Egg Handling and Storage

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ABSTRACT The temperature and relative humidity of storage, as well as the gaseous environment, interact with the fertile egg over time during storage in such a way as to affect the success of incubation either negatively or positively. This interaction occurs both above and below the “physiological zero”, at which embryonic metabolism is minimal. This interaction below physiological zero implies that certain physical aspects of the egg must be affected by the environmental conditions. As the eggshell is a relatively fixed component, changes in albumen, shell membranes, cuticle, yolk, or embryo proper must account for these time- and environment-related effects. It is concluded that the major contributor is the albumen, as it is obviously the most dynamic component below physiological zero and is strategically positioned.

(Key words: egg storage, albumen, hatchability, shell membranes, embryonic development)

INTRODUCTION

The effects of preincubational egg storage on hatchability have been the subject of much research, as indicated by reviews and monographs (Kosin, 1964; Landauer, 1967; Mayes and Takeballi, 1984; Butler, 1991; Wilson, 1991; Meijerhof, 1994), but a comprehensive theory has not been put forth that explains all of the complementary and conflicting information available. For example, Kirk et al. (1980) found that eggs stored for 2 d hatched better when stored at 18 °C than at 15 °C, whereas the opposite was true for eggs stored 8 d; although long-term storage appears to be most successful at or near 12 °C (Olsen and Haynes, 1948; Funk and Forward, 1960). Conversely, Mayes and Takeballi (1984) concluded that the shorter the storage period, the higher the storage temperature required for maximum hatchability. Furthermore, it is commonly recommended (North, 1984) that eggs be collected four times per day to achieve maximum hatchability, yet Kirk et al. (1980) reported reduced hatchability with an increased frequency of collection.

Although relative quiescence of the embryo below physiological zero has been advanced as an explanation for these obvious discrepancies, Meijerhof (1994) and Walsh et al. (1995) discount this as the sole reason. Physiological zero has been reported to be from 19 to 27 °C (Funk and Biellier, 1944; Landauer, 1967; Proudfoot and Hulan, 1976; Decuypere and Michels, 1992). The above-cited effects of storage on hatchability occurred below 19 °C; therefore the presence of an interaction between storage temperature and storage time below physiological zero indicates that embryonic development is not the only variable being influenced by storage conditions (Meijerhof, 1994; Walsh et al., 1995).

These results suggest that one or more dynamic physical components of the fertile egg are major determining factor(s) of successful preincubational storage. The fertile avian egg consists of a cuticle, eggshell proper, shell membranes, albumen, and yolk bearing the blastoderm. Structural details are given by several authors (Romanoff and Romanoff, 1949; Taylor, 1970; Burley and Vadehra, 1989). All of these components exhibit some postovipositional changes except the eggshell proper, and it, as well as the other components, exhibits changes with hen age. The objective of this review is to examine recent work on the dynamic properties of the fertile egg with an eye to the features that could explain existing conflicts and discrepancies in the literature concerning egg handling and storage.

EGG COMPONENTS

Cuticle

During development of the avian egg, a thin protective film of transparent material called the cuticle is applied to the surface of the shell. The cuticle is comprised of nearly
90% protein with some carbohydrate and a small amount of lipid (Simons, 1971). The cuticle dries immediately after oviposition to become a barrier against bacterial invasion and water loss (Simons, 1971). However, immediately before oviposition, fluid transfer through the cuticle and across the shell proper was commonplace. Thus, the cuticle must be transformed from a freely permeable to a semi-permeable barrier by the drying process postoviposition. As the cuticle has been reported to contribute to water conservation differently at different humidities (Board and Halls, 1973), it is likely that this structure changes its properties under the influence of external relative humidity. The functional properties of the cuticle during early incubation have also been reported to change with hen age from being a significant barrier to water loss to facilitating water loss (Peebles and Brake, 1986).

**Eggshell Proper**

The greatest portion of the avian eggshell consists of crystalline calcium carbonate. About 2 to 3% of this calcified layer is an organic matrix comprised mainly of protein (Taylor, 1970). Pores penetrate this crystalline layer to permit diffusion of gases (Burley and Vadehra, 1989). Young hens produce eggs with thicker shells and longer pores than older hens (Britton, 1977; Peebles and Brake, 1987). Maximum hatchability is often observed during the middle of the laying period, when shell thickness may be the lowest and porosity is the highest (Peebles and Brake, 1987). The eggshell generally thins with age (Roland, 1976) but may thicken in very old flocks if egg production decreases relative to calcium intake (Peebles and Brake, 1987). Eggshell porosity also tends to be lowest at the beginning and end of the laying period (Peebles and Brake, 1987) when hatchability is often poorest.

**Shell Membranes**

Inside the eggshell proper are two shell membranes, inner and outer, which are of different thickness and are in close contact except at the broad end, where they separate to form the air cell. The shell membranes consist of a mixture of protein and glycoprotein (Burley and Vadehra, 1989). The shell membranes function to retain the fluid of the albumen and to resist bacterial invasion (Burley and Vadehra, 1989). The membranes exhibit some type of aeration during early incubation when air displaces the water that evaporates from the space between the membranes and appears to be aided by some interaction between the membranes and albumen (Seymour and Piiper, 1988). The process of aeration may begin as an interaction of the colloidal osmotic pressure and pH of the albumen with the shell membranes (Seymour and Piiper, 1988). This aeration must occur because the permeability of fresh eggs to oxygen is not sufficient for later stages of incubation (Kayar et al., 1981). Kayar et al. (1981) showed that most membrane drying was confined to the inner shell membrane during the first 4 d of incubation, as the outer shell membrane was observed to have previously dried. Because the eggshell prior to oviposition must be capable of relatively free passage of fluids, the shell membrane, like the cuticle, must initially be conformationally configured to allow relatively free passage of fluid. During the first 24 h postoviposition, there must be a basic conformational change in the membranes. This membrane change is evidenced by recent difficulties with gene transfer technology.

The dynamic changes in the components of the egg, particularly the shell membrane and albumen, during postoviposition store or early incubation may provide a working hypothesis for improving the success of manipulating the avian egg for gene transfer technologies. Currently, the three approaches to gene transfer in poultry utilize 1) retroviral vectors administered before incubation, 2) direct DNA injection of the newly fertilized egg followed by a three-step surrogate egg culture system, and 3) the transfer of blastodermal cells, which requires manipulation of the unincubated egg and primordial germ cell transfer using eggs incubated for 24 to 55 h (for reviews see Shuman, 1991; Perry and Sang, 1993; Simkiss, 1993). The hatchability of manipulated unincubated eggs has been notoriously and almost universally low (Petitte et al., 1990, 1993; Carsience et al., 1993; Thoraval et al., 1994). The process of compromising the integrity of the egg through “windowing” to gain access to the embryo may interfere with postoviposition changes in the shell membrane and albumen. This possibility is suggested by the observation that the hatchability of windowed eggs is not compromised unless the shell membrane is punctured, i.e., removing the shell alone does not cause a dramatic drop in hatchability. However, when windows are made in eggs incubated for 24 to 35 h, in a manner similar to that required for the transfer of primordial germ cells in unincubated eggs, hatchability improves considerably (Petitte et al., 1991; Vick et al., 1993a). Interestingly, in vitro culture of newly fertilized ova requires a unique culture configuration for Day 1 to 3 embryos consisting of sealing the surrogate shell with gas-impermeable film without trapped air (Perry, 1988; Ono et al., 1994). It is also notable that no chicks have ever hatched using a single-shell culture system (Perry and Mather, 1991). These observations point to a fundamental change in the function of the components of the avian egg within the first day or two after oviposition that have a distinct influence on the subsequent survival of the embryo. Current attempts to circumvent the hatchability problem associated with the manipulation of the unincubated egg have not taken into consideration the implications of the changes in the shell membrane and albumen required for improved hatchability.

**Yolk**

The yolk is formed by the ovary and is comprised of approximately 50% water and 30% lipid, with the
remains largely protein. These constitute most of the nutrients required for embryo development except for the portions derived from the albumen and eggshell. The blastoderm is positioned on the yolk within the nucleus of the pancreas and beneath the perivitelline layer (Romanoff, 1960).

The perivitelline layer surrounding the yolk is comprised of 80 to 90% protein and is divided into three parts: outer layer, continuous membrane, and inner layer (Bell and Freeman, 1971; Burley and Vadehra, 1989). The membrane contains some proteins with antibacterial properties and may represent a physical barrier as well (Back, 1984; Cook et al., 1985; Burley and Vadehra, 1989). The weight and volume of the yolk increases with hen age (Cunningham et al., 1960).

As the egg ages, the perivitelline layer weakens and becomes more elastic, and some components are altered or removed (Fromm, 1967; Fasenko et al., 1995). Changes in perivitelline layer weight and in protein and hexosamine content are associated with an increase in albumen pH, which can be inhibited by oiling the eggshell or enhanced by increasing the rate of rise of albumen pH, (Fromm, 1967). The yolk pH is about 6.0 and contains no carbon dioxide, but the addition of carbon dioxide to the storage environment retards the movement of water from the albumen to the yolk (Romanoff and Romanoff, 1949). Similarly, a decreased storage temperature causes a decrease in water movement from the albumen to yolk (Mueller, 1959). Both temperature and pH affect albumen quality. The decrease in perivitelline layer strength observed during storage has been associated with the dissolution of the chalaziferous layer of the albumen, which occurs during long-term, but not short-term, storage (Fromm, 1967; Heath, 1976).

**Albumen**

The albumen positions the yolk and the blastoderm in the center of the egg away from the shell immediately after lay. The quality of albumen declines with storage (Hurnik et al., 1978) and hen age (Burley and Vadehra, 1989). The protein content of albumen also has been reported to decline with hen age (Cunningham et al., 1960).

At oviposition, the proteins of the albumen possess various nonspecific, anti-microbial, and possibly anti-viral defenses (Burley and Vadehra, 1989) against organisms that may invade immediately after oviposition, before drying of the cuticle, and before the structural changes in the shell membranes have been completed. The albumen pH at oviposition is about 7.6, which is slightly more basic than the uterine fluid (Arad et al., 1989), and rises to about 9.0 during storage as the dissolved carbon dioxide diffuses out (Stern, 1991). The buffering capacity of albumen is weakest between 7.5 and 8.5 (Cotterill et al., 1959), which accounts for the rapid increase as carbon dioxide is lost. This rise in pH probably limits the antimicrobial properties of albumen proteins (Voet and Voet, 1990), but these are replaced by a less favorable pH for bacterial growth, which is best in a narrow pH range between 6.5 and 7.5 (Case et al., 1989). Furthermore, the change in pH might reduce possible detrimental effects of the anti-bacterial proteins against the blastoderm.

Albumen liquefaction probably serves to liberate macromolecules, glucose, and essential ions and to facilitate their movement to the blastoderm (Spratt, 1948; Burley and Vadehra, 1989). Additionally, liquefaction may serve to reduce the barrier to gaseous diffusion imposed by the albumen (Meuer and Baumann, 1988).

**Effects of Carbon Dioxide**

The pH of albumen at oviposition is about that of blood (7.6), which rises to as much as 9.0 to 9.5 with long-term storage (Goodrum et al., 1989; Stern, 1991). Yolk pH is about 6.0. Sometime after oviposition a 1,000-fold ion gradient is established across the dividing epiblast cells of the embryo (Stern, 1991). The establishment of this gradient appears to be necessary for optimum embryo development, as the optimum albumen pH for embryo growth has been estimated to be between 8.2 and 8.8 (Sauveur et al., 1967; Walsh, 1993) before albumen quality declines too far (Sharp, 1929). Furthermore, Becker et al. (1968) found that lowering albumen pH to 7.6 prior to incubation did not improve hatchability; and others have found that holding pH too close to that of the fresh egg during storage results in decreased hatchability (Sauveur et al., 1967; Gillespie and MacHanwell, 1987). This decline in hatchability due to inappropriate albumen pH appears to be related to the time of storage, as Walsh et al. (1995) found that storage of eggs in carbon dioxide increased the number of early dead embryos in eggs held 7 d but increased embryo survival in eggs held 14 d. Generally, albumen quality was improved by the presence of carbon dioxide. It was suggested that albumen quality had not declined enough at 7 d in the presence of carbon dioxide but had declined too much without carbon dioxide at 14 d. Walsh (1993) hypothesized that an optimum albumen quality as well as optimum albumen pH is necessary before incubation should be initiated.

**Storage in Plastic Bags**

Storage of hatching eggs in plastic bags also slows the decline of albumen quality and maintains albumen pH
Temperature and Relative Humidity

Optimum hatchability after long-term storage (>14 d) was achieved when storage temperature is about 12 C (Olsen and Haynes, 1948; Funk and Forward, 1960), but 15 C was better for eggs stored 8 d and 18 C better for those stored 2 d (Kirk et al., 1980). All of these temperatures fall below the so-called “physiological zero”; therefore, significant embryo development during storage should not be a factor. Temperature of storage is directly related to albumen quality changes (Sharp and Powell, 1930; Walsh, 1993), which can explain these time- and temperature-dependent effects. The reason that 12 C is optimum for long-term storage can be surmised from inspection of a psychrometric chart. This temperature is the lowest possible for sufficient moisture holding capacity to prevent dehydration of the embryo. The impact of this dehydration can be demonstrated by simply storing eggs for 2 wk in a home refrigerator at 4 C. Although relative humidity during storage is not extremely critical (Funk and Forward, 1960), dehydration can occur at the low extreme (Proudfoot and Hulan, 1976). It appears that only eggs from older flocks with poorer albumen quality are very sensitive to lower humidity (Walsh, 1993); this fact is probably why Kaufman (1939) concluded that moisture loss was not the reason for high mortality after long-term storage.

Meijerhof et al. (1994) found that a relative increase in temperatures of the nest box, storage area, and presetting area decreased hatchability in eggs from 59-wk-old broiler breeder hens but not 37-wk-old hens during relatively short storage. This result is probably due to differences in albumen quality, as the older flocks start with a lower albumen quality (Walsh, 1993).

Genetics, Flock Age, and Egg Weight

Wilson (1991) indicated that incubation time was positively correlated with egg weight but that eggs from older flocks, which generally are larger, have been reported to hatch earlier (Crittenden and Bohren, 1962; Smith and Bohren, 1975; Lowe and Garwood, 1977). The hatch time can decrease as much as 10 h as hens age (Smith and Bohren, 1975; Shanawany, 1984). We hypothesize that many of these effects are due to a decrease in early embryonic mortality (Crittenden and Bohren, 1962) caused by an increased metabolic rate during the first 2 d of incubation (Crittenden and Bohren, 1962; Mather and Laughlin, 1979) before good oxygen exchange by the embryonic circulation has been established (Cirotto and Arangi, 1989) and the barrier imposed by the thick albumen (Meuer and Baumann, 1988) of pullet eggs has diminished. Vick et al. (1993b) showed clearly that using a lower humidity during incubation could overcome the combined barrier of a thick eggshell and thick albumen in a young flock to reduce early embryonic mortality and increase hatchability. The reduced incubation humidity permits more water to exit the egg and more oxygen to replace it. The rate of carbon dioxide loss could also be increased, which would accelerate albumen liquefaction.

The blastoderm area has been reported to increase in fresh eggs as hens age (Mather and Laughlin, 1979). The longer time in the oviduct due to clutch position or egg weight could be responsible (Bernier et al., 1951), but so could the poorer albumen quality of older hens, which would permit freer gas exchange and oxygen availability within the oviduct. Few data supporting a relationship between clutch position or egg weight and embryo development at 48 h or incubation have been found (Mather and Laughlin, 1979). Obviously, the relatively undifferentiated cells of the preovipositional embryo can grow quite well at a pH in equilibrium with body fluids, so they must need oxygen. If oxygen availability can limit the rate of development during incubation, a similar process must be at work in utero. There are obviously differences among strains with regard to incubation time and hatchability. These differences have been ascribed to unspecified “genetic differences”. An analysis of seven commercial strains of broiler breeders at 47 wk of age under one nutritional and management regimen revealed striking differences in the albumen quality of fresh eggs (Table 1). The strains with greater fresh egg albumen height exhibit good persistency of hatch during storage of eggs from older flocks, but strains with lower fresh egg albumen height do not. In younger flocks, the eggs must be stored longer for strains with the better albumen quality to achieve optimum hatchability (J. Brake, unpublished data). Thus, genetic differences in albumen quality could explain many reports of genetic effects on hatchability.

This difference in albumen quality is strikingly demonstrated by the data of Förster (1993), who showed a relationship between length of storage and candling fertility in two strains of laying hens. As shown in Figure

<table>
<thead>
<tr>
<th>Strain</th>
<th>Albumen height (mm)</th>
<th>Shell density (mg/cm²)</th>
<th>Egg weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES</td>
<td>8.5</td>
<td>106.8</td>
<td>67.8</td>
</tr>
<tr>
<td>BB</td>
<td>8.4</td>
<td>105.8</td>
<td>68.7</td>
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<tr>
<td>AN</td>
<td>8.1</td>
<td>106.8</td>
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<td>NG</td>
<td>7.8</td>
<td>113.4</td>
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<td>7.8</td>
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<td>ER</td>
<td>7.7</td>
<td>104.6</td>
<td>69.4</td>
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<tr>
<td>RO</td>
<td>6.9</td>
<td>109.4</td>
<td>65.2</td>
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We propose a simple explanation. At the beginning of lay, the female is replete with nutrient reserves and is consuming excess nutrients. This nutrient intake level allows the first eggs in the clutch to have high albumen and shell quality. As the clutch proceeds, the hen exhibits anorexia as a prelude to molt (Mrosovsky and Sherry, 1980), and the decrease in nutrient intake would decrease albumen quality. Thus, the eggs laid first in the clutch are environmentally resistant, as shown for young broiler breeder hens, whereas eggs later in the clutch are more environmentally sensitive, as shown for older broiler breeder hens (Walsh, 1993; Meijerhof et al., 1994). After a brief pause at the end of the clutch to allow requisite changes in the last eggs to occur, the hen proceeds to incubate eggs that are now at a similar stage of near optimum albumen quality. Evidence for this is shown by observations of the ostrich. Eggs “robbed” from wild nests and subjected to artificial incubation do not hatch well unless the eggs are taken after the clutch is completed (Bruce Rosseland, personal communication).

Excessive storage is detrimental (Figure 1). Evidence of necrosis and regressive changes in the blastoderm even at 13°C have been reported (Arora and Kosin, 1966; Mather and Laughlin, 1979) and shrinkage of the blastoderm at 10°C has been reported (Funk and Bieller, 1944; Mather and Laughlin, 1979). Kaufman (1939) indicated that moisture loss per se was not the reason for mortality after extended storage. The albumen reaches a pH of 9 to 9.5 after long-term storage (Goodrum et al., 1989), which is much above the optimum. Periodic heating of these long-held eggs maintains hatchability, probably by inducing metabolic production of carbon dioxide to lower tissue pH (Kucera and Raddatz, 1980). However, metabolism also produces ammonia at this stage of development (Needham, 1931), which also reduces albumen quality (Benton and Brake, 1994). It may be that the more highly differentiated cells of the postovipositional embryo are differentially sensitive to the pH gradient and exhibit disproportionate development leading to embryo death. Storage conditions such as high carbon dioxide, plastic bags, and low temperature could delay this senescence and maintain hatchability.

SEQUENCE OF EVENTS DURING POSTOVIPOSITIONAL STORAGE

From the preceding information, one can assemble a probable chronology of postovipositional events. At oviposition the cuticle is hydrated and structured conformationally to allow water passage in utero. Some bacteria penetrate the cuticle to the shell. The cuticle then dries and alters its conformation to prevent further bacterial entry. Bacteria that have penetrated may pass into the albumen, where anti-bacterial proteins are active at the postovipositional pH. The last line of defense is the anti-bacterial properties of the perivitel-line layer. Meanwhile, the outer shell membrane is dehydrating and becoming more of a physical barrier as it binds with the inner shell membrane. This structural change takes about 24 h.
Once initially penetrating bacteria are eliminated, the albumen changes to accommodate embryonic development at high temperature. This change includes the establishment of an optimum pH gradient across the epiblast layer and albumen liquefaction to reduce the barrier to gaseous diffusion and liberate nutrients. This process takes a variable length of time, which is dependent upon albumen quality as influenced genetically, environmentally, and nutritionally. Extended storage allows these changes to pass the optimum point and can only be delayed by a reduced rate of albumen change imposed by environmental factors or periodic induction of metabolism to re-establish the proper gradients.

**PRACTICAL IMPLICATIONS**

Management practices should be evaluated in light of the foregoing and applied appropriately to specific circumstances. A synopsis of the suggestions made by Vick et al. (1993b), Brake et al. (1993), and Meijerhof (1994) is given below. See these papers for greater detail. Very frequent egg gathering will reduce the frequency of cracks and will help maintain hatching quality in older flocks if the eggs are quickly placed in a cooler. Eggs from younger flocks should either be allowed to remain at a warm temperature longer before being placed in the cooler or be allowed to remain in storage for a longer period before setting. Eggs collected from mechanical roll-away nests cool faster than eggs laid in litter nests, especially if the hen remains in the nest. Time between collection and placement in the cooler should account for this difference.

Eggs from younger flocks should be stored at a higher temperature than those from an older flock if they are to be stored for a similar length of time. The storage containers should be designed with enough space and air movement to allow all eggs to cool in a reasonably similar time frame. This timing is particularly important for eggs from older hens.

The relative humidity of storage should be slightly higher for eggs from older flocks or eggs that are to be stored for a long period. Similarly, long-term storage dictates an immediate introduction of the eggs to a 12°C environment. The use of bags, covers, or enclosures will give positive responses only when the flock is very old, the strain has very poor albumen quality, or the length of storage is expected to be very long.

**REFERENCES**


