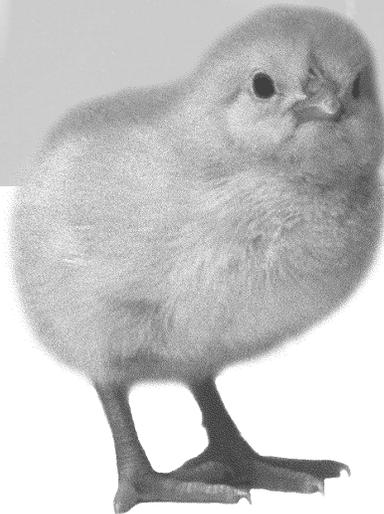


A PRACTICAL MANUAL FOR

Understanding  
*the*  
Shell Structure  
*of*  
Broiler Hatching Eggs

AND MEASUREMENTS OF THEIR QUALITY



MISSISSIPPI AGRICULTURAL & FORESTRY EXPERIMENT STATION + GEORGE M. HOPPER, DIRECTOR  
MISSISSIPPI STATE UNIVERSITY + MARK E. KEENUM, PRESIDENT + GREGORY A. BOHACH, VICE PRESIDENT

# **A Practical Manual for Understanding the Shell Structure of Broiler Hatching Eggs and Measurements of Their Quality**

**E. David Peebles**

Professor

Department of Poultry Science  
Mississippi State University

**Christopher D. McDaniel**

Professor

Department of Poultry Science  
Mississippi State University

# A Practical Manual for Understanding the Shell Structure of Broiler Hatching Eggs and Measurements of Their Quality

## INTRODUCTION

Eggshell quality is associated with hatchability. Major influences on the quality or structure of the eggshell during the reproductive life of the hen are genetic constitution, diet, climate, housing, and age (Simons, 1971). Accurate assessments of eggshell quality may, therefore, allow the producer to vary one or more of these factors that influence eggshell quality in order to improve hatchability.

Traditionally, eggshell quality has been defined in terms of the ability of an eggshell to resist breakage. Consequently, the physical measurement of eggshell strength in commercial table eggs has been used to determine the shell quality of hatching eggs. While growth rates in broilers have increased through breeding, the hatchability of the broiler hatching egg has simultaneously declined. Attempts to improve hatchability by increasing shell thickness or strength often have proven unsuccessful, and conflicting reports indicate that no single physical estimate of the relationship of shell quality to hatchability exists.

Because the structure of the eggshell influences its function as an embryonic respiratory component, it is necessary to examine eggshell quality as it relates to

hatchability in the broiler breeder through a measurement that describes the shell's dynamic respiratory quality. This physiological function of the shell has been best described by its water vapor conductance. Various physical or structural properties of the shell interact to determine porosity, which predisposes the ease with which gases diffuse across the shell; however, water vapor conductance incorporates the interactions of these physical properties and gases, and can be an accurate means of comparing the porosities of eggshells under different environmental conditions.

This bulletin is offered to the poultry husbandman and scientist as a guide to eggshell structure and its relationship to hatchability. It also offers the commercial and academic communities methods by which to monitor the quality of broiler hatching eggshells and the relevance of each method to the functional properties of the shell that control embryonic development and viability. Because water vapor conductance is the most accurate measure of a shell's physiological quality, its relationship to other structural measures of eggshell quality and its means of determination are included in this bulletin.

# EGGSHELL STRUCTURE

The eggshell has been described as a respiratory organ for the developing embryo by regulating water vapor and vital gas exchange. The basic physiological structure of the eggshell includes various components that are potential barriers to the exchange of vital gases and the diffusion of water from the egg (Stewart, 1935; Simons, 1971; Freeman and Vince, 1974; Parsons, 1982). A thin inner film is the most interior barrier of the eggshell, and it is overlaid by the proteinaceous inner and outer shell membranes. The arrangement of the film and inner and outer shell membranes in the eggshell are depicted in Figures 1 and 2. The thin inner film extends over the outermost surface of the vascularized chorioallantois; whereas the much thicker outer shell membrane layer possesses mammillary cores, which serve as epitactic centers for calcite crystal formation in the overlaying shell proper (Creger et al., 1976; Stemberger et al., 1977) (Figure 1). The thin inner film and inner shell membrane together impede oxygen diffusion to the same extent as does the shell proper; whereas, the shell proper is almost totally responsible as the barrier to both carbon dioxide and water vapor diffusion (Rahn et al., 1979).

Calcified mammillary knobs form on the cores, and crystals grow upwards from the knobs and join with adjacent crystals, creating the cones of the mammillary region and crystal columns of the overlaying palisade or spongy layer. Pores in the eggshell proper are formed where the edges of cones or columns fail to meet evenly (Figure 1). Therefore, mammillary core formation and distribution are related to the mechanical strength and respiratory quality of the eggshell (Robinson and King, 1970; Koga et al., 1982). The form and distribution of pores may also vary considerably among avian species (Board et al., 1977; Tullett, 1984).

Covering the outer surface of the eggshell is an uneven organic layer termed the “cuticle” (Figures 1 and 2). The cuticle is composed largely of protein with some polysaccharide and lipid material (Baker and Balch, 1962; Simkiss, 1968; Simons, 1971). The cuticle may either bridge the outer pore openings or extend down into the pore canals, plugging them (Cooke and Balch, 1970; Board, 1982). The cuticle may also link the lumina of pore canals to the egg’s exterior and, thereby, serve as a pathway for gas diffusion (Board and Scott, 1980).

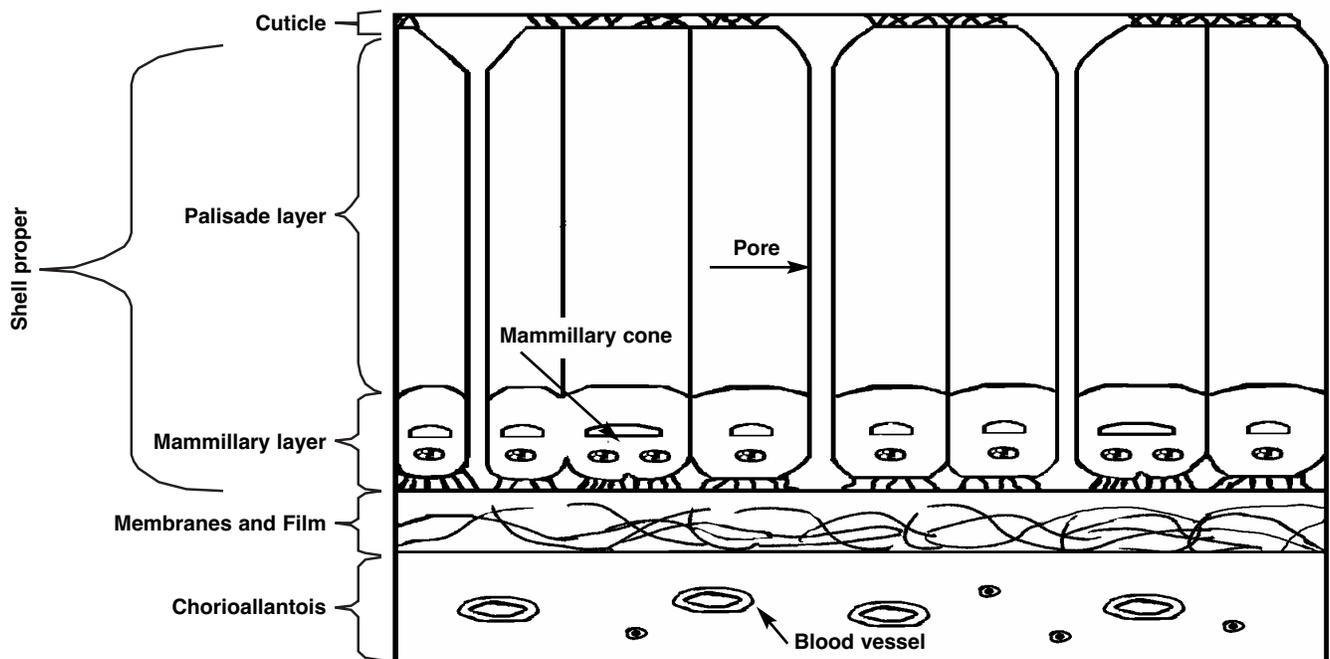
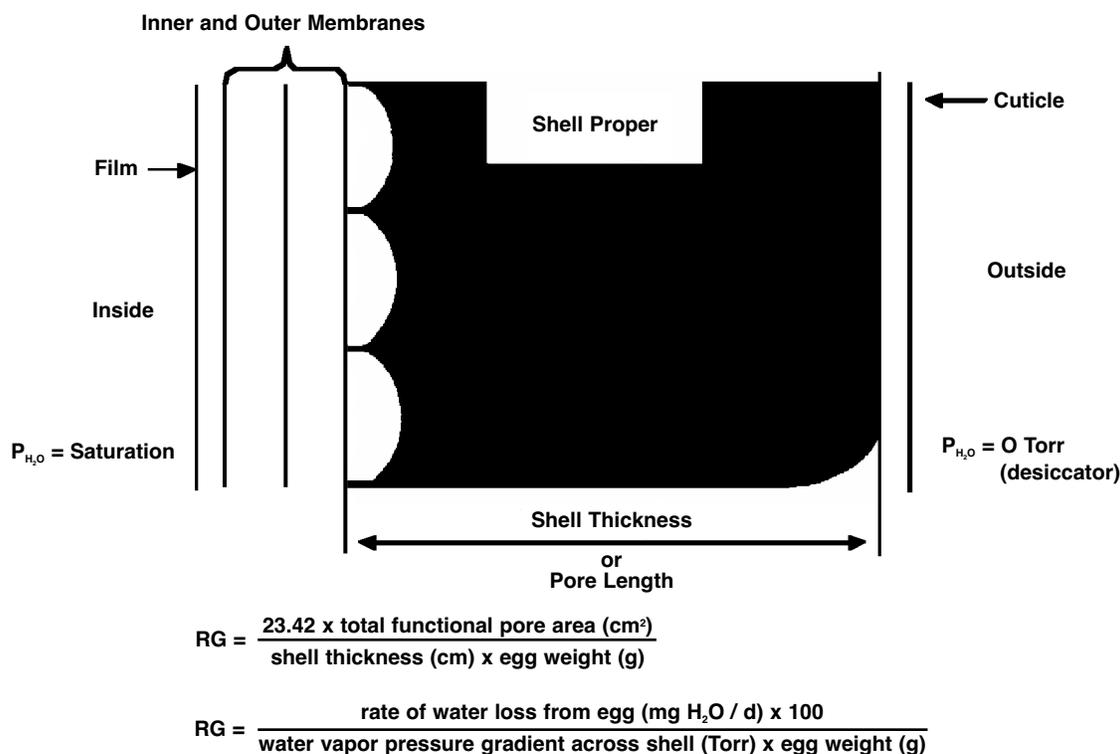


Figure 1. The physiological structure of the eggshell.



**Figure 2. Relative conductance (RG) measurements based on eggshell structural dimensions and the flux of water vapor per unit of tension difference across the shell.**

## MEASUREMENTS OF EGGSHELL QUALITY

It is important to be able to measure the quality of broiler hatching eggshells in relation to their physiological functions. Various methods have been employed to determine eggshell quality. Incubational egg weight loss, eggshell weight per unit of total eggshell surface area, eggshell pore concentration, and relative eggshell water vapor conductance have been proven to most accurately describe the eggshell and its associated membranes as external components of an avian embryonic respiratory organ.

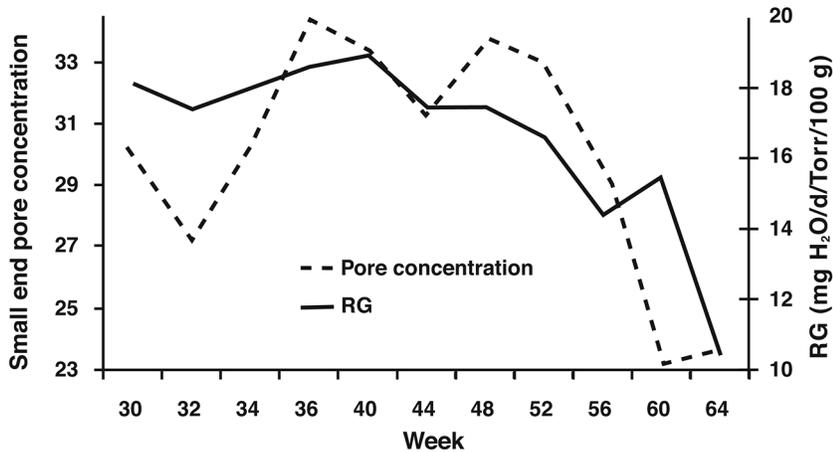
Incubational egg weight loss estimates egg shell porosity and is determined by a much simpler procedure than that for relative eggshell conductance, but it is not suitable for comparing eggs in different geographical locations under different environmental conditions (Peebles et al., 1998). Incubational egg weight loss may be determined easily by hatchery personnel, and it may be used as a relative comparison of the respiratory quality of eggshells for eggs set within a particular incubational environment.

Eggshell weight per unit of surface area is easily determined and is one of the most accurate measures of

shell thickness. Shell thickness without membranes or pore length (described below) and shell weight per unit of surface area are highly positively correlated, as shell thickening leads to an increased shell weight per unit of surface area.

Eggshell pore concentration may be somewhat time-consuming to determine, but it serves as an indicator of overall eggshell porosity. Eggshell pore concentrations at the small end, equator, and large end of eggs have been found to be highly positively correlated with relative eggshell conductance (Peebles and Brake, 1987), and significant reductions in average pore concentrations among all three regions of the eggshell have been found in eggs containing early, late, and pipped embryonic mortalities (Peebles and Brake, 1985). Note the relationship between pore concentration on the small end of the egg and relative eggshell conductance in broiler breeder hen eggs between 30 and 64 weeks of age (Figure 3).

Relative eggshell conductance is a measure of the maximum rate of water loss from the egg and may be used as a standard comparison of the respiratory qual-



**Figure 3. Small end pore concentration (number / 0.25 cm<sup>2</sup>) and conductance adjusted to egg weight (RG) between 30 and 64 weeks of breeder hen age.**

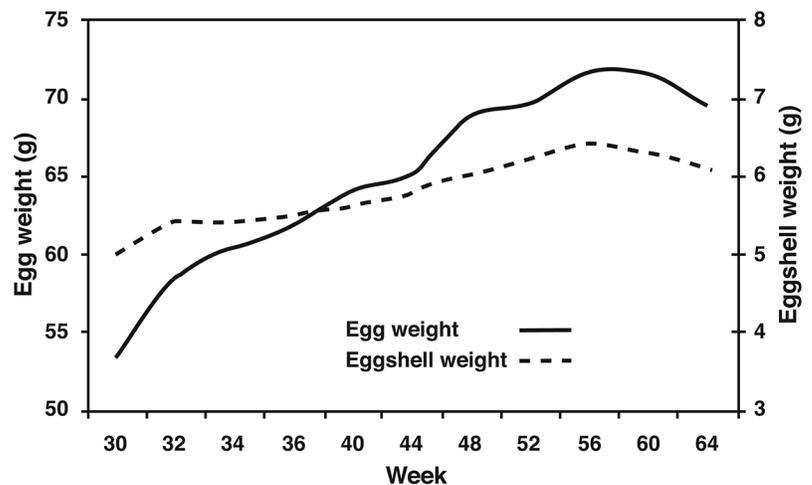
ity of eggshells across geographical location (particularly at different elevations). It is a relative measure because it is adjusted on a constant 100-gram egg weight basis. Its determination is more complex and may be most appropriate for a laboratory setting. Both egg weight and shell weight are known to change with broiler breeder hen age (Figure 4). Also, note that water vapor conductance across avian species is linearly related to egg weight (water vapor conductance =  $0.432 \times [(\text{egg weight})^{0.78}]$ ; Figure 5; Ar et al., 1974) and to egg weight  $\times$  rate of embryonic development (slope = 1.0; Figure 6; Rahn and Ar, 1980). Note the changes in relative eggshell conductance in relation to egg weight with breeder hen age between 30 and 64 weeks (Figure 7). Figure 8 shows the percentage hatchability of fertile eggs for an individual flock between 31 and 63 weeks of age, and Figure 9 shows their associated relative eggshell conductance. These two figures together illustrate that changes in relative eggshell conductance and hatchability with flock age are closely associated.

Three additional methods of estimating eggshell quality are included in this bulletin because of the ease by which they are determined, and because they are related to the four previously described methods. A direct measure of eggshell thickness may be made after the removal of the

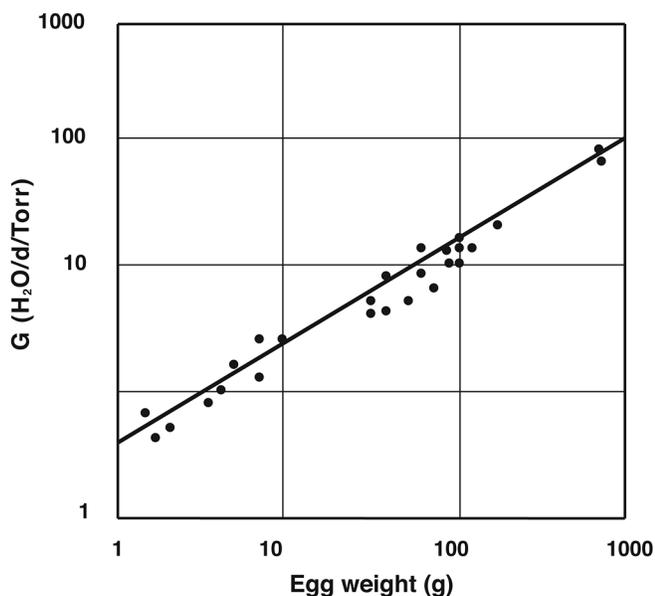
eggshell membranes. This direct measurement of eggshell thickness allows for an approximation of the length of the pores in the eggshell proper. Although shell thickness is generally negatively correlated with relative eggshell conductance (Peebles and Brake, 1987), relative eggshell conductance is controlled by various other factors such as pore concentration and morphology. The relationship between shell thickness and relative eggshell conductance between 30 and 64 weeks of breeder hen age is provided in Figure 10. Furthermore, shell thickness can vary appreciably between different regions of the shell.

Percentage shell is another method for the indirect estimation of shell thickness. Shell thickness markedly affects percentage shell (Asmundson and Baker, 1940), and it is dependent upon egg weight (Wilhelm, 1940). Schoorl and Boersma (1962) reported that percentage shell increases as the shell becomes thicker. However, like shell thickness, percentage shell is only one of various other factors that influence relative eggshell conductance and hatchability.

One of the more traditional and widely used methods of determining eggshell quality in table eggs is egg specific gravity. Specific gravity has commonly been used as a measure of shell thickness (Hamilton, 1982; Thompson et al., 1985) and as an indirect measure of



**Figure 4. Egg weight and eggshell weight between 30 and 64 weeks of breeder hen age.**

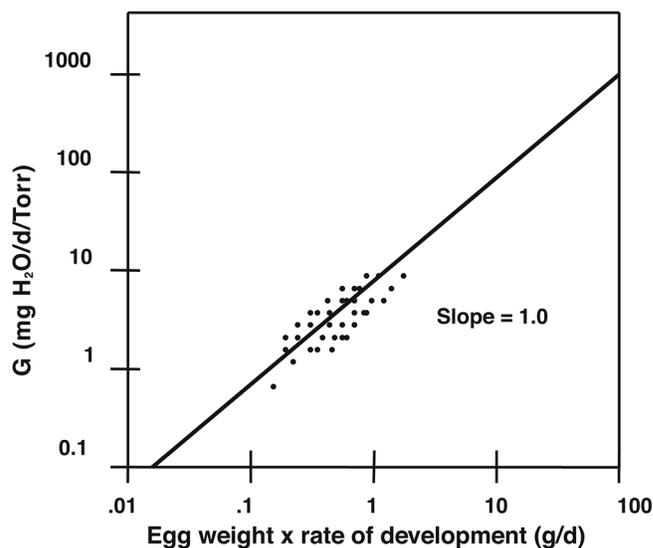


**Figure 5. Relationship between water vapor conductance (G) of eggs and egg weight [Redrawn from: Rahn and Ar (1974)].**

percentage shell in bird eggs (Olsson, 1934). It has been found to be significantly correlated with shell strength. Hamilton (1982) reported that as specific gravity increases, shell thickness concomitantly increases. Two methods used for determining egg specific gravity are included in this bulletin because specific gravity is easy to measure, estimates shell thickness, and may provide some indication as to the relative hatching success of eggs held for the same length of time after being collected from common breeders of the same age. In general, a specific gravity of 1.080 is considered the cutoff point between poor or low shell quality and good or high shell quality. Broiler hatching eggs with a specific gravity less than 1.080 have poor fertility, poor hatchability, and increased embryo mortality as compared with eggs with a specific gravity greater than 1.080 (McDaniel et al., 1981; Bennett, 1992). Approximately 85% of all eggs to be set should have a specific gravity of 1.085 or better for maximum hatchability.

However, specific gravity has not been consistently reported as a reliable indicator of incubational egg weight loss (Brunson and Godfrey, 1953) or hatchability (McDaniel et al., 1979). The specific gravity of an egg is influenced by the evaporation of water through the shell and its replacement by air in the large end of

the egg. Consequently, specific gravity can be largely influenced by air cell size. However, as specific gravity is a measure of total egg density relative to that of a solution in which it is placed, changes in the densities of other egg components including the yolk and albumen can also influence its value. This would be particularly true the closer to lay specific gravity is measured. Similarly, the longer an egg is held after lay, the greater a role the eggshell plays in determining air cell size and subsequently in determining specific gravity. Hen age can affect the relationship between specific gravity and hatchability. Peebles and Brake (1987) found that changes in the specific gravity of eggs with breeder age (Figure 11) have no consistent relationship to their hatchability (Figure 8) or relative eggshell conductance (Figure 9). Therefore, specific gravity may not be a consistent indicator of the hatching success of broiler breeder eggs laid throughout a complete laying cycle. Hamilton (1978) concluded that shell weight per unit of surface area might be a more sensitive indicator of shell quality than specific gravity. A summary of the relationships [positive (+) or negative (-)] among all seven of the eggshell quality parameters described in this bulletin is provided in Figure 12. Those relationships that, to the knowledge of the authors, have not been ascertained are indicated by a question mark.



**Figure 6. Relationship between water vapor conductance (G) of eggs and egg weight x rate of embryonic development [Redrawn from: Rahn and Ar (1980)].**

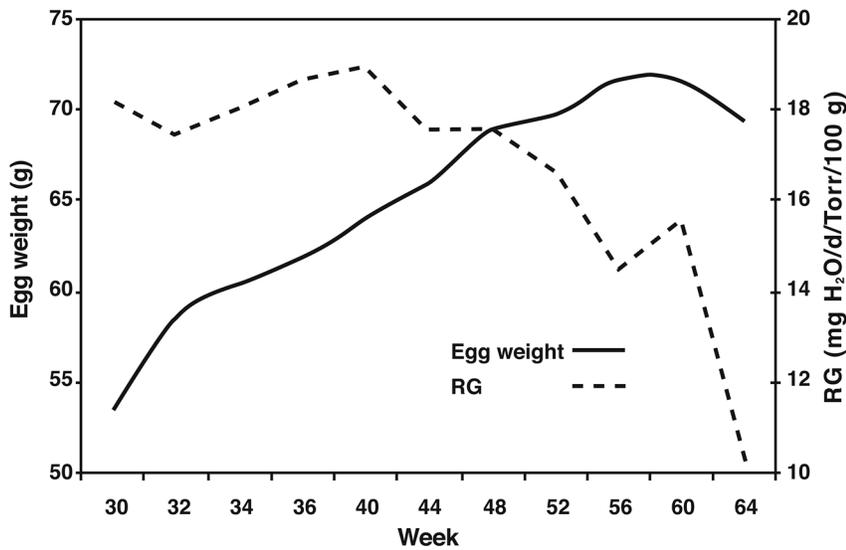


Figure 7. Egg weight and conductance adjusted to egg weight (RG) between 30 and 64 weeks of breeder hen age.

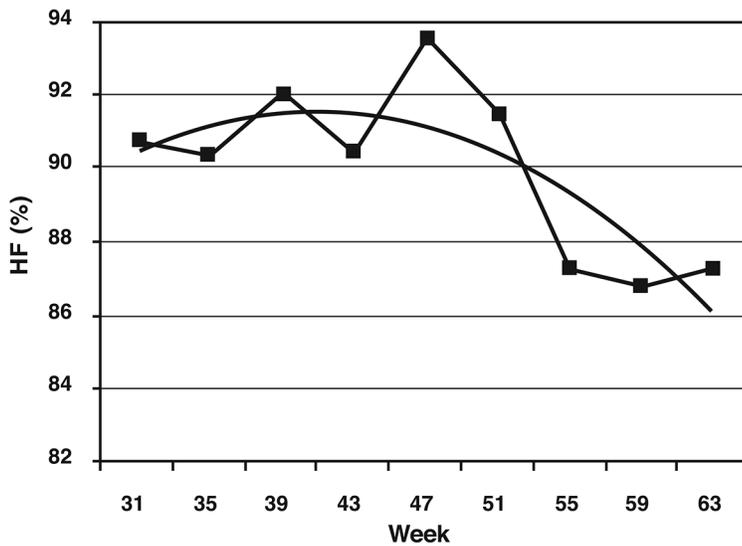


Figure 8. Hatchability of fertile eggs (HF) between 31 and 63 weeks of breeder hen age [Polynomial regression adjusted curve is included to demonstrate the time trend].

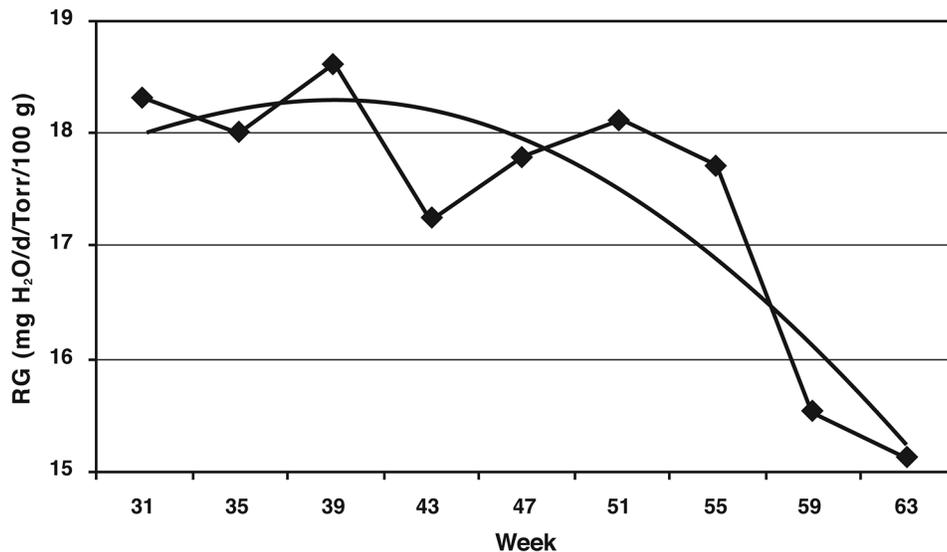


Figure 9. Conductance adjusted to egg weight (RG) between 31 and 63 weeks of breeder age (Polynomial regression curve is included to demonstrate the time trend).

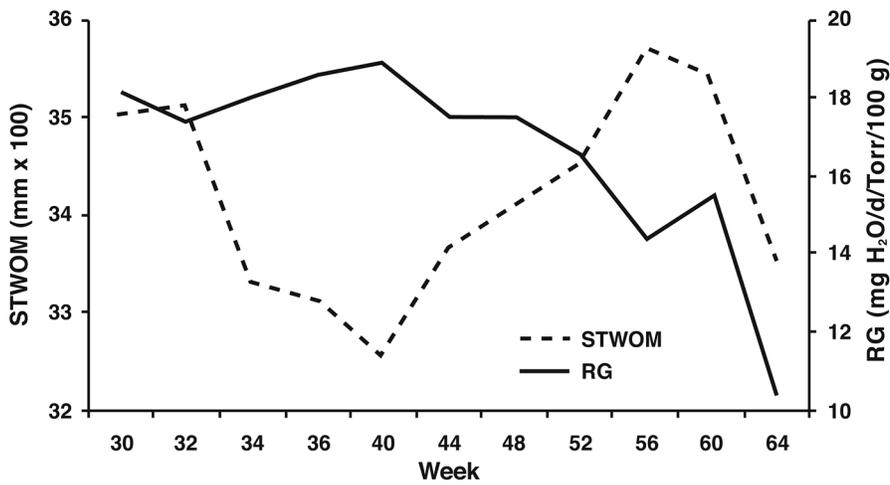


Figure 10. Shell thickness without the membrane (STWOM) and conductance adjusted to egg weight (RG) between 30 and 64 weeks of breeder hen age.

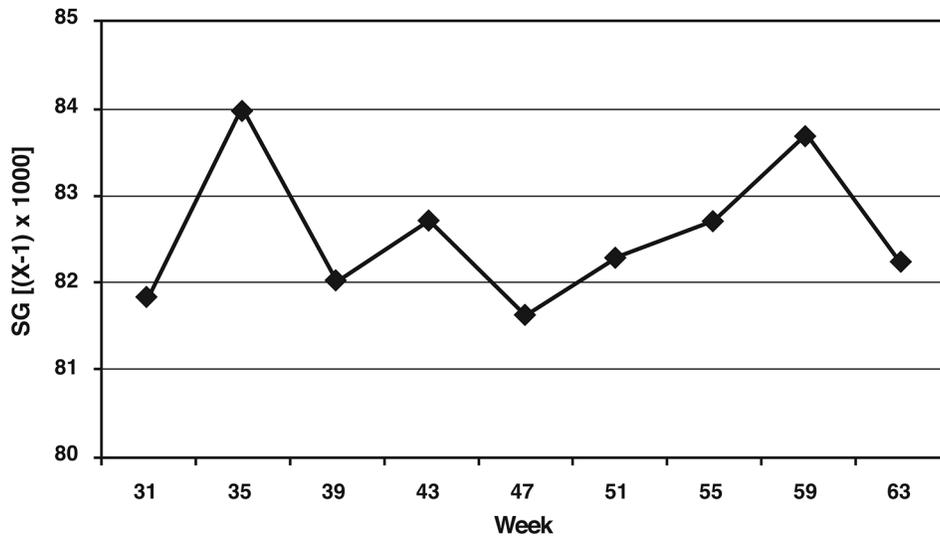


Figure 11. Egg specific gravity (SG) between 31 and 63 weeks of breeder hen age.

	Incubational egg weight loss	Shell weight per unit of surface area	Pore concentration	Relative conductance	Shell thickness without membranes	Percentage shell	Specific gravity
Incubational egg weight loss	—	-	+	+	-	-	-
Shell weight per unit of surface area	-	—	?	-	+	+	+
Pore concentration	+	?	—	+	?	?	-
Relative conductance	+	-	+	—	-	-	-
Shell thickness without membranes	-	+	?	-	—	+	+
Percentage shell	-	+	-	-	+	—	+
Specific gravity	-	+	-	-	+	+	—

Figure 12. Interrelationships among measurements of eggshell quality.

# EGGSHELL QUALITY DETERMINATION

## ***Incubational Egg Weight Loss***

### **Equipment needed**

- (1) A tabletop scale that will weigh up to 15 kilograms with a sensitivity to 0.1 gram for individual and group egg weighing.

### **Procedure**

- (1) Determine pre-set egg weight (grams). Then subtract final egg weight from initial egg weight for a specific time period during incubation. It is recommended that incubational egg weight loss be determined during the first, second, and third weeks of incubation, as well as over the entire incubation period before pipping. Incubational egg weight loss can be determined for individual eggs or groups of eggs.

- (2) Divide the change in egg weight over the specified time period by pre-set egg weight, and then multiply that value by 100. This equals percentage egg weight loss for the period. Normal total percentage incubational egg weight loss for individual eggs from set up until pipping should approximate 12-15%.
- (3) Average daily incubational egg weight loss can further be determined by dividing percentage incubational egg weight loss by the number of days in the incubational period examined.

## ***Shell Weight Per Unit of Total Shell Surface Area***

### **Equipment needed**

- (1) A tabletop scale that will weigh up to 15 kilograms with a sensitivity to 0.1 gram for group egg weighing.
- (2) A small tabletop scale with a sensitivity to 0.01 gram for individual egg or individual and small group eggshell weighing.

### **Procedure**

Eggshell weight per unit of surface area can be calculated by simply knowing fresh egg weight (grams) and dry eggshell weight (milligrams) (Hamilton, 1978). It is important to use a rinsed and dried eggshell weight as this removes variations in weight due solely to residues, and eggshell membrane and eggshell proper water content.

- (1) Obtain a fresh weight of the egg.
- (2) Total eggshell surface area (square centimeters) can be calculated using the following equation by Carter (1975):  $3.9782 \times [(\text{fresh egg weight})^{0.7056}]$ .
- (3) Crack, open, and isolate the entire eggshell after emptying the egg's contents, making sure to retain any shell fragments or pieces. Fragments belonging

to a common shell should be kept together and/or labeled. All eggshell pieces should be rinsed free of external debris or internal egg contents. The external cuticle and internal shell membranes should be retained.

- (4) Eggshells should be dried at 80°C for 2 hours and cooled to room temperature before weighing (Brake et al., 1984). Be sure to allow all internal surfaces of the shell to be exposed during the drying process. Excess water may be blotted from eggshells with an absorbent cloth before drying. Obtain a weight (milligrams) for the eggshell.
- (5) Shell weight per unit of surface area (milligrams per square centimeter) is then calculated by dividing shell weight by surface area. Example: Shell weight per unit of surface area = eggshell weight / total eggshell surface area.
- (6) If shell weight per unit of surface area is determined on a group of eggs together, then divide total egg weight by egg number before surface area calculation, and divide total eggshell weight by number of eggshells before shell weight per unit of surface area calculation. Eggshell weight per egg must be in milligrams before shell weight per unit of surface area calculation.

## **Pore Concentration**

---

### **Equipment needed**

- (1) Small surgical scissors for cutting nickle-sized hole in large end of egg.
- (2) Oven that will heat up to 50°C for rinsed eggshell drying.
- (3) 0.5 gram of 89% methylene blue crystals dissolved in 1 liter of 70% ethanol for production of dye.
- (4) Cheesecloth to filter used dye for reuse and plastic egg flat to hold eggshells.
- (5) One liter-sized bottle attached to calibrated dispensing buret for dye delivery and Pasteur pipet to siphon dye from inside of eggshell.
- (6) Pencil, stiff card with square 0.25-square-centimeter cutout, and illuminated stereo microscope with low power setting

### **Procedure**

Pore concentration at the large and small ends and at the equatorial region of the eggshell may be determined as previously described by Peebles and Brake (1985).

- (1) Cut a small hole in the center of the large end of the eggshell and empty its contents. Gently remove the chorioallantois and rinse out yolk and albumen residues with warm water. The eggshell membranes should be left intact. Blot the eggshell dry on a paper towel and allow it to dry completely overnight at room temperature or for 3 hours in a

50°C oven. Eggshells may be placed in paper flats covered with aluminum foil.

- (2) Fill the eggshell with dye solution up to the opening at the large end after the eggshell membranes have dried. The dye solution is made by dissolving 0.5 gram of 89% methylene blue crystals in one liter of 70% ethanol.
- (3) Allow the dye to remain in the eggshell for 30 minutes and then siphon out the dye into a dispenser for repeated use. The dye may be filtered with cheesecloth. The external surface of the eggshell and holder (plastic egg flat cut for suspension of the egg) must have minimal contact and both must be dry in order to prevent smudging. Allow the dye to dry thoroughly.
- (4) Draw four equally spaced squares around the circumference of the egg at the large end, equator, and small end before counting. These areas marked for pore counting should be larger than 0.25 square centimeter.
- (5) Count pores within the marked areas by using a card with a cut out area measuring 0.25 square centimeter, and a stereomicroscope with low power or a large elevated magnifying lens.
- (6) Average the four counts to one decimal place within each region and record. Do not count dye smudges and drops or incomplete pores. An average pore count per 0.25-square centimeter-area in each eggshell region is recorded for each individual egg.

## **Relative Eggshell Conductance**

---

### **Equipment needed**

- (1) A small tabletop scale with a sensitivity to 0.0001 gram for individual egg weighing.
- (2) Pencil for egg labeling.
- (3) Data sheet (Figure 13) — For set up, see the procedure below for determination of relative eggshell conductance.
- (4) Dry desiccant (indicating silica gel or Drierite™) to fill bottom of desiccator to porcelain base plate.
- (5) Large glass desiccator with lid (no sleeve valve), holed porcelain plate, flange grease, and small plastic egg flat (trimmed for minimal shell contact) for egg containment in the desiccator.
- (6) Instrument for daily stirring of desiccant.

- (7) Barometer (millimeter Hg increments), centigrade thermometer, and large oven for drying (recharging) desiccant (spread thinly on a baking sheet) overnight at 80.0°C and for holding egg-filled desiccator at a constant 25°C for the 24-hour calibration and 96-hour postcalibration weight loss period.

### **Procedure**

- (1) Make a data sheet (Figure 13) with the following column headings: egg ID #, date of collection, fresh egg weight (precalibration), postcalibration egg weights (24 and 96 hours), average weight loss, water vapor conductance, and relative eggshell conductance. At the top of the data sheet, be sure to record the date, temperature, barometric pressure, and time of measurement for the fresh (precalibration), and postcalibration egg weight

measurements. Also, record the date, temperature, barometric pressure, and time at 24 and 72 hours.

- (2) Label and obtain the fresh weight (grams) of each egg before placement in a desiccator (0 hour). Make sure an adequate supply of dried desiccant is placed in the bottom of the desiccator. Desiccant may be dried (recharged) overnight in a hot oven. Then, allow eggs to sit for 24 hours in the desiccator to calibrate before determining weight loss. This allows the removal of any external moisture on the shell's surface before the measurement of internal moisture loss.

- (3) Weigh eggs to a 0.0001-gram accuracy at 24 and 96 hours. Make all recordings as specified above at 24, 48, 72, and 96 hours, but do not weigh eggs at 48 and 72 hours. Desiccant should be stirred at 48 and 72 hours to expose dry desiccant.

- (4) To calculate relative eggshell conductance for each egg, begin by taking the difference in egg weight between 24 and 96 hours and then divide this value by three to get an average daily egg weight loss over the 3-day interval. Average weight loss will be in milligrams per day.

- (5) Average the local temperature and barometric pressure readings at 24, 48, 72, and 96 hours. Record the water vapor pressure of the internal egg (assume egg contents to be water saturated) at the average local temperature from a standard water vapor pressure table (Figure 14). For example, 25°C corresponds to 23.756 millimeters Hg (Torr). Because vapor pressure values are provided in increments of 0.2°C between 10°C and 34°C on the table, temperature averages should be rounded to the nearest 0.2°C to obtain a vapor pressure value. Divide 760 Torr (barometric pressure at sea level)

by the average local barometric pressure to derive a correction factor (ratio). For example,  $760 / 744 \text{ Torr} = 1.02$ . Multiply the barometric pressure correction factor (1.02) with the recorded internal egg water vapor pressure (at calculated average temperature). For example,  $1.02 \times 23.756 \text{ Torr} = 24.231 \text{ Torr}$ . Assume that the water vapor pressure outside of the egg in the desiccator is 0 Torr (Figure 2). Because the water vapor pressure gradient is the difference in water vapor pressure between the inside (water saturated) and outside (desiccated) of the egg, the water vapor pressure gradient is represented by the water vapor pressure (adjusted for barometric pressure) of the internal egg.

- (6) The water vapor conductance for each egg is now calculated by dividing average daily water loss (milligrams per day) by the water vapor pressure gradient (Torr) between the inside and outside of the egg (Figure 2). For example, if average daily water loss = 213.3 mg, then water vapor conductance =  $213.3 \text{ mg daily water loss} / 24.231 \text{ Torr} = 8.80 \text{ mg H}_2\text{O} / \text{d} / \text{Torr}$ .

Relative Eggshell Conductance Record Sheet								
	Readings				Calculations			
	24 h	*48 h	*72 h	96 h	Averages	Water Vapor pressure (Torr) of internal egg (taken from chart using avg. temp.)	Correction factor [760 Torr / avg. barom. press. (Torr)]	Correction factor x water vapor pressure of internal egg (Torr)
Date	2 \ 23 \ 99	2 \ 24 \ 99	2 \ 25 \ 99	2 \ 26 \ 99		23.756	1.02	24.231
Temperature (C°)	26	24	25	25	25			
Barometric Pressure (mm Hg; Torr)	742	746	748	740	744			
Time	1:45 PM	1:00 PM	2:00 PM	1:45 PM				

\* Stir desiccant

		Egg weights					
		Pre-Calibration	Post-Calibration				
Egg ID number	Date of collection	Fresh egg wt(g)	24 h (g)	96 h (g)	Average wt. loss (mg / d)	Eggshell Conductance (mg H <sub>2</sub> O / d / Torr)	Relative Eggshell Conductance (mg H <sub>2</sub> O / d / Torr / 100 g)
1	2 \ 22 \ 99	60.38	60.0960	59.4561	213.3	8.80	14.6
2							
3							
4							

Figure 13. Relative eggshell conductance record sheet.

(7) To remove the influence of egg weight and other subsequent changes in the shell (i.e., shell weight) on water vapor conductance, relative water vapor conductance for each egg may be calculated by dividing water vapor conductance by fresh egg weight, and then multiplying by 100. This is done to remove the effects of egg weight on water vapor conductance and to standardize water vapor conductance to a 100-gram egg weight basis. The unit for relative eggshell conductance is  $\text{mg H}_2\text{O} / \text{d} / \text{Torr} / 100 \text{ g}$ . If water vapor conductance =  $8.80 \text{ mg H}_2\text{O} / \text{d} / \text{Torr}$  and egg weight =  $60.38 \text{ g}$ , then relative eggshell conductance =  $14.6 \text{ mg H}_2\text{O} / \text{d} / \text{Torr} / 100 \text{ g}$ . Relative water vapor conductance may also be expressed as a function of the eggshell's structural dimensions (Figure 2). Through a modification of Fick's First Law of Diffusion, relative eggshell conductance can be expressed as:  $23.42 \times [(\text{total functional pore area (square centimeters)}) / (\text{shell thickness or pore length (centimeters)} \times \text{egg weight (grams)})]$ , where 23.42 is a conversion constant (Wagensteen et al., 1970 / 71; Ar et al., 1974; Paganelli et al., 1975, 1978; Tullett and Board, 1977).

Temp. °C	0.0	0.2	0.4	0.6	0.8
10	9.209	9.333	9.458	9.585	9.714
11	9.844	9.976	10.109	10.244	10.380
12	10.518	10.658	10.799	10.941	11.085
13	11.231	11.379	11.528	11.680	11.833
14	11.987	12.144	12.302	12.462	12.624
15	12.788	12.953	13.121	13.290	13.461
16	13.634	13.809	13.987	14.166	14.347
17	14.530	14.715	14.903	15.092	15.284
18	15.477	15.673	15.871	16.071	16.272
19	16.477	16.685	16.894	17.105	17.319
20	17.535	17.753	17.974	18.197	18.422
21	18.650	18.880	19.113	19.349	19.587
22	19.827	20.070	20.316	20.565	20.815
23	21.068	21.324	21.583	21.845	22.110
24	22.377	22.648	22.922	23.198	23.476
25	23.756	24.039	24.326	24.617	24.912
26	25.209	25.509	25.812	26.117	26.426
27	26.739	27.055	27.374	27.696	28.021
28	28.349	28.680	29.015	29.354	29.697
29	30.043	30.392	30.745	31.102	31.461
30	31.824	32.191	32.561	32.934	33.312
31	33.695	34.082	34.471	34.864	35.261
32	35.663	36.068	36.477	36.891	37.308
33	37.729	38.155	38.584	39.018	39.457
34	39.898	40.344	40.796	41.251	41.710

Figure 14. Pressure of aqueous vapor over water in mm of Hg (Torr) for temperatures from 10 to 34°C at sea level (760 mm Hg).

## Eggshell Thickness Without Membranes

### Equipment needed

- (1) Sodium hydroxide pellets, distilled water, and 1-liter flask.
- (2) Thickness measurer (Ames Co., Waltham, Mass.) with 0.01-millimeter accuracy.
- (3) Pencil for labeling shell.
- (4) Fume hood, hot plate, and large beaker.

### Procedure

- (1) Combine 50 grams of solid NaOH (sodium hydroxide pellets) in a 1-liter flask with 1 liter of distilled water. Dissolution may require 30 minutes with constant stirring. This will make a 5% NaOH solution for membrane removal. The flask may naturally become warm due to the dissolution process.
- (2) After removing the chorioallantois from the shell and leaving the membranes intact, rinse the shell

and allow it to dry completely. Four small pieces of eggshell with intact membranes should be removed from equally spaced areas at the equatorial region.

- (3) Eggshell thickness with membranes may be measured with a 0.01-millimeter accuracy using a thickness measurer (Ames Co., Waltham, Mass.). Average the four measurements at the equator to the second decimal place and record.
- (4) Membrane removal can be accomplished by boiling each piece of eggshell 10 to 15 minutes in a 5% NaOH solution under a fume hood. Eggshells may be labeled with a pencil. Rinse the eggshells and allow them to dry on a paper towel for further measurement. Eggshell thickness without membranes may similarly be measured with a 0.01-millimeter accuracy using a thickness measurer (Ames Co., Waltham, Mass.). Also, average the four measurements at the equator to the second decimal place and record.

## Percentage Shell

### Equipment needed

- (1) A tabletop scale that will weigh up to 15 kilograms with a sensitivity to 0.1 gram for individual and group egg weighing.
- (2) A small tabletop scale with a sensitivity to 0.01 gram for individual egg or individual and small group eggshell weighing.
- (3) Pencil for labeling shell.
- (4) Oven that will heat up to 80°C for rinsed eggshell drying.

### Procedure

- (1) Obtain a fresh weight (grams) of the egg.
- (2) Crack, open, and isolate the entire eggshell after emptying the egg's contents, making sure to retain

any shell fragments or pieces. Fragments belonging to a common shell should be kept together and/or labeled. All eggshell pieces should be rinsed free of external debris or internal egg contents. The external cuticle and internal shell membranes should be retained.

- (3) Eggshells should be dried at 80°C for 2 hours and cooled to room temperature before weighing (Brake et al., 1984). Be sure to allow all internal surfaces of the shell to be exposed during the drying process. Excess water may be blotted from eggshells with an absorbent cloth before drying. Obtain a weight (grams) for the eggshell.
- (4) Percentage shell can be calculated using the following equation: Percentage shell = [eggshell weight / fresh egg weight] x 100.

## EGG SPECIFIC GRAVITY

### Egg Specific Gravity by Archimedes Principle

As suggested by Voisey and Hunt (1974), the specific gravity of eggs should be recorded either at a constant preselected time after lay or after sufficient time for air cell size to stabilize. Eggs with cracked shells should not be tested. Egg specific gravity can be determined through a modification of Archimedes Principle using the weight of an egg in air and in water (Asmundson and Baker, 1940; Richards and Swanson, 1965).

### Equipment needed

- (1) A large container (bucket or trashcan).
- (2) A balance that has the capacity to obtain a hanging weight for the determination of wet weight.
- (3) A basket that is nonbuoyant to obtain wet weight.
- (4) A string to suspend the basket in water.

### Procedure

- (1) Fill a bucket or trash can with water, and allow the temperature of the water to equilibrate with room temperature.
- (2) Form a data table with columns labeled: sample ID # [either individual eggs or groups of eggs (i.e. replicate unit)]; dry weight; wet weight; dry weight – wet weight; and specific gravity.

- (3) Obtain dry weight (grams) of egg(s).
- (4) Obtain the wet weight (grams) of egg(s) in the basket while suspended in a container of water. This weight will be determined using a scale capable of obtaining a hanging weight. For accurate results, the basket must not be buoyant, and the basket and eggs must be totally submerged. Be sure to tare the scale with the basket submerged before obtaining wet egg weight.
- (5) Calculate and record dry weight – wet weight for each egg or group of eggs.
- (6) Calculate and record specific gravity for each egg or group of eggs. The formula for specific gravity is: [dry weight / (dry weight – wet weight)].
- (7) It is very important that the water used for obtaining wet weight is free of debris and at room temperature, and that specific gravity determinations be done shortly after collection (length of holding time affects the accuracy of measurement).

## ***Egg Specific Gravity by Gradational Salt Solution Method***

---

As suggested by Voisey and Hunt (1974), the specific gravity of eggs should be recorded either at a constant preselected time after lay or after sufficient time for air cell size to stabilize. Eggs with cracked shells should not be tested. Egg specific gravity may also be determined by using gradational salt solutions with predesignated densities.

### **Equipment needed**

- (1) Nine 6-gallon (22-liter) plastic buckets with sealable lids.
- (2) Fifty pounds (22.7 kilograms) of feed-grade salt.
- (3) Hydrometer with specific gravity range of 1.050 to 1.100.
- (4) Plastic 2-liter graduated cylinder.
- (5) Plastic egg flats.

### **Procedure**

- (1) Prepare nine salt solutions as follows:
  - Bucket #1 - 4 gallons (15.14 liters) of water + 3.07 pounds (1.39 kilograms) of salt = specific gravity of 1.060.
  - Bucket #2 - 4 gallons of water + 3.31 pounds (1.50 kilograms) of salt = specific gravity of 1.065.
  - Bucket #3 - 4 gallons of water + 3.56 pounds (1.61 kilograms) of salt = specific gravity of 1.070.
  - Bucket #4 - 4 gallons of water + 3.80 pounds (1.72 kilograms) of salt = specific gravity of 1.075.
  - Bucket #5 - 4 gallons of water + 4.06 pounds (1.84 kilograms) of salt = specific gravity of 1.080.
  - Bucket #6 - 4 gallons of water + 4.33 pounds (1.97 kilograms) of salt = specific gravity of 1.085.
  - Bucket #7 - 4 gallons of water + 4.60 pounds (2.09 kilograms) of salt = specific gravity of 1.090.
  - Bucket #8 - 4 gallons of water + 4.87 pounds (2.21 kilograms) of salt = specific gravity of 1.095.
  - Bucket #9 - 4 gallons of water + 5.14 pounds (2.33 kilograms) of salt = specific gravity of 1.100.
- (2) Adjust the exact specific gravity for each solution using a hydrometer. The hydrometer will float on mark of desired specific gravity. Increase the density of the solution by adding concentrated brine solution. Conversely, decrease the density of the solution by adding pure water. The density of the solution is increased by brine rather than crystalline salt because of the extra time required to dissolve crystalline salt.
- (3) Place approximately 1.5 dozen eggs from sample in solution #1 (i.e., specific gravity of 1.060). Remove eggs that float to a plastic flat marked 1.060. Transfer eggs that sink or fail to break the surface to the next solution of higher density (i.e., specific gravity of 1.065).
- (4) Continue this procedure until all eggs have been either moved from the solution in which they float to a correspondingly marked flat or passed to the next higher density solution. Eggs that float by breaking the surface of the solution in which they are placed are given a specific gravity value equal to that of the solution.
- (5) Calculate the percentage of eggs that floated in each solution. Those that passed through all solutions have the best shell quality as measured by specific gravity.

## SUMMARY

Accurate measurements of eggshell quality, therefore, include assessments of pore formation, shell thickness, and passage of gas and water vapors through the eggshell. Determination of eggshell quality can help poultry producers identify problems leading to poor hatchability and poor chick performance. For example, it is well recognized that small eggs hatch sooner than larger eggs, and that vital gas exchange in eggs and the metabolism of embryos and chicks from young hens is compromised. This imbalance in respiratory gas exchange may be due to low relative eggshell conductance or porosity of the shell in eggs from young parents (Tullett and Noble, 1989). Note that the percentage of carbon dioxide and oxygen in the airspace of eggs from young and mature (at peak egg production) flocks may vary considerably throughout incubation (Figures 15 and 16, respectively). After 15 days of incubation, the percentage of oxygen is commonly greater and the concentration of carbon dioxide is commonly lower in the large end airspace of eggs from young flocks when compared with those from mature flocks. Adjustments

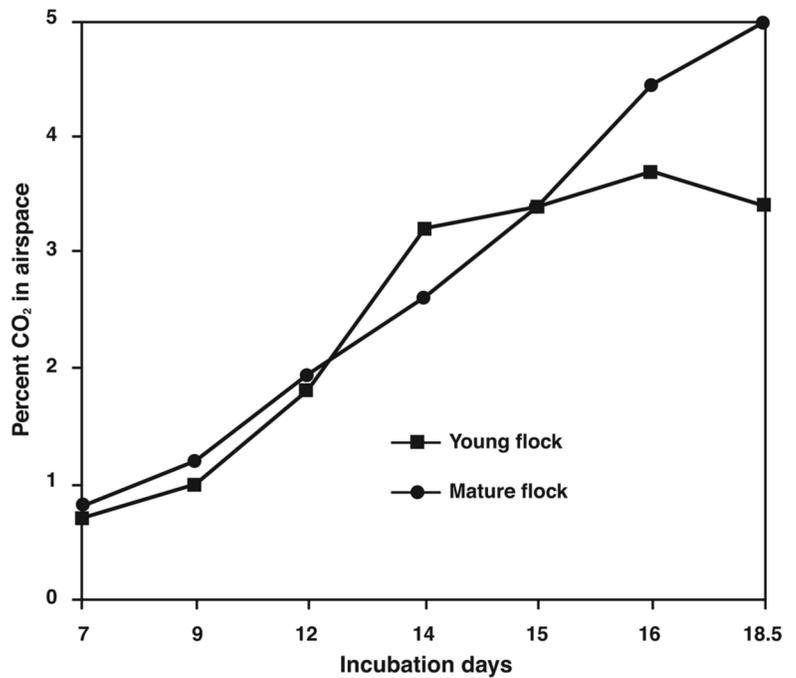


Figure 15. Carbon dioxide concentration in the large end airspace of eggs from young and mature flocks throughout incubation [Redrawn from: Tullett and Noble (1989)].

in incubational conditions, such as relative humidity, may be made to compensate for possible eggshell porosity differences in eggs of a specific size and from a specific age group of hens.

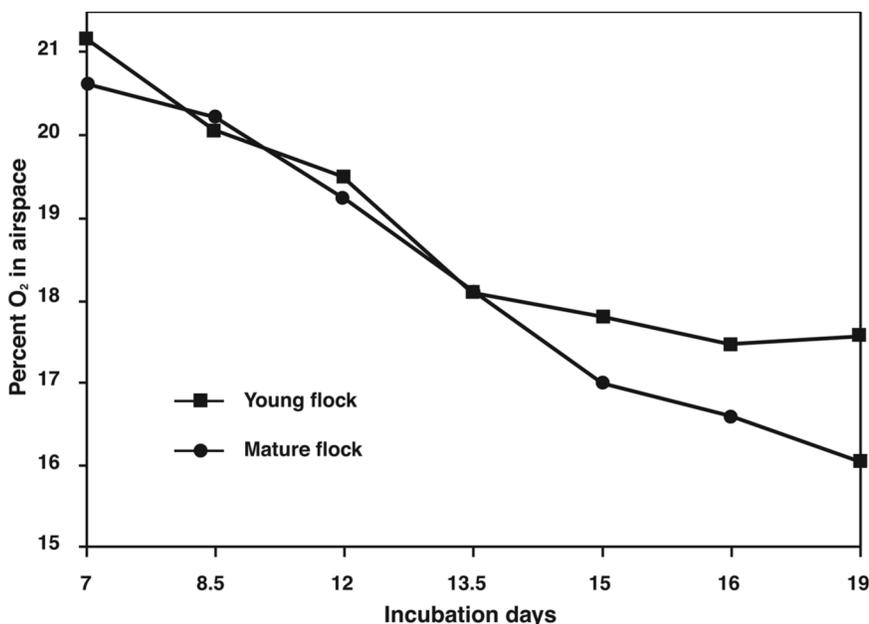


Figure 16. Oxygen concentration in the large end airspace of eggs from young and mature flocks throughout incubation [Redrawn from: Tullett and Noble (1989)].

## LITERATURE CITED

- Ar, A., C.V. Paganelli, R.B. Reeves, D.G. Greene, and H. Rahn.** 1974. The avian egg: Water vapor conductance, shell thickness, and functional pore area. *The Condor* 76:153-158.
- Asmundson, V.S., and G.A. Baker.** 1940. Percentage shell as a function of shell thickness, egg volume and egg weight. *Poultry Sci.* 19:227-232.
- Baker, J.R., and D.A. Balch.** 1962. A study of the organic material of hen's eggshell. *Biochem. J.* 82:352-361.
- Bennett, C.D.** 1992. The influence of shell thickness on hatchability in commercial broiler breeder flocks. *J. Appl. Poultry Res.* 1:61-65.
- Board, R.G.** 1982. Properties of avian eggshells and their adaptive value. *Biol. Rev.* 57:1-28.
- Board, R.G., and V.D. Scott.** 1980. Porosity of the avian eggshell. *Amer. Zool.* 20:349.
- Board, R.G., S.G. Tullett, and H.R. Perrott.** 1977. An arbitrary classification of the pore systems in avian eggshells. *J. Zool. (London)* 1982:251-265.
- Brake, J., J.F. Ort, T.A. Carter, and W.R. Campbell.** 1984. Effect of insect growth regulator CGA72662 (Larvadex®) on broiler breeder production, hatchability, and subsequent chick performance. *Poultry Sci.* 63:910-916.
- Brunson, C.C., and G.F. Godfrey.** 1953. The relationship of egg shape, egg weight, specific gravity, and 21-day incubation weight-loss to hatchability of broad-breasted bronze turkey eggs. *Poultry Sci.* 32:846-849.
- Carter, T.C.** 1975. The hen's egg: Estimation of shell superficial area and egg volume, using measurements of fresh egg weight and shell length and breadth alone or in combination. *Brit. Poult. Sci.* 16:541-543.
- Cooke, A.S., and D.A. Balch.** 1970. Studies of membrane, mamillary cores and cuticle of the hen eggshell. *Brit. Poult. Sci.* 11:345-352.
- Creger, C.R., H. Phillips, and J.T. Scott.** 1976. Formation of an eggshell. *Poultry Sci.* 55:1717-1723.
- Freeman, B.M., and M.A. Vince.** 1974. *Development of the Avian Embryo.* London: Chapman and Hall.
- Hamilton, R.M.G.** 1978. Observations on the changes in physical characteristics that influence eggshell quality in ten strains of White Leghorns. *Poultry Sci.* 57:1192-1197.
- Hamilton, R.M.G.** 1982. Methods and factors that affect the measurement of eggshell quality. *Poultry Sci.* 61:2022-2039.
- Koga, O., N. Fujihora, and Y. Yochimura.** 1982. Scanning electron micrograph of surface structures of soft-shelled eggs laid by regularly laying hens. *Poultry Sci.* 61:403-406.
- McDaniel, G.R., D.A. Roland, Sr., and M.A. Coleman.** 1979. The effect of eggshell quality on hatchability and embryonic mortality. *Poultry Sci.* 58:10-13.
- McDaniel, G.R., J. Brake, and M.K. Eckman.** 1981. Factors affecting broiler breeder performance. 4. The interrelationship of some reproductive traits. *Poultry Sci.* 60:1792-1797.
- Olsson, N.** 1934. *Studies on Specific Gravity of Hen's Eggs. A New Method for Determining the Percentage of Shell on Hens' Eggs.* Leipzig: Otto Harrassowitz.
- Paganelli, C.V., R.A. Ackerman, and H. Rahn.** 1978. The avian egg: *In vivo* conductances to oxygen, carbon dioxide, and water vapor in late development. In *Respiratory Function in Birds, Adult and Embryonic*, ed. J. Piiper, 212-218.
- Paganelli, C.V., A. Ar, H. Rahn, and O.D. Wagensteen.** 1975. Diffusion in the gas phase: the effects of ambient pressure and gas composition. *Resp. Physiol.* 25:247-258.
- Parsons, A.H.** 1982. Structure of the eggshell. *Poultry Sci.* 61:2013-2021.

- Peebles, E.D., and J. Brake.** 1985. Relationship of eggshell porosity to stage of embryonic development in broiler breeders. *Poultry Sci.* 64:2388-2391.
- Peebles, E.D., and J. Brake.** 1987. Eggshell quality and hatchability in broiler breeders. *Poultry Sci.* 66:596-604.
- Peebles, E.D., T. Pansky, S.M. Doyle, C.R. Boyle, T.W. Smith, M.A. Latour, and P.D. Gerard.** 1998. Effects of dietary fat and eggshell cuticle removal on egg water loss and embryo growth in broiler hatching eggs. *Poultry Sci.* 77:1522-1530.
- Rahn, H., and A. Ar.** 1980. Gas exchange of the avian egg: Time, structure, and function. *Amer. Zool.* 20:477-484.
- Rahn, H., A. Ar, and C.V. Paganelli.** 1979. How bird eggs breathe. *Sci. Amer.* 240:46-55.
- Richards, J.F., and M.H. Swanson.** 1965. The relationship of egg shape to shell strength. *Poultry Sci.* 44:1555-1558.
- Robinson, O.S., and N.R. King.** 1970. The structure of the organic mammillary cores in some weak eggshells. *Brit. Poult. Sci.* 11:39-44.
- Schoorl, P., and H.Y. Boersma.** 1962. Research on the quality of the eggshell. A new method of determination. *World's Poultry Congr.*, p. 432-435.
- Simkiss, K.** 1968. The structure and formation of the shell and shell membranes. In *Egg quality: A study of the hen's egg*, ed. T.C. Carter, 4:3-25. Edinburgh: Oliver and Boyd.
- Simons, P.C.M.** 1971. *Ultrastructure of the Hen Eggshell and Its Physiological Interpretation*. Wageningen: Centre for Agricultural Publishing and Documentation.
- Stemberger, B.H., W.J. Mueller, and R.M. Leech, Jr.** 1977. Microscopic study of the initial stages of eggshell calcification. *Poultry Sci.* 56:537-543.
- Stewart, G.F.** 1935. The structure of the hen's eggshell. *Poultry Sci.* 14:24-32.
- Thompson, B.K., R.M.G. Hamilton, and A.A. Grunder.** 1985. The relationship between laboratory measures of eggshell quality and breakage in commercial egg washing and candling equipment. *Poultry Sci.* 64:901-909.
- Tullett, S.G.** 1984. Minireview. The porosity of avian eggshells. *Comp. Biochem. Physiol.* 78A:5-13.
- Tullett, S.G., and R.G. Board.** 1977. Determinants of avian eggshell porosity. *J. Zool. (London)* 183:203-211.
- Tullett, S.G., and R.C. Noble.** 1989. Understanding the chick embryo (III)-Low hatchability problems in young parent stock. *Poultry-Misset*, January:8-9.
- Voisey, P.W., and J.R. Hunt.** 1974. Measurements of eggshell strength. *J. Textile Studies* 5:135-182.
- Wagensteen, O.D., D. Wilson, and H. Rahn.** 1970 /71. Diffusion of gases across the shell of hen's egg. *Resp. Physiol.* 11:16-30.
- Wilhelm, L.A.** 1940. Some factors affecting variations in eggshell quality. *Poultry Sci.* 19:246-253.



**MISSISSIPPI STATE**  
**UNIVERSITY**™



*Printed on Recycled Paper*

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the Mississippi Agricultural and Forestry Experiment Station and does not imply its approval to the exclusion of other products that also may be suitable.

Discrimination based upon race, color, religion, sex, national origin, age, disability, or veteran's status is a violation of federal and state law and MSU policy and will not be tolerated. Discrimination based upon sexual orientation or group affiliation is a violation of MSU policy and will not be tolerated.