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Research Article
Relationship of Eggshell Thickness to Thermal Gradients Across the Shell at the Large and Equatorial Regions of Ross 708 Broiler Hatching Eggs[1,2,3]

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Abstract

Background and Objective: This study was designed to examine the relationship between the thickness and thermal barrier property of the eggshell of embryonated Ross 708 broiler hatching eggs. **Methodology:** On each of 8 levels of a single-stage incubator, 43 eggs were weighed and set. Transponders were implanted in the air cell of 10 eggs per level at 12 d of incubation (DOI) and shell thickness at the large end (LSTH) of those eggs was measured at 19 DOI. Along with air cell temperature (ACT), external surface temperature of the shell was measured daily at the equator (EST) and large end (LST) of the egg by infrared thermometry at 10 AM and 4 PM between 12 and 19 DOI. Thermal gradients across the shell at the equator and large end of the egg were expressed as differences between ACT and EST (TE) and ACT and LST (TL). **Results:** There was a significant ($p<0.0001$) location in the egg × time period interaction for the temperature readings and thermal gradients across the eggshell. At 4 PM on 12 DOI, 10 AM on 13 and 14 DOI and 10 AM and 4 PM on 15-19 DOI, ACT was higher than EST and EST was higher than LST. However, at 4 PM on 13 and 14 DOI, ACT and EST were not significantly different but both were higher than LST. At all time periods examined, TE was lower than TL. Mean EST and LST were positively correlated ($p<0.0001$). However, significant negative correlations were observed between LSTH and TE at 10 AM on 17 DOI and between LSTH and TL at 10AM and 4 PM on 16 and 17 DOI and at 10 AM on 18 DOI. **Conclusion:** Although, EST and LST are positively correlated, EST is more closely related to ACT than is LST and an increase in LSTH, within the range observed in this study, does not increase the shell's function as a thermal barrier in embryonated Ross 708 broiler hatching eggs.

Key words: Air cell, broiler hatching egg, eggshell temperature, eggshell thickness, gradient

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INTRODUCTION

The eggshell plays an important role in the development of the embryo, which includes its role as a major respiratory component. Furthermore, it has been shown that the chicken embryo obtains approximately 80% of its skeletal calcium from the eggshell after 10 d of incubation (DOI)[1,2,3].The rate of vital gas exchange and water vapor loss through the eggshell strongly affects the survival of the developing embryo[4,5]. Although the shell membranes offer minimal resistance to water movement across the eggshell[6], the eggshell itself constitutes a major barrier to gas diffusion[5]. Furthermore, Lourens *et al*.[7] stated that the thermal conductivity of the eggshell is low compared with that of air, eggshell thickness is one of several factors that influences heat transfer by conduction[8].

Total pore area of the eggshell must be sufficient in relation to shell thickness in order to accommodate normal embryonic development and to achieve a successful hatch[9,10]. Peebles and Brake[11] have suggested that eggshell thickness reflects pore length in broiler hatching eggs and that an excessively thick shell may account for some early embryonic deaths. It was also concluded that an abnormal distribution of pores over the eggshell surface may contribute to reduced hatchability and more specifically that optimum hatchability may depend on a proper pore concentration in each eggshell region (large end, equator and small end).

The development and growth rate of the embryo in a domestic avian egg is dependent on several factors. One of particular importance is embryo temperature[12]. Embryonic temperature at the different stages of incubation must be optimized to achieve maximum hatchability. Optimal incubation temperature is considered as the temperature at which hatchability reaches its maximum and chick quality is at its best[13-15]. In various studies, researchers have shown that a developing embryo produces heat and this heat production is usually significant in the second half of the incubation period[16,17]. Likewise, an assessment of the internal temperature of an egg during incubation is important for the determination of the embryo's body temperature, its associated heat production and its level of metabolic activity[18].

Measurement of eggshell surface temperature has been used to estimate internal embryo temperature[13,19]. However, eggshell temperature is dependent on 3 variables. These are the temperature of the surrounding air, the level of heat transfer between the egg and the surrounding environment and the amount of heat produced by the embryo[19]. Therefore, it is expected that internal egg temperature may vary from that of eggshell surface temperature. Peebles *et al*.[20] stated that determination of air cell temperature (ACT), with the use of an implantable transponder in the air cell, was reliable for the accurate detection of the core body temperature of broiler embryos from 14.5 through 18.0 DOI. By circumventing the confounding effects of the thermal barrier properties of the eggshell and the flow of air across its surface, this method detected temperatures that were higher and closer to actual body temperature than eggshell surface temperatures. It was further reported that mean infrared thermometer and transponder (affixed to eggshell) readings of external eggshell surface temperature at the equator (EST) of embryonated eggs were positively correlated and differed only by 0.03°C, which was not statistically significant.

The shell and internal contents of eggs have been significantly altered by genetic selection[21]. Therefore, the first objective of this experiment was to determine ACT, EST and external eggshell surface temperature at the large end (LST) of modern strain commercial broiler hatching eggs (Ross 708). The second objective was to determine the possible relationship between large end shell thickness (LSTH) and the temperature gradient between the interior and exterior of the shell at the equatorial (TE) and large end (TL) regions of the egg. The results of this study will provide information that will further our understanding of the relationships between incubation environment, eggshell and air cell temperatures and subsequently, the relative significance of LSTH as a thermal barrier. This knowledge is basic to the development of a pragmatic and accurate probe system that will record ACT as a means by which to more closely estimate embryo core body temperature.

MATERIALS AND METHODS

Broiler hatching eggs (Ross 708) were collected from a common commercial flock at 36 weeks of age and were held for approximately 72 h under standard conditions before setting. The eggs were weighed individually and only those that were within 10% of the average weight of all eggs collected were marked and included as experimental eggs.

Immediately after weighing, 344 experimental eggs were randomly and evenly distributed among 8 different tray levels in a Jamesway model PS 500 single-stage incubator (Jamesway incubator company Inc, Cambridge, ON, Canada).The incubator was set at 37.5°C dry bulb and 29.4°C wet bulb temperatures. At 10 DOI, the eggs were candled and eggs that were infertile or contained dead embryos were removed. Eighty eggs containing live embryos and level air cells, were selected(approximately 10 eggs on each of the 8 tray levels), weighed and placed on the outer edge of the trays for easy access from inside the incubator. These

80 experimental eggs were incubated among the remaining eggs in the same incubator to maintain an even air flow pattern over the eggs. Percentage egg weight loss (PEWL) for both the 0-10 and 12-19 DOI time periods were measured. According to the procedure of Pulikanti *et al.*[22], PEWL in both time periods was calculated by subtracting egg weight at the end of each period from egg weight at the beginning of each time period. These differences were divided by set (Day 0)egg weight, which was then multiplied by 100. The air cells of all 80 experimental eggs were implanted on 12 DOI with a transponder (Implantable programmable temperature transponder, IPTT-300; Bio Medic Data System Inc., Seaford DE) with an accuracy of ±0.1°C for the determination of ACT. The materials and procedures used for transponder implantation and temperature data recording were similar to those previously described by Pulikanti *et al.*[23] .The eggs were again weighed after transponder implantation. The LST and EST of the eggs were also recorded from inside the incubator using an infrared thermometer (Braun Thermoscan thermometer, IRT 4520, Kronberg, Germany) with an accuracy of ±0.2°C. All temperature recordings were made by a single observer after entering, shutting the door and remaining in the incubator until it returned to its set temperature. The ACT, EST and LST measurements were only used from 71 eggs that contained live embryos and which had shells that were not cracked through the end of the experiment. Calculation of TE was the difference between ACT and EST and calculation of TL was the difference between ACT and LST. Calibrations and accuracy measurements of the transponder and infrared thermometer were performed by the manufacturer. In addition, 2 wireless data logger temperature nodes (HOBO ZW series wireless, Onset Computer Corporation, Bourne, MA) were located on opposite sides of the middle trays inside the incubator to record temperature and relative humidity. The ACT, EST and LST readings from each egg were recorded twice daily (10 AM and 4 PM), at the same time each day, until 4 PM on 19 DOI.

Approximately 1.0 cm from the center of the large end of the egg, a circle containing 4 equidistant dots were drawn. On 19 DOI, a circular cut was made below the marked section on the large end of the eggshell with the outer shell membrane intact. The region above the cut was gently removed and rinsed in warm water to remove excess albumen and debris from the shell membrane. After removal of excess water, the shell samples were allowed to dry for 24 h at room temperature. The LSTH of each egg was measured to a 0.01 mm accuracy with a micrometer (Ames thickness measurer, Waltham, MA) at each of the 4 dots on the circle. The LSTH of each egg was based on the mean of the 4 measurements.

Statistical analysis: All procedures of data analysis used were of SAS software (Version 9.4, SAS[24]). A mixed model ANOVA employing PROC MIXED was used to analyze the temperature and thermal gradient data. Fixed effects in the model were location and time. Random effects included tray level, egg within tray level and level by time interaction. A first order autoregressive correlation structure was used to account for repeated measurements of temperature on the eggs at given locations. Least-squares means were separated by least significant difference[25]. Partial correlations among variables were analyzed by Multivariate ANOVA of PROC GLM. Correlation coefficients and differences in least-squares means were considered significant at $p \leq 0.05$.

RESULTS

There was a significant ($p<0.0001$) location in the egg×time period interaction for the recorded temperature readings (Table 1). At 4 PM on 12 DOI, 10 AM on 13 and 14 DOI and 10 AM and 4 PM on 15, 16, 17, 18 and 19 DOI, ACT was significantly higher than EST and EST was significantly higher than LST. However, at 4 PM on 13 and 14 DOI, ACT and EST were not significantly different but both were significantly higher than LST. Mean ACT, EST and LST at each individual time of day within each DOI are also provided graphically in Fig. 1. Mean ACT, EST and LST across the 10 AM and 4 PM time periods within the 12-19 DOI interval, were 38.73, 38.61 and 38.27°C, respectively. There was a significant ($p<0.0001$) location in the egg×time period interaction for the thermal gradients across the eggshell (TE and TL) (Table 2). The thermal gradient between the air cell and eggshell at the equator (TE) was significantly lower than the thermal gradient between the air cell and eggshell at the large end of the egg (TL) at each of the 10 AM and 4 PM time periods within the 12-19 DOI interval. Mean TE and TL at each individual time of day within each DOI are also provided graphically in Fig. 2. Mean TE and TL across the 10 AM and 4 PM time periods within the 12-19 DOI interval were 0.111 and 0.458°C, respectively.

When correlation analysis was performed based on the design of the experiment, only EST and LST recordings taken across the 10 AM and 4 PM time periods within the 12-19 DOI interval were significantly ($p<0.0001$) positively correlated (Table 3). It was observed that mean LSTH at 19 DOI was 0.349±0.0026 mm, with minimum and maximum LSTH measurements being 0.290 and 0.400 mm, respectively.

Table 1: Mean air cell temperature and shell temperature at the equator and large end of the egg at 4 PM on Day 12 and at10 AM and 4 PM on Days 13, 14, 15, 16, 17, 18 and 19 of incubation[1]

	Temperature (°C)			
Day of Incubation	Time of day	Air cell	Equator of eggshell	Large end of eggshell
12	10 AM	----	----	----
	4 PM	38.33[a]	38.18[b]	38.00[c]
13	10 AM	38.30[a]	38.24[b]	38.02[c]
	4 PM	38.35[a]	38.34[a]	38.09[b]
14	10 AM	38.60`[a]	38.56[b]	38.20[c]
	4 PM	38.50[a]	38.49[a]	38.18[b]
15	10 AM	38.77[a]	38.67[b]	38.31[c]
	4 PM	38.81[a]	38.72[b]	38.36[c]
16	10 AM	38.94[a]	38.87[b]	38.44[c]
	4 PM	38.92[a]	38.74[b]	38.33[c]
17	10 AM	38.90[a]	38.74[b]	38.31[c]
	4 PM	38.89[a]	38.81[b]	38.40[c]
18	10 AM	38.79[a]	38.66[b]	38.26[c]
	4 PM	38.90[a]	38.70[b]	38.31[c]
19	10 AM	38.90[a]	38.67[b]	38.32[c]
	4 PM	39.00[a]	38.84[b]	38.49[c]
Mean[2]		38.73[a]	38.61[b]	38.27c

[a-c]Means within a row with no common superscript differ significantly ($p \leq 0.05$). [1]71readings were used to calculate the means for each location at each time of day within day of incubation. Pooled SEM: 0.035. [2]1,065 readings (71 eggs × 15 time periods) were used to calculate the means for each location. Pooled SEM: 0.031

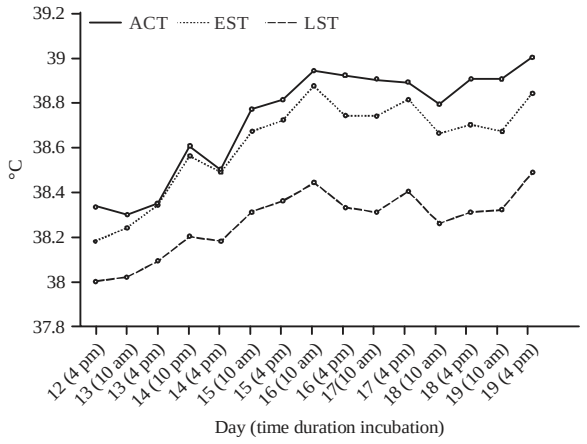

Fig. 1: Mean air cell (ACT) and equator (EST) and large end (LST) eggshell temperature readings from 4 PM at 12 days of incubation through 4 PM at 19 days of incubation

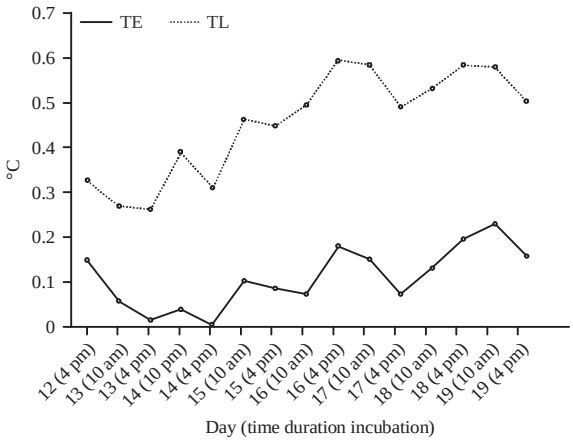

Fig. 2: Mean temperature reading differences between the air cell and eggshell equator (TE) and between the air cell and eggshell large end (TL) from 4 PM at 12 days of incubation through 4 PM at 19 days of incubation

French[13] suggested that the transfer of heat between the egg and incubator may be influenced by the design of the incubator. Other studies have also reported that air speed within the incubator may vary between tray locations and result in temperature differences between the trays[13,18,26]. However, there was no significant ($p = 0.094$) incubator tray level effect on LSTH. Furthermore, there was no significant incubator tray level effect on PEWL for eggs that were (12-19 DOI) and were not (0-10 DOI) subjected to transponder implantation ($p = 0.354$ and $P = 0.693$, respectively). Across the 10 AM and 4 PM time periods within the 12-19 DOI interval, LSTH was not significantly correlated with ACT ($p = 0.227$), EST ($p = 0.586$), LST ($p = 0.084$), or TE ($p = 0.075$) but was significantly ($p = 0.030$) negatively correlated ($r = - 0.272$) with TL. There was only a significant negative correlation between LSTH and TE at 10 AM on 17 DOI. However, there were significant negative correlations between LSTH and TL at 10 AM and 4PM on 16 and 17 DOI and at 10 AM on 18 DOI (Table 4). Furthermore, across the 10 AM and 4 PM time periods within the 12-19 DOI interval, TE and TL were significantly ($p<0.0001$) positively correlated ($r = 0.945$).

Table 2: Mean TE and TL at 4 PM on Day 12 and at 10 AM and 4 PM on Days 13, 14, 15, 16, 17, 18 and 19 of incubation[1,2]

Day of Incubation	Time of day	TE	TL
12	10 AM	----	----
	4 PM	0.151[b]	0.330[a]
13	10 AM	0.060[b]	0.273[a]
	4 PM	0.017[b]	0.265[a]
14	10 AM	0.041[b]	0.393[a]
	4 PM	0.007[b]	0.313[a]
15	10 AM	0.106[b]	0.465[a]
	4 PM	0.088[b]	0.451[a]
16	10 AM	0.075[b]	0.499[a]
	4 PM	0.182[b]	0.596[a]
17	10 AM	0.153[b]	0.589[a]
	4 PM	0.075[b]	0.494[a]
18	10 AM	0.132[b]	0.536[a]
	4 PM	0.198[b]	0.588[a]
19	10 AM	0.234[b]	0.584[a]
	4 PM	0.160[b]	0.509[a]
Mean[3]		0.112[b]	0.459[a]

[a,b]Means within a row with no common superscript differ significantly ($p \leq 0.05$). [1]71readings were used to calculate the means for each location at each time of day within day of incubation. Pooled SEM: 0.0267. [2]TE: Thermal gradient between the air cell and eggshell at the equator of the egg and TL: Thermal gradient between the air cell and eggshell at the large end of the egg. [3]1,065 readings (71 eggs×15 time periods) were used to calculate the means for each location. Pooled SEM: 0.0156

Table 3: Partial correlation coefficients (p-values) between broiler hatching egg variables across the 10 AM and 4PM time periods between 12 and 19 days of incubation[1,2]

Item	EST	LST
ACT	0.041	0.026
	(0.220)	(0.4330)
EST	----	0.2610
		(0.0001)

[1]A total of 1,065 observations were used for each partial correlation coefficient. [2]ACT: Air cell temperature, EST: Eggshell temperature at the equatorial region of the egg and LST: Eggshell temperature at the large end of the egg

Table 4: Partial correlation coefficients (p-values) of LSTH at 19 days of incubation with TE and TL at 4 PM on Day 12 and at 10 AM and 4 PM on Days 13, 14, 15, 16, 17, 18 and 19 of incubation[1,2]

Day of Incubation	Time of day	TE	TL
12	10 AM	----	----
	4 PM	-0.127 (0.318)	-0.113 (0.376)
13	10 AM	-0.139 (0.274)	-0.190 (0.133)
	4 PM	-0.113 (0.375)	-0.120 (0.345)
14	10 AM	-0.084 (0.510)	-0.044 (0.730)
	4 PM	0.002 (0.990)	-0.066 (0.606)
15	10 AM	-0.035 (0.784)	-0.071 (0.580)
	4 PM	-0.051 (0.688)	-0.032 (0.801)
16	10 AM	-0.163 (0.199)	-0.272 (0.029)
	4 PM	-0.155 (0.222)	-0.258 (0.039)
17	10 AM	-0.315 (0.011)	-0.329 (0.008)
	4 PM	-0.224 (0.075)	-0.300 (0.016)
18	10 AM	-0.178 (0.159)	-0.268 (0.033)
	4 PM	-0.157 (0.217)	-0.197 (0.119)
19	10 AM	-0.137 (0.281)	-0.139 (0.275)
	4 PM	-0.194 (0.127)	-0.157 (0.219)
Overall		-0.224 (0.075)	-0.272 (0.030)

[1]A total of 71 observations were used for each partial correlation coefficient. [2]LSTH: Eggshell thickness at the large end of the egg, TE: Mean difference between air cell and equator eggshell temperature and TL: Mean difference between air cell and large end eggshell temperature.

DISCUSSION

Peebles *et al.*[20] concluded that ACT readings using a transponder is more reliable and accurate in determining embryo core body temperature than measuring eggshell temperature with an infrared thermometer. This is largely because ACT using a transponder evades the effects of the thermal barrier properties of the eggshell and particularly the flow of air across its surface. Ozcan *et al.*[27] have more specifically reported that average eggshell temperature is primarily influenced by air temperature and velocity at the inlet of an incubator, as well as the metabolic heat that the embryo produces and to a smaller extent by the thermal conductivity and emissivity of the egg. The results of the current study have shown that in the 12-19 DOI time interval, ACT was higher than ESTat 13 of the 15 time periods and that EST was higher than LST at all 15 time periods. This would further support the contention that eggshell surface temperatures are highly influenced by air temperature and the airflow rate across the egg[26,13]. These relationships are also in agreement with previous studies by Peebles *et al.*[20]. Additionally, Peebles *et al.*[20] demonstrated that ACT readings using a transponder were significantly higher than EST readings using either a transponder or infrared thermometer and that transponder and infrared EST readings were similar and not significantly different.

The heat produced by the embryo is associated with its level of metabolism during development[16]. During the first half of incubation, the embryo absorbs heat from the circulating air inside the incubator so that embryo temperature is either below or the same as that of incubation temperature[28]. During the second half of incubation the embryo undergoes rapid growth and development and subsequently produces metabolic heat. Pulikanti *et al.*[29] has documented a progressive increase in ACT between 10.5 and 18.0 DOI that is commensurate with an increase in embryo metabolism and temperature. The observation that ACT was not significantly correlated with EST and LST is indicative of the inability of EST and LST to fully detect the metabolic heat produced by the embryo throughout the12-19 DOI period. These data likewise suggest that because EST is consistently higher than LST between 12 and 19 DOI, a larger proportion of the metabolic heat produced by the embryo is transferred to the shell surface at the equator than at the large end and would be further due to the closer proximity of the embryo to the eggshell surface at the equator than at the large end. The large end represents a greater barrier for the transfer of heat from the embryo to the shell surface than does at the equatorial region. Transponders used for ACT readings do not

have direct contact with the eggshell but rather lie on the inner air cell membrane, to which the embryo likewise has direct contact. The lower coefficient for the partial correlation between ACT and LST in comparison to that between ACT and EST, supports the contention that the air space in the air cell acts as a large buffer zone that interferes with the transfer of metabolic heat from the embryo to the eggshell at the large end and further reduces the relationship between ACT and eggshell temperature. The results of work by Abbasnezhad *et al.*[30] have shown that the air cell acts as a heat insulator and that an increase in air cell volume results in a decrease in the rate of heat transfer. Nevertheless, the significant positive correlation between EST and LST indicates that both share similar responses to changes in factors that influence them and although EST is higher than LST, their responses fluctuate similarly.

The lack of significant correlations between LSTH and ACT, EST, or LST across the 2 time periods in the 12-19 DOI interval would imply that the range of differences observed in LSTH at the large end of the egg in this study had no impact on the shell surface temperatures recorded at either the large end or equator. The overall significant negative correlation between LSTH and TL across the 2 time periods in the 12-19 DOI interval would likewise advocate that an increased LSTH in the range examined does not lead to a greater difference between ACT and LST. However, this negative relationship could indicate that a thinner shell would allow for a greater increase in ACT due to the increased assimilation of heat derived from the incubator in addition to that produced by the embryo itself, thereby increasing the difference between ACT and LST. This negative relationship may be reduced and become non-significant ($p = 0.075$) at the equator (for correlation of LSTH and TE) due to a subsequent increase in the shell's absorption of heat emanated from the embryo, as a result of the closer proximity of the embryo to the shell. Nevertheless, because mean TL (0.458°C) was higher in comparison to that of TE (0.111°C), these results indicate that the eggshell itself poses as a significant thermal barrier[20]. Although TE and TL were positively correlated, the greater TL value in comparison to that of TE is further confirmation that the dead air space in the air cell is a contributing factor to this difference.

CONCLUSION

In conclusion, the results of this study suggest that the eggshell poses as a thermal barrier and the use of transponders for measuring ACT will help to evade or minimize this effect. Nevertheless, further study is needed to better determine the possible differential roles of equator and large end eggshell thickness as thermal barriers in broiler hatching eggs. More so, EST and LST, as well as ACT may be used as viable means of estimating embryo temperature during incubation. However, ACT is the most accurate means of determining actual embryo temperature when compared to EST and LST, because it evades the confounding effects of the eggshell and the flow of air across the surface of the eggshell. A higher level of temperature sensitivity is likewise achieved with the use of transponders in the air cell because of their close proximity to the developing embryo. This will help in the development and design of thermistor probes and the timing of their implantation in the air cell for determining accurate embryo temperature.

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