such as *Salmonella* have the potential to penetrate the shell and membranes of an intact shell egg. Research by Stokes et al. (1956) showed that when there is a temperature differential between an egg and the environment in addition to moisture present, egg shells are more likely to be penetrated by bacteria. The area of the egg most prone to penetration is the air cell, because it responds more quickly to changes in temperature (Vadehra et al., 1970a).

The combination of refrigeration and low relative humidity has the potential to enhance *Salmonella* survival. According to Messens et al. (2006), *S.* Enteritidis survived longer at 10 °C compared to 15 °C and 23 °C when the % RH was lowered from 97% to 75%. *Salmonella* can probably survive longer in these conditions because the disadvantageous conditions may induce slower metabolism in the bacteria (Radkowski, 2002). Alternatively, when previously sweated wet eggs are placed in refrigeration, moisture retention on the shell may account for greater survival of *Salmonella* at low temperatures (Rizk et al., 1966). Storing eggs in refrigerated conditions causes drying and flaking of the cuticle layer. The negative pressure created due to the temperature differential between previously sweated eggs and a cold environment could enhance the movement of bacterial-laden moisture into the contents of eggs (Haines and Moran, 1940).

Ernst et al. (1998) concluded that sweating did not increase *S.* Enteritidis numbers in the eggs they tested. They divided shell eggs into intact and cracked groups, and stored them at 7 °C.

Half of the eggs were inoculated by immersion with 10^6 CFU/mL *S.* Enteritidis, air dried, and refrigerated. The remaining eggs were uninoculated. Half of each treatment group was removed from refrigeration and allowed to sweat for 3 h, 95% RH at 32 °C. The variables of storage time after sweat were also evaluated (0, 8 and 14 days) to determine if additional storage after sweat impacted *Salmonella* penetration. It was concluded that sweating did not increase *S.* Enteritidis numbers in the intact eggs. However, dramatically higher numbers of *S.* Enteritidis were found in both the sweated and non-sweated cracked eggs (63.6% and 77.3%, respectively) compared to 5.7% and 2.8% in the intact sweated and non-sweated intact eggs.

In contrast Fromm and Margolf (1958) observed *Salmonella* penetration more frequently in both washed and contaminated eggs allowed to sweat for 3 or 5 h. Specifically, the albumens were contaminated in all groups of eggs that were permitted to sweat for 1, 3, and 5 h at 22 °C, 80-85% RH. Also, the incidence of contamination increased when the eggs were sweated on days 1 and 8 of storage. The yolks were contaminated in groups that were allowed to sweat 3 and 5 h. The methods of this study differed from the previous study in that wet eggs were immediately returned to storage, while the eggs in the study by Ernst (1998) were dried before being returned to storage. Another difference is the method of egg washing. Fromm and Margolf (1958) used the immersion method with 48.9 °C wash water for 3 min. Ernst (1998) obtained eggs from a commercial plant. Present day egg washing laws do not allow the use of immersion method washing (USDA, 2007).

De Reu et al. (2006) inoculated the shells of intact shell eggs and agar-filled eggs with 10^3-10^4 CFU of *S.* Enteritidis. Half of the eggs were stored for 21 days in a climate chamber at 20 °C and 60% RH. The other eggs were stored for 24 h in a refrigerator at 6 °C and 70-85% RH, then stored for 20 days at 20 °C, 60% RH. Condensation was observed forming on these

eggs for 30 min. A significantly higher average of 62% of the agar-filled egg shells with condensate were penetrated compared with 43% for the control group ($P < 0.01$). No significant difference in whole egg contamination was found (18%, 22%).

III. Moisture Content and Moisture Quantity of Sweated Chicken Eggs in Two Storage Environments

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SUMMARY

There are instances where shell eggs may be moved from refrigeration into ambient temperature with high humidity such as before wash and during transportation. Under these conditions it is of concern that bacteria on wet eggs can grow and migrate through the shell pores into the egg. Objectives of this experiment were: 1) to compare 3 methods of quantifying condensate on eggs and 2) to quantify condensate on refrigerated shell eggs at two temperatures (22 ^{o}C and 32 ^{o}C). For objective 1, 270 fresh shell eggs (3 replications, 90 eggs per replication) were stored at 4 ^{o}C, 60% relative humidity (RH), then placed at 22 ^{o}C, 60% RH for 1 h. After this time, 30 pre-weighed eggs were randomly selected and weighed. Thirty eggs were thoroughly wiped with pre-weighed paper towels to collect condensate. Thirty eggs were evaluated with a pinless moisture meter for quantifying egg condensate, which was found to be an ineffective method. There was no difference in quantifying egg condensation by egg weight or weight of moisture absorbed on a paper towel (0.2% vs. 0.19% percentage gain mL condensation/egg surface area) ($P > 0.05$). For objective 2, 104 fresh eggs formed condensation at two temperatures (22 ^{o}C and 32 ^{o}C, 60% RH). Each egg weight was continuously recorded from the beginning of condensation formation to the point where the egg reached a constant weight. There was a difference found in the time it took for an egg to reach maximum condensation (11 min at 32 °C, 17 min at 22 °C), as well as completely dry (25 min at 32 °C, 34 min at 22 °C) between the two temperatures ($P < 0.05$).

DESCRIPTION OF PROBLEM

Salmonella Enteritidis (SE) continues to be of concern to the egg industry. Current models have estimated that *S.* Enteritidis contamination in US-produced shell eggs is 1 in

20,000, or 0.005% [1]. *Salmonella* is estimated to be the number one cause of bacterial foodborne illness in the United States [2]. Egg condensation is an issue faced by the egg industry that may increase *Salmonella* penetration into eggs. Refrigerated eggs that are transferred from a cold environment to room temperature or higher may "sweat" due to condensation formed on the egg from the moisture in the air. Egg condensation formation is common in the egg industry, when refrigerated eggs are set out at room temperature from anywhere from 4 to 36 h to increase their temperature before wash. When the eggs are placed back in refrigeration after wash, i. e., when there are temperature and moisture differentials between the egg and the environment, the subsequent cooling causes the egg contents to contract, which creates a negative pressure that helps to draw bacteria through the shell pores [3]. When eggs are placed in refrigeration wet, moisture retention on the shell may account for greater survival of *Salmonella* at low temperatures [4].

Fromm and Margolf [5] observed *Salmonella* penetration more frequently in both immersion washed and feces-contaminated eggs allowed to sweat for 3 and 5 h. Ernst et al. [6] concluded that sweating at 32 °C for 3 h did not increase *S.* Enteritidis numbers in the eggs they tested. De Reu et al. [7] inoculated the shells of whole eggs and agar-filled eggs with 10^3-10^4 CFU of SE, and allowed eggs to form condensation for 30 min at 20 ^{o}C, 60% relative humidity (RH). Sixty-two percent of the egg shells with condensate were penetrated compared to 43% in the control group ($P < 0.01$). No significant difference in whole egg contamination was found.

The primary environmental factors that affect condensation formation on eggs are the air temperature, air humidity, and air velocity (speed and direction) [8]. The material factors include egg surface area, roughness, and composition; also presence of cuticle, if the egg is oiled, film packaging, and the type of egg carton. Water may form at different rates on a hydrophobic and

hydrophilic surface (e.g., oiled and non-oiled eggs). On hydrophilic surfaces, a thin liquid film forms on the high surface energy substrate and acts as a conduction barrier for heat transfer. On hydrophobic surfaces, condensation forms in droplets [9]. During the condensation of water vapor, heat is released, causing an increase in temperature at the point of condensation. If the measured surface temperature is equal to or lower than the determined dew point, condensation forms [8].

There is no standard method to measure condensation on eggs. There are some instruments available to measure dew formation such as lysimeters and wood moisture meters. Lysimeters are used to measure dew on soil and grass [10]. However, the drainage-like structure of the apparatus does not fit the requirements needed to collect condensate on a shell egg surface. A piece of equipment known as a pinless moisture meter is normally used to measure wood surface moisture, but could be applied to egg surfaces. This study is the first to examine the use of a pinless moisture meter to characterizing egg sweating. The objectives of this experiment were: 1) to compare three methods of quantifying condensate on sweated eggs and 2) to quantify moisture content on refrigerated shell eggs allowed to form condensation at two temperatures (22 ^{o}C and 32 ^{o}C).

MATERIALS AND METHODS

Experiment 1

The first portion of this study was conducted in the month of May in Auburn, Alabama, and compared three methods of quantifying moisture on eggs allowed to form condensation. An experimental unit was one egg. A completely randomized design was used with three treatments: (1) weighing an egg before and after maximum condensation formation, (2) weighing a paper towel with condensate wiped from an egg after maximum condensation formation, and (3) a

pinless moisture meter on eggs before and after maximum condensation formation. Three replications of the study were conducted over three days with fresh eggs each replication. Eggs were randomized to treatment within each replication, with 270 eggs total, and 30 eggs per treatment. Two hundred and seventy fresh nest run (63.26 ± 6.51 g), unwashed eggs were randomly selected from the Auburn University Poultry Farm, and stored for 24 h in plastic flats with one egg in each hole, stacked at 4 °C, 60% RH. Eggs with visible cracks were not selected. Immediately after eggs were taken from the refrigerator, eggs from treatments 1 and 2 were individually weighed on a scale (Mettler-Toledo-Thermo Fisher Scientific, Waltham, Mass). Pinless moisture meter readings were taken on the air cell end of the egg facing upwards on the treatment 3 eggs. Next, all eggs were returned to plastic flats and allowed to form condensation for 1 h. at 22 °C, 60% RH, which was previously determined as the time required for maximum condensation to form on eggs at 22 °C, 60% RH in a preliminary study, conducted in the month of May in Auburn, Alabama [11]. After 1 h., thirty of the eggs that were previously weighed were weighed again to determine condensation weight, lifting the egg by portions with minimal condensation to retain maximum moisture on the egg. Moisture was wiped from the surface of the next 30 eggs with pre-weighed paper towels. The wet paper towels were immediately re-weighed. Thirty of the eggs were measured with a pinless moisture meter in duplicate on the top air cell end of the egg [12]. This location was selected for its ease of access. After these measurements, all eggs were returned to a 4 °C refrigerator for 24 h. This series of readings was replicated in different orders (weight method first, then paper towel method, then moisture meter; or paper towel method, then moisture meter, then weight method, etc.) on different eggs randomly selected before being placed in flats three times over 3 d.

Experiment 2

The next portion of the study was conducted in the month of September in Auburn, Alabama, and involved exposing unwashed, refrigerated eggs (4 °C, 60% RH) to two different temperatures of 22 °C, 60% RH and 32 °C, 60% RH. A completely randomized design was utilized, with two treatments (22 °C and 32 °C), with 17 eggs per treatment, and three replications of the experiment, with fresh eggs each replication utilized [13]. One hundred and four unwashed fresh nest run (64.26 ± 6.56 g) eggs were randomly selected from the Auburn University Poultry Farm and stored for 24 h in plastic flats with one egg in each hole, stacked at 4 °C, 60% RH. Eggs with visible cracks were not selected. One egg at a time was removed from refrigeration and tared on a scale inside an incubator at a 32 °C, 60% RH, or a room temperature environment (22 °C, 60% RH). The weight of the egg was recorded every 3 min over the time period it took for the egg to form maximum condensation and then to completely dry (return to original weight).

Statistical Methods

Experiment 1 weight of condensation data from treatments 1 and 2 were converted from grams condensation to milliliters condensation formed (1 mL = 1 g). The density of water is approximately 1 g/mL at 22 °C and 32 °C. Egg surface area was calculated with the following equation:

Surface Area=3.9782(Weight)$^{.7056}$ [14]

Data from Treatment 3 were compiled as average percentage increase in moisture meter reading from the initial (dry) and final (wet) egg readings. A percent increase in egg weight due to condensation (Y) was calculated from treatment 1 and 2 data using the following equation:

$$Y = \frac{\frac{(mL\ moisture\ on\ wet\ egg)}{(egg\ surface\ area)} - \frac{(mL\ moisture\ on\ dry\ egg)}{(egg\ surface\ area)}}{\frac{(mL\ moisture\ on\ dry\ egg)}{(egg\ surface\ area)}} \text{ x } 100$$

Treatments 1, 2, and 3 were subsequently compared with ANOVA using proc glm of SAS [15] and means were separated with the Tukey method [16]. No treatment by replication interaction was found, therefore, the interaction was removed from the statistical model. Treatments 1 and 2 were compared using the Bland Altman procedure using SAS software [17]. Linear regression was utilized in SAS to generate a correlation coefficient between percent increase milliliters condensation formation/egg surface area (Treatment 1) and percent increase in moisture meter reading (Treatment 3) for each replication [18]**.** No interaction occurred between replications, and the three regression lines were combined into one.

The data from experiment 2 were converted to milliliters condensation formed/egg surface area using the previously stated equation. Due to the limitations in scale precision, data points less than a 0.0001% increase milliliters condensation/cm^2 were considered a "dry" egg. Data were analyzed by ANOVA using proc glm of SAS [15] with the main factors being temperature and treatment, and the repeated measure being time (min). There was a temperature by treatment interaction ($P < 0.0001$), therefore, each time point was analyzed for differences in egg surface condensation due to temperature using an independent sample t-test [19]. The maximum condensation amount formed for each treatment, the average time for the egg to reach maximum condensation, and the subsequent average time for the egg to completely dry were calculated. Independent sample t-tests were then used to compare the means [19]. The proc glm procedure using repeated measures ANOVA higher order trend analysis was performed on the 22 °C, 60% RH curve to determine the best fitting curve equation, thus explaining changes in egg condensation weight over time. This utilizes a regression approach by specifying higher-order effects in the MODEL statement [20]. Once the correct representative polynomial order

was determined, the proc glm procedure was applied to determine the curve equation [20]. This procedure was repeated with the 32 °C, 60% RH curve.

RESULTS AND DISCUSSION

The results of experiment 1, shown in Table 1, indicate that there was no difference in quantifying egg sweat by egg weight (0.2% increase from dry weight) or weight of moisture absorbed on a paper towel (0.19% increase from dry weight) ($P = 0.4$). The pinless moisture meter generated a different result (52% percentage increase in reading, $P < 0.001$). The Bland-Altman analysis, which plots the difference in the weight gain and the paper towel moisture absorption technique moisture weight readings against the average of the 2 technique readings, agrees that the first two methods are comparable, with the majority of the plotted points falling in the 95% confidence interval. There is no *P* value for this type of analysis, and a visual inspection is sufficient [17]. Weighing the egg before and after sweat is most likely the best method, since the paper towel method allows for the chance that some moisture can be missed when wiping off the egg. This is the first study to quantify moisture on eggs in these ways. Richards [21] used absorbent paper on grass by first pressing lightly to avoid run-off from the grass to the ground, and then harder to absorb the rest of the dew. The absorbent paper was then sealed in a plastic bag of a known weight, and the whole sample and container weighed, with the weight compared with the weight of dry paper. A problem with this approach is that not all condensation water is absorbed, making the measured amount an underestimate of the true amount, which, according to Monteith [22], can amount to as much as 50–100%.

The moisture meter is not a good measure of quantifying egg surface moisture. The correlation coefficient between percent increase milliliters condensation/cm^2 on eggs with condensation and percent moisture meter reading increase for the pooled replications was 0.06

(Figure 1). The regression line was not different from a slope of zero (P = 0.9860). A rise in moisture meter reading given from a known dry product indicates moisture. The moisture meter ranges in readings from 5 to 72, with 72 representing liquid water [23]. The readings on dry eggs ranged from 28 to 42. The readings on the wet eggs ranged from 40 to 65. The average percent increase in reading was 52.42%. A factor influencing the meter reading is surface temperature [24]. The meter measures the wettest portion of the material from the surface to its lowest penetration point, which is 20 mm into the material [25]. An egg shell is approximately 0.4 mm thick. Therefore, the moisture meter could have picked up moisture content underneath the egg shell**.** The meter works using electromagnetic wave technology, which measures the dielectric constant, or the measure of potential energy per unit stored in the material in the form of electric polarization in a 3 dimensional field underneath a measuring pad [24]. Most pinless meters are automatically calibrated to represent Douglas-fir wood. Based on these results, to design a capacitance-based moisture meter for an egg surface, the instrument power would need to be turned down or an additional polymer layer would need to be added to the instrument measurement tip. This would in effect lessen the depth of measurement, with the target depth 1 mm or less.

The results of experiment 2, shown in Figure 2, Table 2, and Table 3, show that the 22 °C temperature caused a slightly higher maximum condensation percent increase (0.0008 mL/cm^2) compared to the 32 °C temperature (0.0007 mL/cm^2). Temperature and treatment were main effects (P = 0.0009 and P < 0.0001). There is no previous research quantifying condensation on eggs in any environment, therefore, there is no suitable material to compare this result.

The linear regression equation of the 22 °C condensation formation and drying curve (Figure 2) was: mL/surface area= -0.0005 + 0.0005min - 0.00007min^2 + 0.000003min^3 –

$0.00000005min^4$. The mean square error term for the curve fitting model was 0.00000015. The linear regression equation of the 32 °C condensation formation and drying curve (Figure 2) was: mL/surface area= $-0.00017 + 0.0004min - 0.000063min^2 + 0.000003min^3 - 0.00000006min^4$. The mean square error term for the curve fitting model was 0.00000003. Since a time and temperature interaction was found ($P < 0.05$), times at which egg weight were recorded were analyzed individually for differences in condensation weight due to differences in temperature (Table 2). At 3 and 6 min, the 32 °C environment had a higher percent increase condensation weight/egg surface area (mL/cm^2) ($P = 0.0002$, $P = 0.0418$, respectively). At 9 min the two treatments were not different ($P > 0.05$). At 12, 15, 18, 21, and 24 min, the 22 °C environment had a higher percent increase of condensation amounts ($P < 0.05$). After 24 min, the percent increase in milliliters condensate/egg surface area was less than 0.0001 in the 32 °C environment, and the 22 °C environment eggs continued to have a higher percent increase condensate amounts until the eggs dried to 0.0001 mL condensate/cm^2 at 42 min ($P < 0.05$).

As shown in Table 3, it was found that there was a difference in the time it took to form maximum condensation on the eggs at 22 °C and 32 °C ($P < 0.0001$), with the eggs in the 32 °C environment forming maximum condensate faster than the 22 °C environment (11.08 min versus 17.16 min). The dew point of 32 °C, 60% RH air is 23 °C. The dew point of 22 °C, 60% RH air is 13 °C. Condensation occurs when the dew point temperature of the room air is higher than the temperature of the egg surface [8]. At 32 °C, a more dramatic temperature differential between the air and egg surface occurred more quickly, accounting for the faster condensation rate.

It was found that there was a difference in the time to return to original weight after maximum condensation formation on eggs in the warm and room temperature environments, with the room temperature eggs taking longer to dry (33.81 min at 32 °C versus 25.04 min at 22

°C) ($P < 0.0001$). Evaporation rate is impacted by the egg surface structure, surface area, the temperature of the environment and egg, and relative humidity.

Overall, there was a difference in the time required for desorption compared to the adsorption time in the hot and room temperature environments ($P < 0.0001$). As mentioned previously, the large difference between the cold egg temperature and hot air account for the rapid adsorption rate. Based on these results shown in Table 3, cold eggs that are exposed to room temperature could have condensation sitting on the surface for 51 min. In addition, eggs that have formed condensation while sitting in flats accumulate water underneath the surface, which sometimes is not able evaporate, which could have microbial implications.

CONCLUSIONS AND APPLICATIONS

1. Weighing an egg before and after condensation formation is a sound method to quantify moisture formation. A wood moisture meter is not a good substitute for this, but further calibration experiments could be conducted.
2. Eggs forming condensation at room temperature (22 °C, 60% RH) formed statistically more condensation than eggs at a 32 °C, 60% RH environment ($P < 0.05$).
3. Eggs taken from a cold environment to a 32 °C environment form condensation significantly faster than eggs placed in a 22 °C environment. Overall, the time required for desorption was higher than the adsorption time in both the 22 and 32 °C environments.
4. There was a difference between the 22 °C and 32 °C environments in the time required for the eggs to completely dry from the point of maximum condensation formation, with the eggs in the 32 °C environment drying faster. Eggs that are exposed to condensation-forming room conditions will likely have water sitting on the surface for a minimum of

51 min. In addition, eggs sitting in flats accumulate water underneath the eggs, which is unable to evaporate from underneath the egg surface, which could have microbial implications.

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TABLES AND GRAPHS

Table 1. Average percent increase in condensate on dry eggs allowed to form maximum condensation measured by a direct weight method, a condensation absorption method, and a moisture meter

Condensation quantification method	Percent increase	Dry egg moisture wt/surface area[2] (mL/cm^2) or moisture meter reading	Condensation wt/dry egg surface area (mL/cm^2) or moisture meter reading
Condensate weight dry/wet (mL/cm^2)	0.2%[B]	0[B]	0.002[B]
Condensation absorption paper towel (mL/cm^2)	0.19%[B]	0[B]	0.0019[B]
Moisture meter % reading increase dry/wet egg	52.42%[A]	34.92[A]	53.1[A]
Pooled SEM	723.6	272.42	634.64

[AB] means within a column with no common superscript differ significantly ($P < 0.001$, F=631.11, MSE=126.7). N=270, 3 replications, N=90 fresh eggs per replication, 30 eggs per treatment. Eggs removed from 4 °C, 60% RH environment immediately before condensation formation at 22 °C for 60 min.

[2]Egg Surface Area $cm^2=3.9782(Weight)^{.7056}$ [14]

Table 2. Percent increase in milliliters egg condensation/surface area[1] over time as egg formed condensation and dried at 22 °C, 60% or 32 °C, 60% RH[2]

Time (min)	% Increase in mL condensation/egg surface area at 22 °C, 60% RH	% Increase in mL condensation/egg surface area at 32 °C, 60% RH
0	0	0
3	0.00033^{B}	0.00042^{A}
6	0.00054^{B}	0.00062^{A}
9	0.00067^{A}	0.0007^{A}
12	0.00076^{A}	0.00061^{B}
15	0.0007^{A}	0.00049^{B}
18	0.00068^{A}	0.0004^{B}
21	0.00063^{A}	0.00022^{B}
24	0.00052^{A}	0.00013^{B}
27	0.00042^{A}	$<0.0001^{B}$
30	0.0003^{A}	$<0.0001^{B}$
33	0.00023^{A}	$<0.0001^{B}$
36	0.00017^{A}	$<0.0001^{B}$
39	0.00013^{A}	$<0.0001^{B}$
42	0.0001^{A}	$<0.0001^{B}$
45	$<0.0001^{A}$	$<0.0001^{A}$
48	$<0.0001^{A}$	$<0.0001^{A}$
51	$<0.0001^{A}$	$<0.0001^{A}$
54	$<0.0001^{A}$	$<0.0001^{A}$

^{AB}Denotes a significant difference of $P < 0.05$ between percent increase in mL condensation/egg surface area at 22 °C and 32 °C.

[1]Egg Surface Area $cm^2=3.9782(Weight)^{.7056}$ [14]

[2]N=51, 3 replications, N=17 fresh eggs per replication. Eggs removed from 4 °C environment immediately before condensation formation at 22 °C or 32 °C temperature, egg weight recorded every 3 min. Egg condensation weight converted to mL/dry egg surface area (cm^2). Independent sample t-tests performed at each time point comparing % increase mL condensation/surface area between 22 °C and 32 °C.

Table 3. Average maximum condensate/surface area on shell eggs at 2 temperatures, time for maximum condensate to form at 2 temperatures, time for condensate to evaporate on eggs allowed to form condensation at two temperatures

Environment conditions	Maximum condensation amount (mL/surface area[4] $cm^{2)}$	Time at maximum condensate (min)	Time to evaporate condensate (min)	P value, F value, MSE
Room temp (22 °C, 60% RH)	0.0008^{A}	17.16^{Ab}	33.81^{Aa}	$P < 0.0001$[1] F=42.75 MSE=53.29
Hot incubator (32 °C, 60% RH)	0.0007^{B}	11.08^{Bb}	25.04^{Ba}	$P < 0.0001$[1] F=24.04 MSE=20.4
Pooled SEM	0.00003	0.51	0.93	
P value, F value, MSE	$P = 0.0396$[2] F=4.35, MSE=0.0000001	$P < 0.0001$[2] F=56.48 MSE=16.19	$P < 0.0001$[2] F=28.68 MSE=65.04	

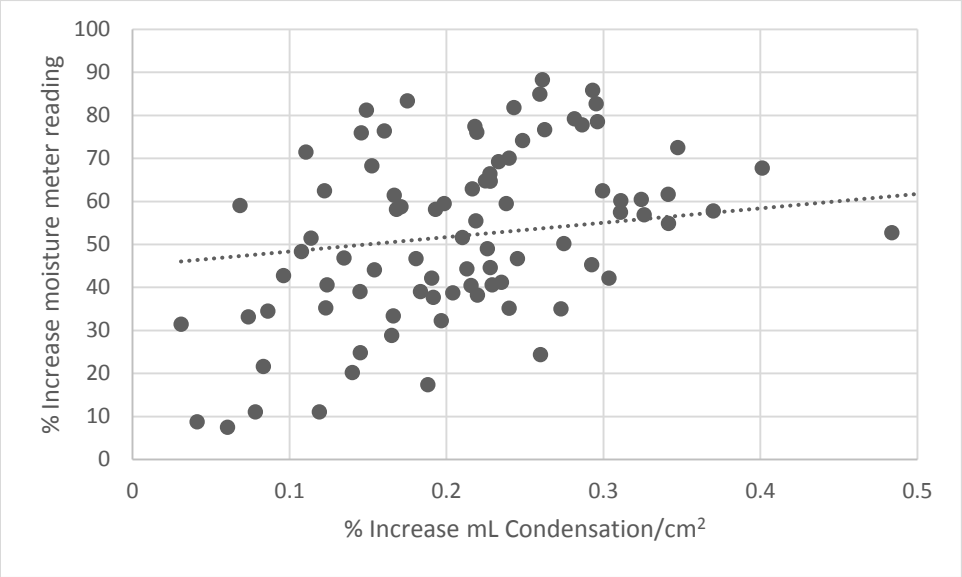
[1,ab]P values, F values, and MSE compare time at maximum condensate and time to evaporate condensate across a row.

[2,AB]P values, F values, MSE, and SEM compare maximum condensation amount, time at maximum condensate, and time to evaporate condensate across a column.

[3]N=51, 3 replications, N=17 fresh eggs per replication. Eggs removed from 4 °C immediately before condensation formation.

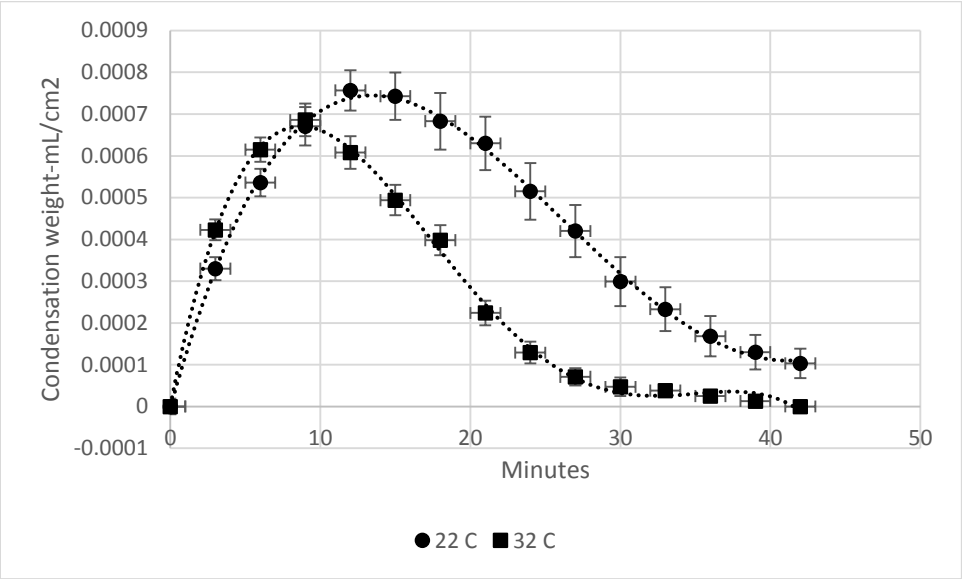
[4]Egg Surface Area $cm^2=3.9782(Weight)^{.7056}$ [14]

Figure 1. Average percent increase mL condensation formed/surface area on eggs allowed to form maximum condensation versus percent increase in moisture meter reading on dry and wet eggs[1]



[1]N=90, 3 replications, N=30 fresh eggs per replication. Eggs removed from 4 °C immediately before condensation formation for 60 min. *P* = 0.9860 that H_0=slope=0. Line equation: y=33.451x + 45.013. R^2=0.06

Figure 2. Average weight gain due to condensation in milliliters per surface area[1] egg on eggs allowed to form condensation at 22 °C, 60% RH and 32 °C, 60% RH[2]



[1]Egg Surface Area $cm^2=3.9782(Weight)^{.7056}$ [14]

[2]N=51, 3 replications, N=17 fresh eggs per replication. Eggs removed from 4 °C immediately before condensation formation.

IV. Assessing the impact of sweating on *Salmonella* Enteritidis penetration into shell eggs

Assessing the impact of egg sweating on *Salmonella* Enteritidis penetration into shell eggs

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ABSTRACT

Salmonella Enteritidis (SE) prevalence in eggs is a major concern to the egg industry. Some research has shown that egg sweating can increase *Salmonella* penetration into egg contents when refrigerated eggs are moved to a warmer temperature. This occurs when eggs are tempered before wash, to minimize thermal cracks from occurring. The objective of the study was to assess the effect of egg sweating on *S.* Enteritidis penetration into shell eggs over a six week storage period at 4 °C. A 2x2 factorial of *S.* Enteritidis inoculation and egg sweating was utilized. Treatments included (SES) nalidixic acid (NA)-resistant *S.* Enteritidis inoculated and sweated, (SENS) NA-resistant *S.* Enteritidis inoculated and not sweated, (NSES) buffered peptone water (BPW) inoculated and sweated, and (NSENS) BPW inoculated and not sweated. Inoculated eggs were exposed to 10^8 *S.* Enteritidis. Eggs formed condensation for approximately 17 min in a 32 ° C incubator. Shell rinse, shell emulsion, and egg contents were sampled then enumerated and assessed for prevalence of *S.* Enteritidis over a 6 wk storage period at 4 °C. After wk 1, the shell rinse from the *S.* Enteritidis inoculated/non-sweated treatment had higher *Salmonella* counts (0.32 $\log_{10}$ CFU/mL) than the other three treatments, where no *S.* Enteritidis was enumerated. A significant week by treatment interaction was found for the shell rinse *S.* Enteritidis detection ($P < 0.05$). In subsequent wks, no *S.* Enteritidis counts from any treatment were obtained from the egg shell rinse, shell emulsion, or egg contents. The SENS treatment shell rinses had significantly higher *S.* Enteritidis prevalence than the sweated and inoculated treatment (SES) in wks 1 (100% vs. 34.3%), 2 (57.6% vs. 22.2%), and 3 (38.2% vs. 11.1%) ($P < 0.05$). In samples from wks 4, 5, and 6, there was no difference in *S.* Enteritidis prevalence between the SES and SENS treatment. Egg sweating did not increase *S.* Enteritidis penetration into the shell emulsion across treatment or week ($P < 0.05$). The decreasing trend of *S.*

Enteritidis prevalence obtained over the six week period indicate that refrigeration is a very effective method to inhibit *Salmonella* growth. These results indicate that egg sweating occurring under common US egg handling practices is not harmful to egg safety.

Key words: Shell egg, egg safety, *Salmonella* Enteritidis, sweating, condensation

INTRODUCTION

Egg safety is of paramount importance in the egg industry, with the presence of *Salmonella* being of particular concern to consumers. *Salmonella* can be introduced into an egg through some 10,000 pores in the shell (Yamamoto, 1997) and entry could be facilitated by "egg sweating" or the formation of condensation on shell eggs, when they are moved from a cold to a warm environment with a minimum relative humidity. Egg sweating occurs at many points during processing and distribution in the egg industry when previously cooled eggs are set out to warm before wash, as well as before transportation.

In 2012, the FoodNet by the CDC identified 7,842 human illnesses of *Salmonella*. Among these, 1,239 illnesses were serotype Enteritidis (CDC, 2014) with 29.3% of patients hospitalized. Of 10,319 outbreak-related illnesses caused by a single confirmed etiologic agent in 2012, *Salmonella* resulted in the most outbreak-related hospitalizations (449, 64%) (CDC, 2012). Sixty-eight percent of *Salmonella* Enteritidis cases are associated with eggs or egg products (WHO, 2001). While various serotypes have been isolated from egg shells, *Salmonella* Enteritidis has been isolated primarily from egg contents (Saeed, 1998). Models have estimated that *S.* Enteritidis contamination in US-produced shell eggs is 1 in 20,000, or 0.005% (Ebel et al., 2000). Salmonella has been estimated to be the number one cause of bacterial foodborne illness in the United States (Scallan et al., 2011). USDA risk assessment data suggests that cooling eggs to 7° C or below within 12 h of lay would reduce food-borne illness in eggs by 78% (FSIS,

2005). Czarick and Savage (1992) and Anderson et al. (1992) reported that eggs cased in cardboard cases required almost one week to cool from 27 °C to 7 °C. Cooling is delayed if eggs are packaged immediately after processing.

Previous research to exploring if egg sweating promotes *Salmonella* penetration into egg contents is scant and contradictory. Ernst et al. (1998) assessed both intact and cracked shell eggs and concluded that sweating did not increase *S.* Enteritidis numbers in the intact eggs tested. However, in contrast, Fromm and Margolf (1958) observed *Salmonella* penetrated more frequently in both washed and feces-contaminated eggs that were allowed to sweat for 3 and 5 h. De Reu et al. (2006) allowed condensation to form on agar-filled eggs for 30 min, while other eggs remained in storage at 20 °C. The frequency of *Salmonella* egg shell penetration was observed in the agar-filled eggs when condensation was allowed to form, although this effect was not observed using intact shell eggs.

The FDA Egg Rule, which went into effect in 2010, allows previously refrigerated nest run or eggs from off-line production facilities to be tempered at processing room temperatures before wash for up to 36 h (FDA, 2009). During this warming period, if the relative humidity (RH) is at a required minimum level, condensation will form on the eggs. However, at this stage the contents of the eggs are in an expansion mode and this creates a positive pressure in the egg which does not allow for movement across the surface to the contents. The bigger issue is when eggs are allowed to condense moisture on the surface and are then moved from a warm to a cold environment: this creates a negative pressure, which permits unevaporated moisture and bacteria on the surface to potentially translocate into the egg contents (Bruce and Drysdale, 1994). This has been demonstrated with a mechanical vacuum that simulated a negative pressure, resulting in bacterial penetration of a partial egg shell (Haines and Moran, 1940). According to Braun et al.

(1999), the level of *S.* Enteritidis penetration into the egg contents increases with temperature and relative humidity. This situation caused by egg sweating is widely believed to be a cause of bacterial penetration (Fromm and Margolf, 1958). The instances when eggs might sweat include while sitting on belts before being collected for in-line processing, when being tempered before wash, and before or after cold truck transportation if the ambient temperature and RH permit. If eggs sweat at different time points during egg processing, unwanted *Salmonella* penetration into egg contents may occur.

The objective of this study was to assess the effect of egg sweating on *Salmonella* Enteritidis penetration into shell eggs stored over a six week period. Eggs were exposed to maximum condensation that mimicked industry conditions. Published data by the authors quantified the maximum amount of condensation that formed on sweated eggs (Gradl et al., 2016). The goal of the current study was to assess whether the occurrence of egg sweating is harmful to egg safety.

MATERIALS AND METHODS

A completely randomized block design study was conducted with a total of 1,100 refrigerated fresh unwashed eggs from an average of 65 wk old hens from a commercial eggplant. Eggs were randomly divided into 4 treatment groups with 84 eggs/trt. Three replications of the study were conducted simultaneously with 336 eggs/rep. One egg was considered an experimental unit. Eggs were stored at 3-4 °C in pulp paper flats overnight in the available cold room. Treatments included (SES) NA-resistant *Salmonella* Enteritidis inoculated and sweated, (SENS) NA-resistant *S.* Enteritidis inoculated and not sweated, (NSES) buffered peptone water (BPW) inoculated and sweated, and (NSENS) BPW inoculated and non-sweated.

A 200 ppm nalidixic acid-resistant *Salmonella* Enteritidis suspension was utilized**.** An inoculum for each of 3 the replications was prepared with concentrations ranging from 1.2 – 9.45 x 10^8 CFU/mL (Table 1). Turbidity of the inoculum was determined with a spectrophotometer set at 660 nm (Thermo Electron Corporation Spectronic 20D+, Rochester, NY) with approximate concentration calculated from known standard curves**.** A sample of the inoculum was removed aseptically and plated in duplicate at various dilutions on 200 ppm brilliant green sulfur-nalidixic acid (BGS-NA) to confirm concentration. Eggs were allowed to equilibrate at room temperature overnight in pulp paper flats. Inoculums and BPW controls were taken from a 37 °C incubator before inoculation. Eggs from corresponding replications were inoculated with 25 µL 10^8 CFU/mL 200 ppm nalidixic acid resistant *S.* Enteritidis in buffered peptone water (BPW) on the side of an egg in a horizontal position in an egg. The inoculum was spread over the egg with a sterile loop into an approximately 2.5 cm diameter oval shape. Control treatment eggs were spread with 25 µl BPW using the same procedure described previously. Eggs were allowed to completely dry before being placed air cell side up in plastic flats and returned to a 3-4 °C cold room overnight.

The next day, eggs in the sweated treatment were allowed to form condensation in a 32 °C, approximately 60% relative humidity (RH) incubator for 17 min. Twelve min was previously determined to be the time required for eggs to form maximum condensate (an approximate 0.0007% weight gain (mL)/egg surface area (cm^2) in moisture) (Gradl et al., 2016). The total time the eggs were in the incubator (RH 40-75%) was 77 min, because the large number of cold eggs put into the incubator at once required the humidity level to be adjusted with additional sterile water to allow the eggs to sweat**.** When the cold eggs were placed in the incubator, the action of opening the incubator door caused the RH to drop to an average of 40% for about 60

min. When the RH did not increase, sterile water was sprayed inside the incubator, which raised the RH to 75% for the remaining 17 min. Week 0 samples were taken immediately after sweat. The remaining eggs were stored while still wet in plastic flats in stacks of three in half-case cardboard boxes in a 3-4 °C cold room. Samples were taken weekly in the consecutive weeks 1 through 6.

The egg shell surface was sampled by the shell rinse method by Jones et al. (2002), using 10 mL of 42 °C sterile phosphate buffered saline (PBS). Shell rinse contents were diluted as necessary and 0.1 mL was plated in duplicate on (BGS-NA) plates and incubated for 37 °C for 24 h before enumeration. Remaining shell rinse contents were pre-enriched with 1 mL sterile 10X buffered peptone water (BPW) at 37 °C for 24 h. Rappaport Vassiliadis broth (RV) was inoculated with 0.1 mL of the pre-enriched egg shell rinse and incubated at 42 °C for 24 h. A loopful of the enriched samples was streaked onto BGS-NA and incubated at 37 °C for 24 h. A positive or negative result was recorded.

Eggs were dipped, twelve at a time on plastic flats, in 70% ethanol and allow to air dry. The shell matrix and membrane contamination was determined by the shell crush method of Musgrove et al. (2005). A single egg was cracked on a sterile beaker. Remaining adhering albumen was rinsed out of the egg shell with sterile 42 °C phosphate buffered saline (PBS). The shell and membranes from a single egg were placed in a sterile conical 50-mL centrifuge tube with 10 mL 42 °C sterile PBS and macerated for 1 min with a sterile glass rod. Egg shell emulsions were diluted as necessary and 0.1 mL was plated in duplicate on BGS-NA plates and incubated for 37 °C for 24 h before enumeration. Remaining egg shell emulsions were pre-enriched with 1 mL sterile 10X BPW at 37 °C for 24 h and previously described *Salmonella* prevalence procedures were followed.

The contents from each egg were placed in a 7 oz. sterile sampling bag and then stomached for 1 min at high speed. A 0.25 mL aliquot of contents was duplicate plated BGS-NA plates and incubated for 37 °C for 24 h before enumeration. Remaining egg contents were pre-enriched with 5 mL sterile 10X BPW at 37 °C for 24 h and previously described *Salmonella* prevalence procedures were followed.

Statistical methods

A 2x2 factorial completely randomized design was utilized. Enumeration data was analyzed using the general linear model procedure of SAS (SAS, version 9.4, SAS Institute Inc., Cary, NC). The *S.* Enteritidis inoculum concentrations for each replication were treated as covariates. *S.* Enteritidis counts were transformed $\log_{10}$. A two-way repeated measures ANOVA was conducted that examined the effect of egg sweating and *S.* Enteritidis inoculation on *S.* Enteritidis enumeration counts over time. The main effects were inoculation and storage time. Least squares means were presented and compared using the LSMEANS/PDIFF option when interaction effect was significant ($P < 0.05$).

Data for the enrichment aspect of the study was dichotomized as negative or positive for *S.* Enteritidis presence. The egg content *Salmonella* presence was not analyzed, since there were no positive results. Due to the small number of eggs per treatment, multiple Fisher tests were conducted using the FREQ command of SAS for each replication comparing the treatment results across a single week ($P \leq 0.05$). Multiple Fisher tests were conducted comparing week results across a treatment ($P \leq 0.05$) Replication results across a single treatment that were statistically similar were combined. Results are reported as percent positive prevalence.

RESULTS AND DISCUSSION

Table 2 presents the *S.* Enteritidis shell rinse enumeration data from Table 3 as a factorial arrangement of treatments (sweated, *S.* Enteritidis inoculated). From the shell rinses, the main effects were inoculation ($P = 0.0013$) and storage time ($P < 0.0001$). The error term was defined as inoculation*sweating*replication. Only the egg shell rinse counts were analyzed with this method. No *S.* Enteritidis counts were detected from the egg shell emulsion or contents for the duration of the study. The effect of inoculation on *S.* Enteritidis recovery over the course of 7 wks was highly significant ($P < 0.01$). The effect of sweating on *S.* Enteritidis recovery over 7 wks was not significant ($P > 0.05$). Replications (R) were significant at wk 0 and over the entire 7 wks ($P < 0.05$). The sweating x replication (S X R) interaction was not significant at wk 0, wk 1, or over the 7 wks ($P > 0.05$). The inoculation x replication (I x R) interaction was significant at wk 0 ($P < 0.05$), and not significant at wk 1, or over the 7 wks ($P > 0.05$). The main effect for shell rinse *S.* Enteritidis recovery was *S.* Enteritidis inoculation ($P < 0.01$) at week 0. *S.* Enteritidis inoculation was not a main effect during wk 1. Whether an egg was inoculated (yes) or not (no) was a significant effect ($P < 0.01$) during wks 0 and 1. Week (storage time) (W) was a main effect ($P < 0.01$). The interaction between week, replication, and *S.* Enteritidis inoculation was also significant ($P < 0.01$).

The *S.* Enteritidis recovery counts at wk 0, wk 1, and over the entire 7 wk study, categorized by sweated and *S.* Enteritidis inoculated, and summarized over 3 replications, are shown in Table 3. Shell rinse recovery counts of *S.* Enteritidis (SE) in wk zero resulted in the SENS treatment having higher *S.* Enteritidis counts than the SES treatment (3.63 $\log_{10}$ CFU/mL and 2.05 $\log_{10}$ CFU/mL). A higher inoculum concentration (Table 1) resulted in a trend of less SE being recovered from the shell rinse in the SES treatment, but not the SENS treatment, where no trend was detected. No growth was detected from the BPW inoculated treatments (NSES and

NSENS) in wk 0. In week 1, the shell rinse SENS treatment had higher *Salmonella* counts (0.32 $\log_{10}$ CFU/mL) than the other three treatments, where no *S.* Enteritidis was enumerated. After week 1, no *Salmonella* counts were obtained from the egg shell rinse, shell emulsion, or egg contents. On the egg shell rinse, the SES and SENS treatments had higher *S.* Enteritidis counts during week zero (3.63 $\log_{10}$ CFU/mL and 2.05 $\log_{10}$ CFU/mL) compared to week 1, where 0.32 $\log_{10}$ CFU/mL was enumerated from the SENS treatment (Table 3). No *S.* Enteritidis was detected on the shell rinses during wks 2-6.

The shell rinse, shell emulsion, and egg content *S.* Enteritidis-NA prevalence are shown in Tables 4 and 5. Eggs that were cracked before sampling were not included in the data analysis. In week 0, all of the *Salmonella* inoculated shell rinses (SES and SENS) were positive for *Salmonella*, with the NSES and NSENS shells having significantly fewer positive results ($P <$ 0.0001). The positive *S.* Enteritidis prevalence of the control treatment (NSES and NSENS), 6/36 (16.7%) and 1/36 (2.8%) shells were due to contamination. Contamination could have been due to improper aseptic technique when switching between sampling from the *S.* Enteritidis inoculated eggs to the BPW inoculated eggs. Other causes could have been the NSES treatment becoming contaminated during the sweating process, or previously existing contamination on the unwashed eggs. During week 1, the shell rinse SENS treatment had significantly higher *Salmonella* prevalence (100%) than the SES (34.3%) ($P <$ 0.0001), NSES (0%) and NSENS (0%) treatments ($P <$ 0.0001). During week two, the shell rinse SENS treatment had significantly higher *S.* Enteritidis prevalence (57.6%) compared to the SES treatment (22.2%) ($P =$ 0.0034), the NSES treatment (0%), and NSENS treatment (3.1%) ($P <$ 0.0001). Again, the contamination in the NSENS treatment was most likely due to improper aseptic technique when sampling. In week 3, the shell rinse SENS treatment had significantly higher *S.* Enteritidis prevalence (38.2%)

compared to the SES treatment (11.1%) ($P = 0.0117$) and the NSES and NSENS treatments (0%) ($P < 0.0001$). During week four, none of the shell rinse treatments were significantly different in *S.* Enteritidis prevalence, with the only positive *S.* Enteritidis result on the SENS treatment (5.9%), while the rest of the treatments were negative ($P > 0.05$). In week five, none of the shell rinse treatments were significantly different in *S.* Enteritidis prevalence, with the NSENS treatment positive for *S.* Enteritidis (2.9%), while the rest of the treatments were negative ($P > 0.05$). In week six, none of the shell rinse treatments were significantly different in *S.* Enteritidis prevalence, with the SES treatment 5.55% *S.* Enteritidis positive, the SENS treatment was 2.8% *S.* Enteritidis positive, and the NSES and NSENS treatments were negative ($P > 0.05$).

Week 0 had significantly higher prevalence of *S.* Enteritidis compared to week 1 (100% prevalence compared to 34.3%) on the shell rinses from the SES treatment ($P < 0.001$). Week 1 was not significantly different in *S.* Enteritidis prevalence than week 2 (34% and 22.2%) on the shell rinses from the SES treatment ($P = 0.3$). Week 2 was not significantly different in *S.* Enteritidis prevalence on the shell rinses during week 3 (22.2% and 11.1%) ($P = 0.34$). Weeks 3, 4, 5, and 6 were not significantly different from each other in *S.* Enteritidis prevalence on the shell rinses (11.1%, 0%, 0%, and 5.5%, respectively) ($P > 0.05$).

From the shell rinse *Salmonella* prevalence of the SENS treatment, weeks 0 and 1 were not significantly different (100% and 100%) ($P > 0.05$). Weeks 1 and 2 were significantly different in *S.* Enteritidis prevalence on the shell rinses (100% and 57.6%) ($P < 0.0001$). Weeks 2 and 3 were not significantly different in *S.* Enteritidis prevalence on the shell rinses (57.6% and 38.2%) ($P = 0.1449$). Weeks 3 and 4 were significantly different in *S.* Enteritidis prevalence on the shell rinses (38.2% and 5.9%) ($P = 0.0026$). Weeks 4, 5, and 6 were not significantly

different from each other in *S.* Enteritidis prevalence on the shell rinses (5.9%, 2.9%, 2.8%) ($P > 0.05$).

From the shell rinse *Salmonella* prevalence of the NSES and NSENS treatments, none of the prevalence values were significantly different across weeks 0-6 with the exception of the contamination issues mentioned previously ($P > 0.05$).

There were no significant differences in shell emulsion *Salmonella* prevalence across treatments or weeks ($P > 0.05$). A single positive shell emulsion *S.* Enteritidis result occurred in the SES treatment during week 0. One positive shell emulsion from the SENS treatment occurred in weeks 0, 1, and 5. No *S.* Enteritidis was enriched from the egg contents throughout the duration of the study (Table 2).

These results suggest that sweating eggs does not increase *Salmonella* Enteritidis prevalence in the egg contents over time. However, these results disagree with Fromm and Margolf (1958), who determined the contents of inoculated eggs which were permitted to sweat, especially the "dirty" eggs were contaminated. The results of the current study agree with Ernst et al. (1998), who inoculated eggs with *Salmonella* via immersion and found sweating did not increase *Salmonella* penetration in intact eggs. However, the study by Ernst et al. (1998) and others differed from Fromm and Margolf (1958) in that both dirty washed and unwashed eggs were dried after sweating before being returned to storage and sampled the next day. In the current study, inoculated eggs were sampled the same day they were sweated (wk 0). These differences in results could also be due to the change in chicken egg genetics between 1958 and present day (Anderson et al., 2013).

The fact that the sweated eggs were in the incubator for 77 min could have possibly impacted the results of the study. The large number of eggs placed in the incubator at once

caused the humidity levels to drop, which made manual adjustment of the RH necessary. Previous experiments by the authors have shown that a single egg takes approximately 12 min to form maximum condensation at 32 °C, 60% RH (Gradl et al., 2016). Ideally, multiple treatments with different time, temperature, and relative humidity combinations would be tested to overcome this. Given the presented data, however, it can be concluded that sweating eggs does not increase *S.* Enteritidis penetration into shell eggs. *Salmonella* penetration into the egg membrane may occur with increased storage time, but the addition of sweating as a treatment did not impact this. Sweating did not impact the shell rinse prevalence of inoculated *Salmonella* during weeks 0, 1, 2 and 3 of the study. It is possible that the refrigerated storage conditions resulted in *Salmonella* cells to become injured or slow in growth. It has previously been shown that dry egg shells (45% RH) in refrigerated conditions do not provide a good growth environment for *Salmonella* (Messens et al., 2005). It should be noted that *S.* Enteritidis might survive for up to four weeks on shells contaminated with feces, from which the pathogen can obtain its required nutrients (Schoeni et al., 1995; Braun et al., 1999; Little et al., 2007); however, this growth pattern was not the case with the current findings, as some of the eggs in our study had some adhering fecal matter. The current findings suggest that egg sweating is not a significant risk factor in promoting *Salmonella* penetration into shell eggs. Further research should be conducted with more temperature, humidity, and time sweating combinations to more completely assess the problem.

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TABLES

Table 1. Average nalidixic-acid resistant *Salmonella* Enteritidis egg shell inoculum concentrations per replication in CFU/mL and $\log_{10}$ CFU/mL[1]

Inoculum 1	Inoculum 2	Inoculum 3	Average Concentration
9.45×10^8 (8.98)	1.2×10^8 (8.08)	8.85×10^8 (8.94)	6.5×10^8 (8.81)

[1]A 200 ppm nalidixic acid-resistant *Salmonella* Enteritidis suspension was created. Turbidity of the inoculum was spectrophotometrically determined**.** Concentration was confirmed by plating on 200 ppm BGS-NA agar. Inoculated eggs received 25 µL of an average 10^8 CFU/mL nalidixic acid resistant *Salmonella* Enteritidis.

Table 2. Analysis of variance of sweated, nalidixic-acid resistant *Salmonella* Enteritidis inoculated shell egg rinses over individual weeks and entire experiment[1]

		7 week model	Week 0	Week 1
Source	df	MS	MS	MS
Total		816	143	143
Sweated(S)	1	0.2	0.27	0.02
SE Inoculated (I)	1	0.72**	4.44**	0.02
yes	1		36.15**	1.86**
no	1		$5.1x10^{-28}$	0
Replication (R)	1	0.28*	1.73*	0.009
SxI	1	0.02	0.27	0.02
IxR	1	0.284	1.73*	0.02
SxR	1	0.057	0.53	0.009
SxIxR (Error)	3	0.057	0.93	0.009
Week (W)	6	0.622**		
WxRxS	6	0.08		
WxRxI	6	0.24**		
WxRxIxS	18	0.13**		

*$P < 0.05$
**$P < 0.01$

[1]Shell rinse, shell emulsion, and egg content samples taken weekly. Sweated eggs formed condensation for approx. 17 min in a 32 °C incubator. Inoculated eggs received 25 µL of an

average 10^8 CFU/mL nalidixic acid resistant *Salmonella* Enteritidis. Uninoculated eggs were spread with 25 µl BPW.

Table 3. Average nalidixic-acid resistant *Salmonella* Enteritidis counts in $\log_{10}$ CFU/mL on *S.* Enteritidis inoculated, sweated egg shell rinses over a six week period[1]

Week	Sweated+ Inoculated (SES)	Non-sweated+ Inoculated (SENS)	Sweated+ Uninoculated (NSES)	Non-sweated+ Uninoculated (NSENS)
0	2.05±0.201	3.46±0.06	ND[2]	ND
1	ND	0.32±0.08	ND	ND
2	ND	ND	ND	ND
3	ND	ND	ND	ND
4	ND	ND	ND	ND
5	ND	ND	ND	ND
6	ND	ND	ND	ND

[1] Shell rinse, shell emulsion, and egg content samples taken weekly. Sweated eggs formed condensation for approx. 17 min in a 32 °C incubator. Inoculated eggs received 25 µL of an average 10^8 CFU/mL nalidixic acid resistant *Salmonella* Enteritidis. Uninoculated eggs were spread with 25 µl BPW.

[2]ND=None detected

Table 4. Number of *Salmonella* Enteritidis inoculated, sweated, enriched egg shell rinses positive for *Salmonella* Enteritidis over a six week period[1]

Week	Sweated+ Inoculated (SES)			Non-sweated+ Inoculated (SENS)			Sweated+ Uninoculated (NSES)			Non-sweated+ Uninoculated (NSENS)		
Replication	1	2	3	1	2	3	1	2	3	1	2	3
0	$36/36^{Aa}$			$36/36^{Aa}$			$6/36^{Ba}$			$1/36^{Ca}$		
0*	$12/12^{A}$	$12/12^{A}$	$12/12^{A}$	$12/12^{A}$	$12/12^{A}$	$12/12^{A}$	$0/12^{B}$	$2/12^{B}$	$4/12^{B}$	$0/12^{B}$	$1/12^{B}$	$0/12^{B}$
1	$12/35^{Bb}$			$35/35^{Aa}$			$0/35^{Cb}$			$0/35^{Ca}$		
1*	$6/11^{B}$	$4/12^{B}$	$2/12^{B}$	$11/11^{A}$	$12/12^{A}$	$12/12^{A}$	$0/11^{C}$	$0/12^{B}$	$0/12^{B}$	$0/12^{C}$	$0/11^{B}$	$0/12^{B}$
2	$8/36^{Bbc}$			$19/33^{Ab}$			$0/35^{Cb}$			$1/32^{Ca}$		
2*	$4/12^{A}$	$4/12^{A}$	$0/12^{B}$	$7/12^{A}$	$7/12^{A}$	$5/9^{A}$	$0/12^{B}$	$0/11^{B}$	$0/12^{B}$	$0/9^{B}$	$1/12^{B}$	$0/11^{B}$
3	$4/36^{Bbc}$			$13/34^{Ab}$			$0/35^{Bb}$			$0/36^{Ba}$		
3*	$3/12^{AB}$	$0/12^{A}$	$1/12^{AB}$	$6/12^{A}$	$3/12^{A}$	$4/10^{A}$	$0/12^{B}$	$0/12^{A}$	$0/11^{B}$	$0/12^{B}$	$0/12^{A}$	$0/12^{B}$
4	$0/32^{Ac}$			$2/34^{Ac}$			$0/34^{Ab}$			$0/36^{Aa}$		
5	$0/35^{Ac}$			$1/35^{Ac}$			$0/35^{Ab}$			$0/35^{Aa}$		
6	$2/36^{Ac}$			$1/36^{Ac}$			$0/33^{Ab}$			$0/33^{Aa}$		

[A,B] Values in rows that share no common upper-case superscripts are significantly ($P < 0.05$) different.

[a,b] Values in columns that share no common lower-case superscripts are significantly ($P < 0.05$) different.

*Denotes values from row above separated into three replications. [AB] Means separated within the same replication across a single row (i. e. replication 1 treatment means compared across replication 1 only)

[1]Shell rinse samples taken weekly. Sweated eggs formed condensation for approx. 17 min in a 32 °C incubator. Uninoculated eggs were spread with 25 µl BPW. Inoculated eggs received 25 µL of an average 10^8 CFU/mL nalidixic acid resistant *Salmonella* Enteritidis

Table 5. Number of *Salmonella* Enteritidis inoculated, sweated enriched shell emulsions and egg contents positive for *Salmonella* Enteritidis over a six week period[1]

	Shell Emulsion				Contents			
Week	Sweated+ Inoculated (SES)	Non-sweated+ Inoculated (SENS)	Sweated+ Uninoculated (NSES)	Non-sweated+ Uninoculated (NSENS)	Sweated+ Inoculated (SES)	Non-sweated+ Inoculated (SENS)	Sweated+ Uninoculated (NSES)	Non-sweated+ Uninoculated (NSENS)
0	2/36^{Aa}	1/36^{a}	0/36^{a}	0/34^{a}	0/35^{Aa}	0/36^{a}	0/36^{a}	0/36^{a}
1	0/33^{A}	1/35	0/35	0/36	0/33^{A}	0/35	0/35	0/36
2	0/36^{A}	0/33	0/35	0/32	0/36^{A}	0/33	0/35	0/32
3	0/36^{A}	0/34	0/35	0/36	0/36^{A}	0/34	0/35	0/36
4	0/32^{A}	0/34	0/33	0/36	0/32^{A}	0/34	0/33	0/36
5	0/35^{A}	1/32	0/32	0/35	0/35^{A}	0/32	0/32	0/35
6	0/36^{A}	0/36	0/32	0/33	0/36^{A}	0/36	0/32	0/33

^{A,B} Values in rows that share no common upper-case superscripts are significantly ($P < 0.05$) different.

^{a,b} Values in columns that share no common lower-case superscripts are significantly ($P < 0.05$) different.

[1]Shell emulsion and egg content samples taken weekly. Sweated eggs formed condensation for approx. 17 min in a 32 °C incubator. Uninoculated eggs were spread with 25 µl BPW. Inoculated eggs received 25 µL of an average 10^8 CFU/mL nalidixic acid resistant *Salmonella* Enteritidis.

V. SUMMARY AND CONCLUSIONS

The first objective was to design and perform an experiment assessing if egg sweating increased *Salmonella* Enteritidis penetration into shell eggs, three methods of quantifying condensate on sweated eggs were compared. The second objective was to quantify condensate on refrigerated shell eggs at two temperatures (22 ^{o}C and 32 ^{o}C). For objective 1, 270 fresh shell eggs were stored at 4 ^{o}C, 60% relative humidity (RH), then placed at 22 ^{o}C, 60% RH for 1 h. After this time, 30 pre-weighed eggs were randomly selected and weighed. Additional eggs were thoroughly wiped with pre-weighed paper towels to collect condensate. A pinless moisture meter was evaluated for quantifying egg condensate, and was found to be an ineffective method. There was no difference in quantifying egg condensation by egg weight or weight of moisture absorbed on a paper towel (0.2% vs. 0.19% percentage gain mL condensation/egg surface area) ($P > 0.05$). For objective 2, 104 fresh eggs formed condensation at two temperatures (22 ^{o}C and 32 ^{o}C, 60% RH). Each egg weight was continuously recorded from the beginning of condensation formation to the point where the egg reached a constant weight. There was a difference found in the time it took for an egg to reach maximum condensation (11 min at 32 °C, 17 min at 22 °C), as well as completely dry (25 min at 32 °C, 34 min at 22 °C) between the two temperatures ($P < 0.05$). Eggs that are exposed to condensation-forming room conditions will likely have water sitting on the surface for a minimum of 51 min In addition, eggs sitting in flats accumulate water underneath the eggs, which is unable to evaporate from underneath the egg surface, which could have microbial implications. The third objective was to assess the effect of egg sweating on *S.* Enteritidis penetration into shell eggs over a six week storage period at 4 °C. A 2x2 factorial of *S.* Enteritidis inoculation and egg sweating was utilized. Treatments included (SES) nalidixic

acid (NA)-resistant *Salmonella* Enteritidis inoculated and sweated, (SENS) NA-resistant *S.* Enteritidis inoculated and not sweated, (NSES) buffered peptone water (BPW) inoculated and sweated, and (NSENS) BPW inoculated and not sweated. Inoculated eggs were exposed to 10^8 *S.* Enteritidis. Eggs formed condensation for approximately 17 min in a 32 ° C incubator. Shell rinse, shell emulsion, and egg contents were sampled then enumerated and assessed for prevalence of *S.* Enteritidis over a 6 wk storage period at of 4 °C. After wk 1, the shell rinse from the *S.* Enteritidis inoculated/non-sweated treatment had higher *Salmonella* counts (0.32 $\log_{10}$ CFU/mL) than the other three treatments, where no *S.* Enteritidis was enumerated. A significant week by treatment interaction was found for the shell rinse *S.* Enteritidis detection ($P < 0.05$). In subsequent wks, no *S.* Enteritidis counts from any treatment were obtained from the egg shell rinse, shell emulsion, or egg contents. The SENS treatment shell rinses had significantly higher *S.* Enteritidis prevalence than the sweated and inoculated treatment (SES) in wks 1 (100% vs. 34.3%), 2 (57.6% vs. 22.2%), and 3 (38.2% vs. 11.1%) ($P < 0.05$). In samples from wks 4, 5, and 6, there was no difference in *S.* Enteritidis prevalence between the SES and SENS treatment. Egg sweating did not increase *S.* Enteritidis penetration into the shell emulsion across treatment or week ($P < 0.05$). The decreasing trend of *S.* Enteritidis prevalence obtained over the six week period indicate that refrigeration is a very effective method to inhibit *Salmonella* growth. These results indicate that egg sweating occurring under common US egg handling practices is not harmful to egg safety. Since the FDA Egg Rule (FDA, 2009) allows eggs that were previously refrigerated to remain for up to 36 h at room temperature conditions, further experiments might be conducted testing eggs left at room temperature for varying periods of time. The current

experiment only had one sweat treatment. Other temperature and relative humidity combinations might also be assessed.

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APPENDIX

Extended Discussion: Assessing the impact of sweating on *Salmonella* Enteritidis penetration into shell eggs

There are several instances when eggs could form condensation on the surface in an egg processing environment. The first could occur when eggs are taken from a 7 °C environment to a room temperature environment (22 °C) to temper before wash. The next possible instance is when eggs stored at 7 °C are placed on refrigerated trucks for transportation. Personal communication with Brian Joyer of Sparboe Farms, Litchfield, Minnesota, confirmed this. The final instance is when the eggs from the refrigerated trucks are loaded onto retail store docks. The severity of egg condensation is related to temperature, relative humidity, how controlled an environment is, the number of eggs, and air circulation. It is important to point out that the eggs in this experiment were exposed to a 32 °C, 60% RH environment in a scientific incubator, which is much smaller and controlled than the environments where eggs may sweat at an egg processing plant. In addition, the distribution of eggs in the incubator on shelves is subject to unequal heat distrubution throughout the incubator. After eggs are processed, they are placed in cartons or flats, placed into cases, and then palletized. With 30 dozen eggs per cardboard case, this slows down the rate of egg temperature change. The eggs in the center case of a pallet can take 7-10 d to reach 7 °C (Anderson et al., 1992). If forced-air cooling techniques were utilized with eggs packed in cartons with view windows or fiber trays, cooling to 7.2 °C would be achieved in 2-4 hours (Thompson and Knutson, 2000). A sudden environmental temperature increase when the eggs are loaded into refrigerated trucks will cause condensation on the egg surfaces. Damron et al. (1994) found that eggs will not continue to cool in refrigerated trucks. A

study by Anderson et al. (2008) found that in the summer, the average ambient refrigerated truck temperatures was 8.5 °C, the average egg temperature was 16.9 °C, and average case temperature was 16.6 °C. This illistrates the insulative power of packaging, however, Bell (2002) states that if eggs are moved from refrigerated conditions to 16 °C, sweating will occur if the relative humidity is 82% or higher.

Condensation will occur on the surface of the egg as long as the surface temperature is lower than the dew point temperature of the surrounding air. Condensation occurs on cold eggs because warm air can hold more moisture than cold air. The surface temperature, heat and mass transfer coefficients of an individual egg may vary on a single egg surface and from egg to egg (Linke and Geyer, 2013). Therefore, both condensation formation and atmospheric evaporation could simultaneously occur. In our experiment, approximately 500 eggs were taken from 4 °C and placed in a 32 °C incubator. Uniform increase in egg temperature depends on a uniform level of air flow around the eggs. A large number of eggs will create a barrier to air flow, which might cause it to flow to an area in the incubator with less resistance. According to Spotila et al. (1981), air flow has negligible effects on evaporative water loss from the surface of eggs, therefore, there is no apparent limit to increasing air flow to control temperature. The uniformity of air flow within an incubator determines how easily air can move between eggs (French, 1997). An incubator can have gravity or forced convection technology. The temperature distribution of gravity convection is due to warm air moving upwards. Forced-air incubators have a fan that evenly distributes heat throughout the incubator. This type of incubation also encourages evaporation. For the current study, a forced-air incubator would allow even temperature distribution, but this type of environment is not representative of an egg processing floor with

variable air flow. Regardless of the incubator type, there are areas in an incubator where heat is not evenly distributed. A study by Walker et al. (2013) found significant differences in temperature between two of the same make and model incubators set at 37 °C on the top, middle, and bottom shelves, as well as the front and back of the shelves. Incubators use natural convection to heat the substances inside (Rathore and Kapuno, 2011). Many commercial incubators are unable to maintain a uniform temperature around an egg due to uneven air flow in the machine. Mauldin and Buhr (1995) reported that egg surface temperatures can vary between 0.4 and 3.1 °C above the set incubator temperature. Therefore, it is unknown if all 500 eggs sweated in our experiment received the same level of heated air flow, because natural convection is impacted by gravity.

Condensation formation on an egg is a result of unsteady state heat transfer, or when the rate of heat transfer varies with time because of a change of the internal energy of the system (Rathore and Kapuno, 2011). Maximum condensate will form on a single egg in a 32 °C, 60% RH incubator in approximately 12 minutes. By introducing a large number of eggs to the same environment, this caused the RH in the incubator to drop, possibly impacting the results of the study. The RH in the incubator was manually adjusted after eggs were observed to be condensate-free after humidity levels remained constant at 40% for an hour (dew point=17 °C). After this time, eggs were observed to visibly sweat for 17 min according to Hillerman (1955) the eggs may have been warm after 60 min. However, a temperature differential was still present between the eggs and the environment, which allowed condensation to occur with the introduction of additional water to raise the RH in the incubator to 60%.

All studies that have assessed *Salmonella* penetration into shell eggs up to this point have

not taken into account egg packaging. During the instances where eggs are placed in refrigerated trucks or taken out of the trucks, the packaging should theoretically slow down the rate of heat transfer to eggs that causes condensation. In a packed layer of eggs, outer layers would have different amounts of moisture (condensate) absorption (more or less would depend on material properties, such as cardboard or plastic wrap) than the inner layers.

The choice to allow eggs to form condensation for the minimum time required to reach peak condensation amounts was made because other studies looking at egg sweating used various time and temperature combinations with no rationale. Calculating the exact time for an egg to form maximum condensation and completely dry at 22 °C or 32 °C was the most accurate way to determine this minimum condensation time. Since the FDA egg rule allows eggs to temper in a situation where they could increase in temperature for up to 36 hours, a longer sweat time could be tested. However, the other two situations when eggs could sweat when being transported into or out of delivery trucks would only allow a small window of time for eggs to sweat. In addition, if eggs are left out for long periods of time to sweat, the condensate will eventually evaporate. The speed of condensation formation and evaporation depends on the number of eggs, the temperature, and the RH. For example, Ernst et al. (1998) sweated 64 eggs for 3 h at 32 °C, 95% RH in a forced air incubator. Eggs were then allowed to dry at 22 °C for 5 h. Fromm and Margolf (1958) sweated 6 eggs at a time for 1, 3, or 5 hours at 22 °C, 80-85% RH. De Reu et al. (2006) stored 50 eggs for 21 days in a climate chamber at 20 °C and 60% RH. The other 50 eggs were stored for 24 h in a refrigerator at 6 °C and 70-85% RH, then stored for 20 days at 20 °C, 60% RH. Condensation was observed forming on these eggs for 30 min. The remaining 20 days of storage, the eggs heated to room temperature, which could promote *S.*

Enteritidis growth. De Reu's study is more applicable to European egg storage regulations, where eggs are less often refrigerated. All three of these studies chose arbitrary sweating and drying times that might only apply to a single situation in industry.

Another point that might be brought up about the conditions of our study is that eggs were stored in a 4 °C cold room, while regulations require a 7 °C storage temperature. Ernst et al. (1998) found comparable results to ours and also stored eggs before sweat at 4 °C. Chen et al. (2005) compared the storage of table eggs at 4 °C, 10 °C, and 22 °C. The whole eggs were inoculated with 10^2, 10^4, and 10^6 *S.* Enteritidis cells. At 22 °C, for all concentrations of inoculum, *S.* Enteritidis was able to grow, while at 4 °C and 10 °C, its growth was inhibited, regardless of the initial inoculum concentrations used. Both 4 °C and 7 °C environments are restrictive to the survival of *Salmonella* cells, which may explain why most *S.* Enteritidis in our study was only detected after enrichment. *Salmonella cells* stop replicating at 7.2 °C (Kim et al., 1989). Factors such as temperature, relative humidity, incubator air flow, number of eggs, and egg storage temperature could have possibly impacted the results of our experiment. Additional factors present during egg processing, storage, and transportation, such as cooling rate, packaging, and variable environmental conditions must also be considered when evaluating the results of our experiment.