Research & Reviews: Journal of Agriculture and Allied Sciences

e-ISSN:2347-226X
p-ISSN:2319-9857

Broiler Hatching Egg Air Cell: Air Cell Size Profile and its Relationship to Shell Temperature and Weight Loss in Ross 708 Broiler Hatching Eggs between 3 and 12 Days of Incubation

Durojaye OA[1], To SDF[2], Elliott KEC[1], Gerard PD[3] and Peebles ED[1]*

[1]Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762, USA
[2]Department of Agricultural and Biological Engineering, Mississippi State University, Mississippi State, MS 39762, USA
[3]Department of Mathematical Sciences, Clemson University, Clemson, SC 29634, USA

Research Article

Received date: 29/09/2018
Accepted date: 17/12/2018
Published date: 24/12/2018

***For Correspondence**

E. David Peebles, Professor, Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762, USA

E-mail: d.peebles@msstate.edu

Keywords: Air cell, Broiler embryo, Eggshell temperature, Incubation, Water loss

ABSTRACT

Air cell size profile during incubation and its relationship with eggshell temperature (EST) and cumulative percentage egg weight loss (CPEWL) in Ross 708 broiler hatching eggs was determined. On each of the 4 middle tray levels of a single-stage incubator, 72 eggs were weighed and set. On each level, 7 different eggs were randomly selected at the same time each d between 3 and 12 days of incubation (doi) to measure egg weight, EST, air cell depth (ACD), total egg volume, and air cell volume (ACV). Daily CPEWL and relative ACV (RACV; percentage of total egg volume) of each egg was calculated. Mean daily CPEWL (R^2=0.8687), EST (R^2=0.4757), ACD (R^2=0.7106), ACV (R^2=0.8424) and RACV (R^2=0.8447) increased, whereas egg weight (R^2=0.2722) decreased between 3 and 12 doi. Between 3 and 12 doi, ACD increased by 0.43 cm, which averaged approximately 0.048 cm per day. Mean ACD and ACV, respectively, increased from 0.87 cm and 0.97 mL on 3 doi to 1.30 cm and 4.53 mL on 12 doi. Moreover, across 3 and 12 doi, CPEWL was positively correlated with ACD, ACV, and RACV, and EST was positively correlated with RACV. Within doi, CPEWL was observed to have a significant positive correlation with ACD from 3 to 5 and 7 to 12 doi, and with ACV and RACV from 4 to 12 doi. To maintain their close proximity to the inner shell membrane, the dimensions of temperature sensing devices and the timing of their insertion into the air cells of broiler hatching eggs should be commensurate with the described changes in ACD and ACV between 3 and 12 doi. Furthermore, the relationships described between CPEWL, EST, and air cell size (ACD, ACV, and RACV) confirm that CPEWL is the major factor in determining air cell size profile throughout incubation, and that the additional influence of EST can become manifest through its effect on RACV.

INTRODUCTION

The air cell is the air-filled area occupying the space at the large end of the egg, which results from the separation of the inner and outer shell membranes as the liquid contents of the egg contract [1]. Eggshell porosity and the temperature and humidity of the surrounding environment influence embryonic metabolism as well as the size and vital gas [oxygen (O_2) and carbon dioxide (CO_2)] contents of the air cell [2-8].

Sufficient egg water loss and exchange of O_2 and CO_2, by passive diffusion across pores in the shell, are required to ensure proper air cell formation for facilitation of the hatching process and the subsequent initiation of pulmonary respiration [9-13]. When the hatchling pips internally, air from the air cell fills its lungs and air sacs [14], so that diffusion replaces convection as the means

e-ISSN:2347-226X
p-ISSN:2319-9857

of gas exchange between the bird and its environment. The interval of time between internal and external pipping is dependent upon the partial pressures of O_2 and CO_2 in the air cell [8], with CO_2 acting as a stimulant for external pipping [15].

According to Romijn and Roos [1], the properties of the air cell that change during incubation include its volume (**ACV**), and the composition and pressure of the gases it contains. These authors further added that the ACV of a hen's egg gradually increases with its loss of water. Hasselbalch [16] observed that ACV increases as the period of incubation proceeds, and that there is a rectilinear relationship between them. Needham [17] mentioned that the greatest increase in ACV is between 6 and 13 days of incubation (**doi**), and that different eggs on the same day may have a different ACV. Differences in eggshell permeability among different breeds may contribute to this effect.

Upon comparing the eggs from 1955 Athens Canadian Random Bred (**ACRB**) meat-type chickens with those from 2013 Cobb 500 broiler breeder hens, Collins et al. [18] have shown that the shell and internal contents characteristics of broiler hatching eggs have been altered by genetic selection. Collins et al. [18] observed that percentage egg weight (**EW**) loss (**PEWL**) after 18 doi was 2.7% greater in the ACRB eggs, and that eggshell conductance values adjusted or not adjusted for EW, as well as eggshell conductance constants, at 12 and 18 doi were greater in the ACRB eggs. Moreover, ACRB chicks had smaller residual yolk sacs at hatch and hatched 6 h later. It was concluded that Cobb 500 eggs experienced a lower rate of gas exchange across their eggshells during incubation, which was a contributing factor to an earlier hatch and decreased yolk utilization. However, there are no published reports in the literature documenting the air cell size profile of modern commercial broiler hatching eggs during incubation. Furthermore, air cell size in relation to the eggshell temperature (**EST**) and PEWL have not been reported for those eggs during incubation. Therefore, the primary objective of this experiment was to determine the air cell size profile of incubating Ross 708 broiler hatching eggs and its relationship to EST and PEWL. The results of this study will provide information that will further our understanding of the changes in the size of the air cells of modern commercial broiler hatching eggs as incubation progresses.

It has been reported that the air cells of Ross 308 broiler hatching eggs may be implanted with temperature transponders (1.0 cm long, 2.0 mm wide) between 12 and 14 doi without negatively affecting the porosity of the shell or the development of the embryo [19]. Pulikanti et al. [20] also reported that air cell implantation of temperature transponders in those eggs could be used to successfully determine embryo temperature and for the accurate calculation of absolute and relative eggshell conductance. Nevertheless, it was concluded that increased embryo survivability, through improvements in the implantation procedure, would make temperature transponder use more pragmatic [20]. Consequently, the development of minimally intrusive thermistor probe networks that can extend into the air cell, upon insertion through the shell and its outer membrane early in incubation, are likewise in developmental stages [21]. As incubation progresses, the monitoring of air cell depth (**ACD**) as well as ACV is particularly important, as the air cell must be able to accommodate either temperature transponders or inserted probes whose lengths should be adjusted to accommodate changes in ACD. This study was initiated at 3 doi when air cell depth could be more accurately assessed.

Therefore, the knowledge gained from this current research is also basic to the development of pragmatic and accurate probe systems that can be used in the laboratory as well as commercially to record air cell temperature as a means by which to more closely estimate the core body temperature of embryos throughout incubation. The closer a probe tip lies in proximity to the embryo without penetrating the inner eggshell membrane, the more accurate will be a core body temperature reading. Upon accurately monitoring embryo temperature, more precise adjustments in incubational conditions can be made to optimize the metabolism and development of the embryo.

MATERIALS AND METHODS

Incubation

Broiler hatching eggs (Ross 708) were collected from a common commercial broiler breeder flock at 36 weeks of age and were held for approximately 72 h under standard conditions before being set. Only those eggs that were not cracked, misshapen, or dirty and that were within 10% of the average weight of all eggs collected, were marked and included as experimental eggs. A total of 288 experimental eggs were weighed individually and 72 were immediately and randomly set in a vertical position with the large end up on each of 4 middle tray levels in a calibrated Natureform incubator (Model NMC-1080, Natureform, Jacksonville, FL). The incubator was set at 37.5 °C dry bulb and 29.4 °C wet bulb temperatures.

Data collection

Beginning on 3 doi and continuing through 12 doi, EW, EST, ACD, total egg volume, and ACV readings were recorded once daily at the same time (4:00 PM) each day for 7 eggs that were randomly selected on each tray level (approximately 28 eggs in total). Measurements were only recorded for those eggs containing live embryos. Daily cumulative PEWL (**CPEWL**) values within the 3 and 12 doi period were calculated by determining the difference between set EW and EW on that particular d (3, 4, 5, 6, 7,

e-ISSN:2347-226X
p-ISSN:2319-9857

8, 9 10, 11, or 12), dividing that difference by set EW, and then multiplying that value by 100. The EST values were recorded using infrared thermometry (Braun Thermoscan thermometer, IRT 4520, Kronberg, Germany) with an accuracy of ± 0.2 °C. In addition, 1 wireless data logger temperature node (HOBO ZW series wireless, Onset Computer Corporation, Bourne, MA) was used to record air temperature within the incubator.

The ACD of eggs was determined using a principle similar to that designated by Ragni et al. [22], in which the length of a line extending between the middle point of the inner eggshell membrane and a point on the opposite end of the air cell at the large end of the egg was measured. The values for ACD were measured using a white sewing thread dipped in melted paraffin wax, which was then allowed to re-solidify or cool before use. A hole was made at the center of the large end of the egg with a hand-drilling tool (Dremel® micro Inc., Racine, WI), and the thread was inserted through the hole until its tip touched the center of the inner eggshell membrane. A pen or marker was used to make a mark on the thread, where it touched the outer surface of the eggshell at the hole, and it was then cut at the mark. A ruler was used to measure the length in cm of the cut thread.

Similar to the method employed by Rush et al. [23], total egg volume was determined by placing the incubated egg in a graduated glass cylinder, and then by measuring the resultant volume (mL) of the water that was displaced by the egg after complete immersion. The ACV values were measured using 1 mL, 3 mL, or 10 mL syringes, and the needle sizes used were 18 or 21 gauge. To avoid the effects of water surface tension, a surfactant (Triton™ X-100 Laboratory grade, Sigma-Aldrich Co., St. Louis, MO) was used (one drop per 500 mL of water) at all time periods in the water that was dispensed into the air cells of eggs. In the process of dispensing the water from the needle and syringe into the air cell, precaution was taken to not allow the needle to puncture the inner eggshell membrane. The amount of water dispensed by the syringe was calculated by subtracting the water left in the syringe from the initial amount of water in the syringe before use. Furthermore, the weight of the egg before the water was added was subtracted from the weight of the egg with the added water. The difference in weight was converted to a unit of volume using the estimate that a 1 mL volume of water is equal to 1 g of weight. This calculation was performed as a means to confirm the accuracy of the measurement of ACV by the previous method. Relative air cell volume (**RACV**) was calculated by dividing ACV by total egg volume, which was then multiplied by 100.

Statistical analysis

A completely randomized experimental design was used, with individual egg serving as a replicate unit for the analyses of EW, CPEWL, EST, ACD, ACV, and RACV. The 9.4 version of SAS [24] was employed for the following analyses: One-way ANOVA of PROC MIXED for effects of doi on ACD, ACV, and RACV; Multivariate ANOVA of PROC GLM for partial correlation of CPEWL with EST, and for partial correlations of CPEWL and EST with ACD, ACV, and RACV across doi; Multivariate ANOVA of PROC GLM for partial correlations of CPEWL with EST, ACD, ACV, and RACV within doi; and PROC REG for linear regression of all variables. Individual egg values were used for each variable included in the regression and partial correlation data analyses, and tray level served as a random factor in the PROC MIXED procedure. Graphs and linear trend lines based on the means at each doi for all variables were generated using Excel version 2016 [25]. A P-value less than or equal to 0.05 was used to test for the significance of the Pearson's Product-Moment partial correlation coefficients, and to test whether the dependent or response variables varied significantly with time (independent variable) in the linear regression analyses.

RESULTS

Across the 3 to 12 doi period, CPEWL exhibited highly significant (P<0.0001) positive correlations with ACD, ACV, and RACV. Furthermore, there was a significant (P ≤ 0.030) positive correlation between EST and RACV across the 3 to 12 doi period **(Table 1)**. However, within doi **(Table 2)**, CPEWL was observed to have a significant positive correlation with ACD from 3 to 5 doi (P ≤ 0.028) and 7 to 12 doi (P ≤ 0.024), and with ACV and RACV from 4 to 12 doi (P ≤ 0.045). A positive correlation between CPEWL and EST also approached statistical significance at 5 (P=0.063) and 8 (P=0.059) doi. The slopes, and R^2 and P values for the linear regression analyses of the individual egg values for EW, CPEWL, EST, ACD, ACV, and RACV, from 3 to 12 doi, are displayed in **Figures 1-7**, respectively. The trend lines for the doi means of each of the variables are likewise provided in those figures. Between 3 and 12 doi, EW decreased (R^2=0.2722, P<0.0001), whereas CPEWL (R^2=0.8687, P<0.0001), EST (R^2=0.4757, P<0.0001), ACD (R^2=0.7106, P<0.0001), ACV (R^2=0.8424, P<0.0001), and RACV (R^2=0.8447, P<0.0001) increased with doi.

Table 1. Partial correlation coefficients (P-values) between broiler hatching egg variables across the 3 to 12 d of incubation period[1,2].

Item	CPEWL	ACD	ACV	RACV
CPEWL	------- (-------)	0.629 (< 0.0001)	0.691 (< 0.0001)	0.725 (< 0.0001)
EST	0.093 (0.138)	0.051 (0.416)	0.096 (0.126)	0.137 (0.030)

[1]A total of 265 observations were used for the determination of each correlation coefficient.
[2]CPEWL = cumulative percentage egg water loss; EST = eggshell temperature; ACD = air cell depth, ACV = air cell volume; and RACV = relative air cell volume.

e-ISSN:2347-226X
p-ISSN:2319-9857

Table 2. Daily partial correlation coefficients (P-values) between cumulative percentage egg weight loss and other broiler hatching egg variables from 3 to 12 days of incubation[1,2].

Day of Incubation	EST	ACD	ACV	RACV
3	-0.298 (0.148)	0.439 (0.028)	-0.021 (0.920)	-0.028 (0.895)
4	- 0.169 (0.440)	0.889 (< 0.0001)	0.681 (0.0003)	0.684 (0.0003)
5	0.385 (0.063)	0.583 (0.003)	0.822 (<0.0001)	0.860 (<0.0001)
6	0.278 (0.188)	0.285 (0.177)	0.678 (0.0003)	0.682 (0.0002)
7	0.255 (0.241)	0.468 (0.024)	0.471 (0.023)	0.421 (0.045)
8	0.400 (0.059)	0.667 (0.0005)	0.807 (<0.0001)	0.790 (<0.0001)
9	0.053 (0.809)	0.759 (< 0.0001)	0.572 (0.004)	0.639 (0.001)
10	-0.077 (0.722)	0.733 (< 0.0001)	0.887 (<0.0001)	0.873 (<0.0001)
11	0.061 (0.793)	0.710 (0.0003)	0.859 (<0.0001)	0.919 (<0.0001)
12	0.163 (0.436)	0.613 (0.001)	0.845 (<0.0001)	0.941 (<0.0001)

[1]Observations (N) by day (d) for the determination of each daily correlation coefficient: 3(d) = 28 (N); 4(d) = 26 (N); 5(d) = 27 (N); 6(d) = 27 (N); 7(d) = 26 (N); 8(d) = 26 (N); 9(d) = 26 (N); 10(d) = 27 (N); 11(d) = 24 (N); 12(d) = 28 (N).

[2]EST = eggshell temperature; ACD = air cell depth; ACV = air cell volume; and RACV = relative air cell volume.

In the 3 and 12 doi period, EST increased from a low of 37.5 ± 0.03 to a high of 38.1 ± 0.03°C, and EW decreased from 61.3 ± 0.66 to 55.2 ± 0.66 g. During that same period of time, CPEWL was 7.25 ± 0.188%, and mean PEWL per d was 0.606 ± 0.0102%. The daily ACD values and their differences from 3 to 12 doi, are further displayed in bar graph form in **Figure 4** for more detailed reference. Mean ACD increased from 0.87 ± 0.015 cm at 3 doi to 1.30 ± 0.019 cm at 12 doi, with significant increases occurring between 3 and 5, 5 and 6, 6 and 8, and 8 and 11 doi. The significant increases in ACD between those doi intervals represented respective numerical increases of 0.077, 0.078, 0.063, and 0.165 cm. Overall, ACD values increased by 0.43 cm across the 3 to 12 doi period, which averaged approximately 0.048 cm per day.

In association with the observed increase in ACD between 3 and 12 doi, mean ACV increased from 0.97 ± 0.073 mL at 3 doi to 4.53 ± 0.111 mL at 12 doi, with significant increases occurring between 4 and 5, 5 and 6, 6 and 7, 7 and 8, 8 and 9, 10 and 11, and 11 and 12 doi. The significant increases in ACV between those doi intervals represented respective numerical increases of 0.881, 0.335, 0.354, 0.351, 0.305, 0.564, and 0.434 mL. Overall, ACV values increased by 3.56 mL across the 3 to 12 doi period, which averaged approximately 0.396 mL per d. In addition, mean RACV increased from 1.68 ± 0.162% at 3 doi to 8.09 ± 0.162% at 12 doi, with significant increases also occurring between 4 and 5, 5 and 6, 6 and 7, 7 and 8, 8 and 9, 10 and 11, and 11 and 12 doi. The significant increases in RACV between those doi intervals represented respective numerical increases of 1.518, 0.640, 0.678, 0.622, 0.571, 0.890, and 0.898%. Overall, RACV values increased by 6.41% across the 3 to 12 doi period, which averaged approximately 0.712% per day.

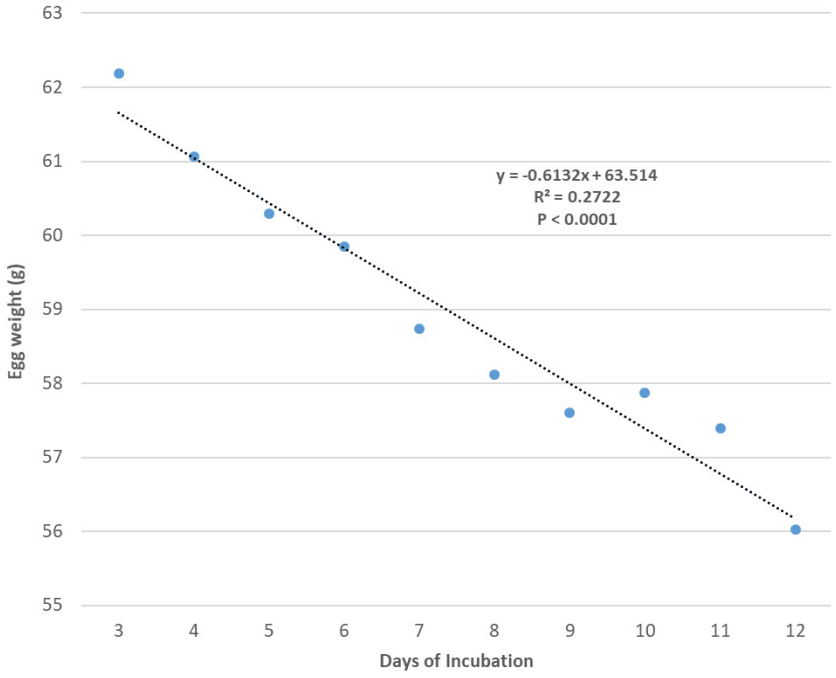


Figure 1. Daily mean egg weight values from 3 to 12 days of incubation.

e-ISSN:2347-226X
p-ISSN:2319-9857

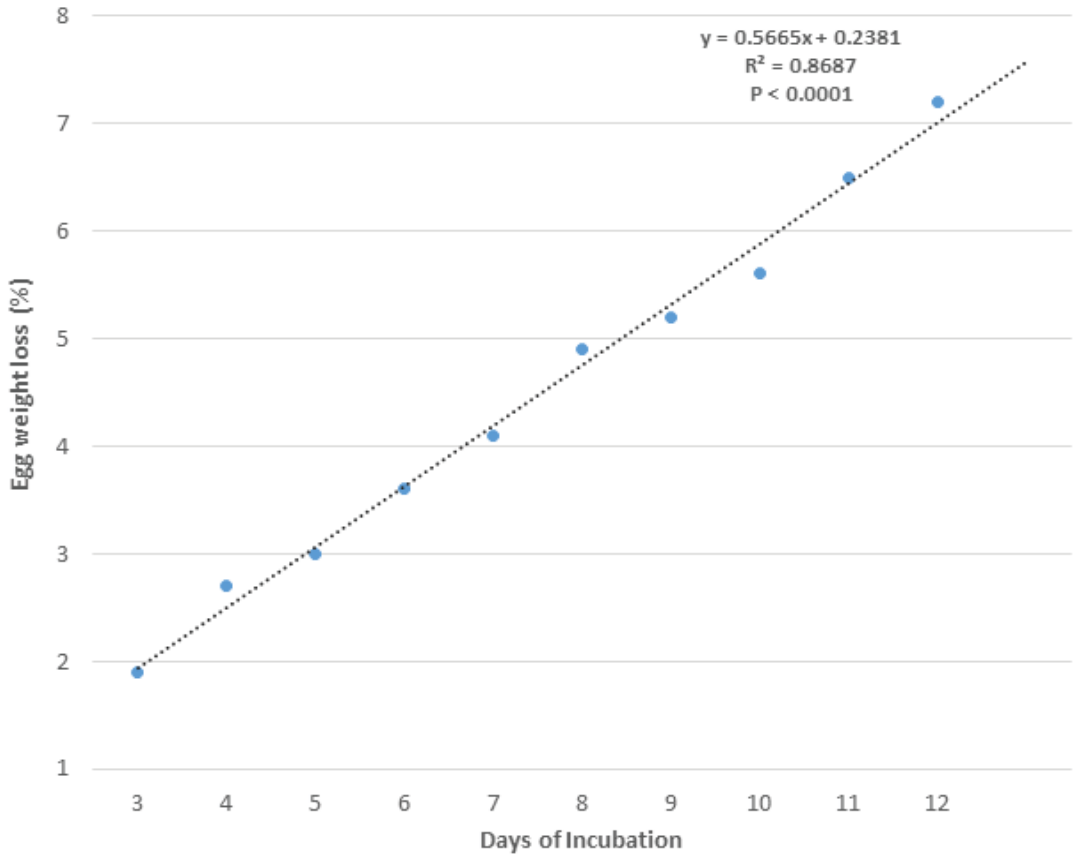


Figure 2. Daily mean cumulative percentage egg weight loss values from 3 to 12 days of incubation.

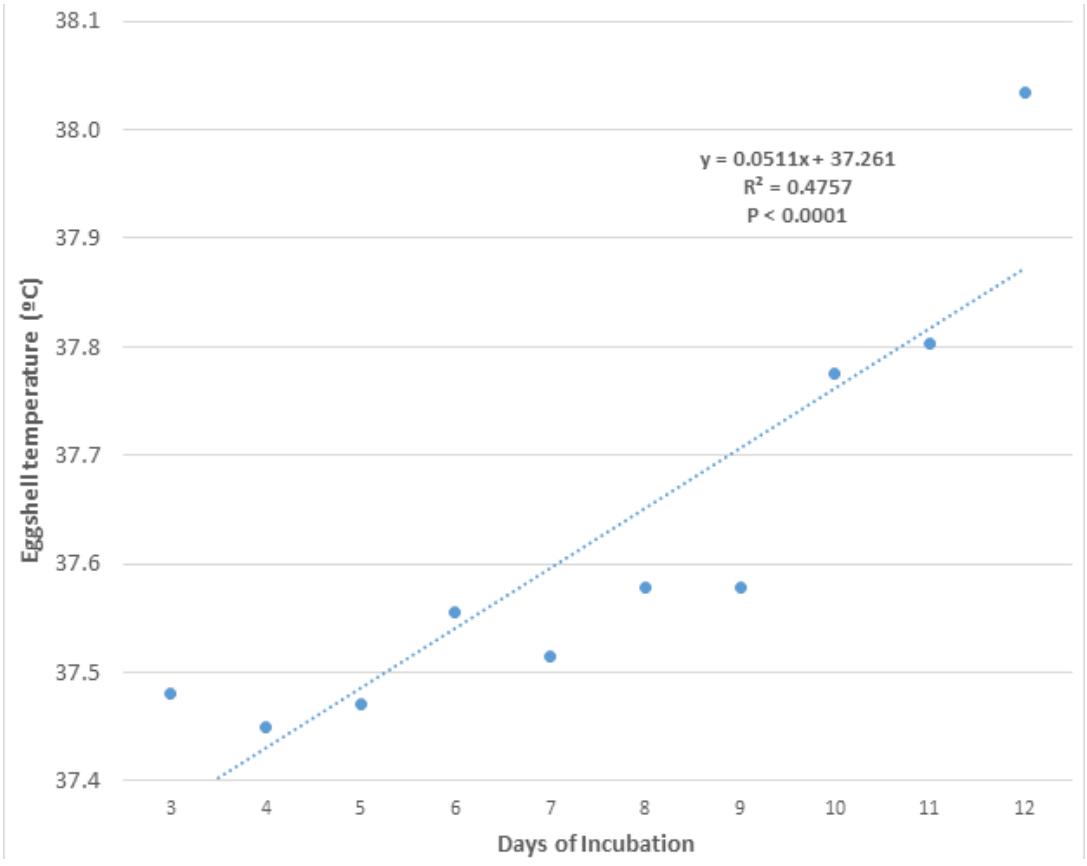
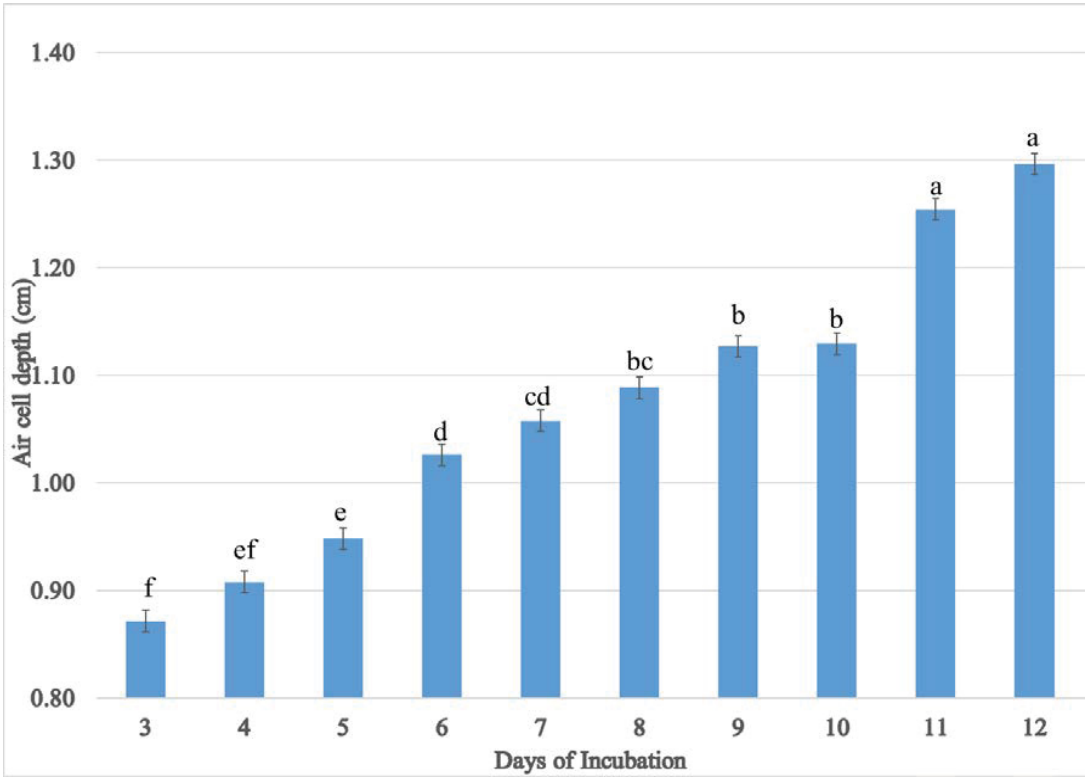


Figure 3. Daily mean eggshell temperature readings from 3 to 12 days of incubation.

e-ISSN:2347-226X
p-ISSN:2319-9857



[a-f]Means with no common superscript differ significantly (P ≤ 0.05).

Figure 4. Daily mean air cell depth readings from 3 to 12 d of incubation.

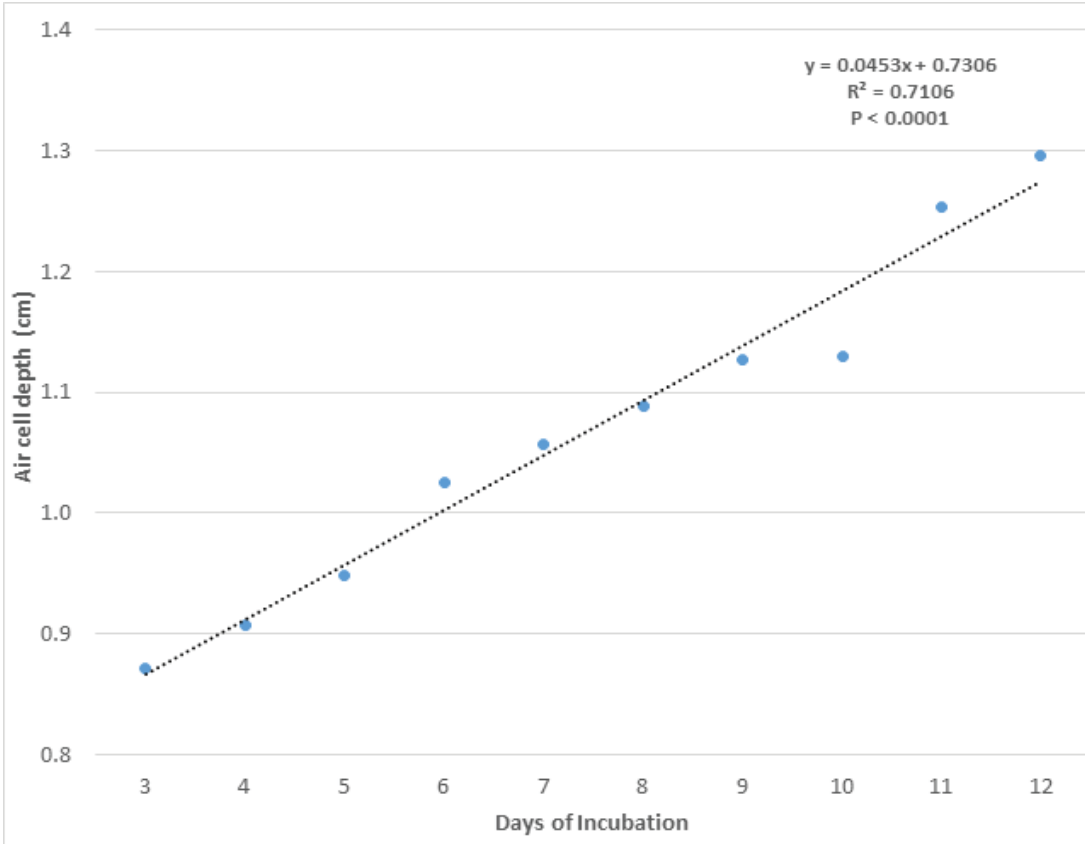


Figure 5. Daily mean air cell depth values from 3 to 12 days of incubation.

e-ISSN:2347-226X
p-ISSN:2319-9857

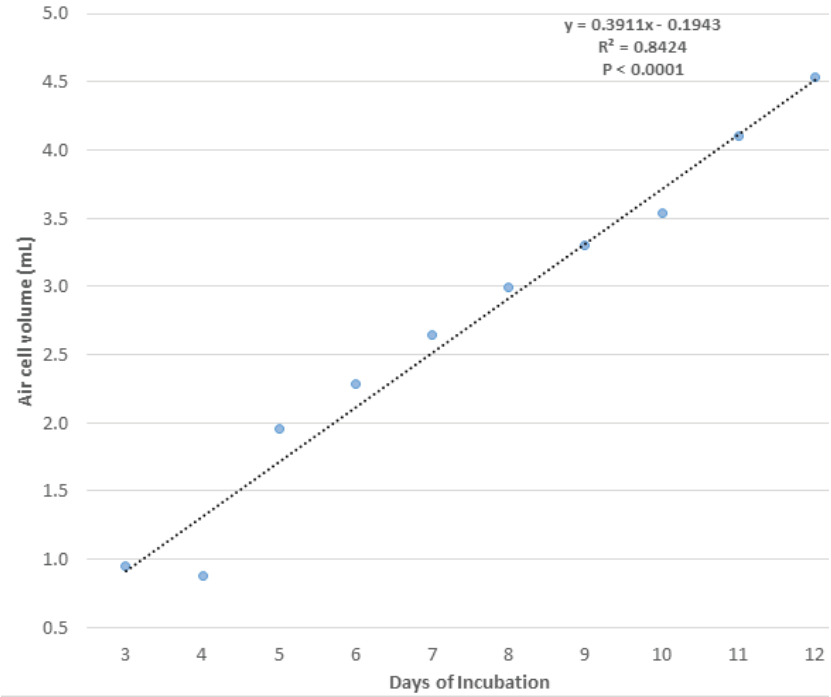


Figure 6. Daily mean air cell volume values from 3 to 12 days of incubation.

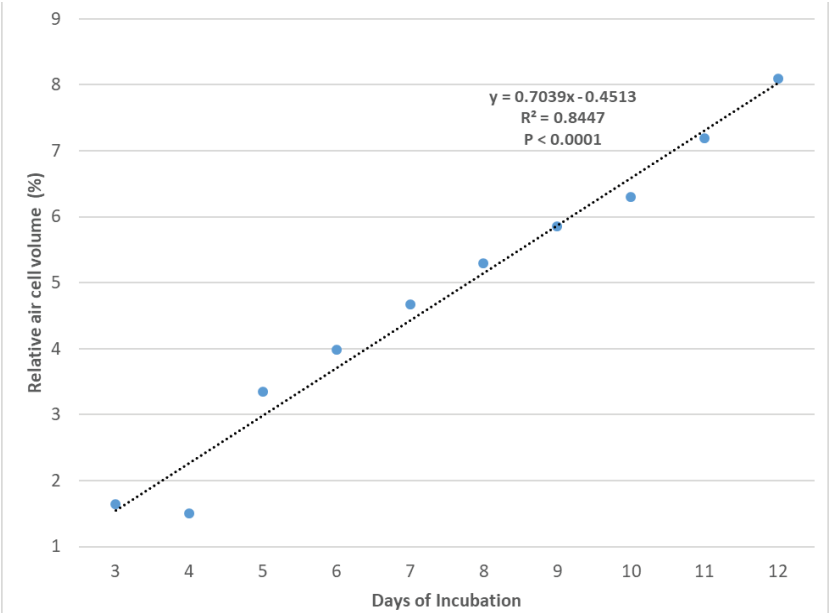


Figure 7. Daily mean relative air cell volume values from 3 to 12 days of incubation.

DISCUSSION

Incubation temperature is known to affect egg weight loss [26], embryo metabolism and development [27,28], hatchability [29], hatching BW [30], and posthatch performance [30,31]. Heat generated by the incubator and developing embryo contribute to EST [32,33], and metabolic heat liberated by the developing embryo contributes to the increase in EST during the last half of incubation [26,34,35]. Nevertheless, upon examining the relationship between CPEWL and EST across and within doi, a statistically significant positive correlation between the 2 variables was not observed in this study. Although EST can contribute to CPEWL through its effects on the water vapor pressure gradient across the eggshell [20,32,36,37], the lack of a significant positive correlation between the 2 variables indicates that EST itself plays a more minor or indirect role than that of other factors in dictating CPEWL in the 3 to 12 doi period. Other more potentially influential factors would include environmental humidity and eggshell structure. However, a perceptible influence of EST on CPEWL became manifest through its ultimate positive correlation with RACV. It is noteworthy that EST was correlated with air cell volume only when it was expressed as a proportion of total egg volume. Therefore, the size of the egg can confound the relationship between EST and air cell size or volume. Nevertheless, further information is needed to more systematically define the precise relationship between EST and the air cell profile of broiler hatching eggs throughout the entire incubational period, including the relative involvements of CPEWL and embryo metabolism in that relationship.

e-ISSN:2347-226X
p-ISSN:2319-9857

The air cell in avian eggs is known to increase in size as water vapor is lost from the egg during incubation [2,38]. Likewise, mean ACD, ACV and EST of the Ross 708 broiler hatching eggs in this study were shown to increase with doi, and as would be expected, the progressive increase in ACV with doi corresponded with a progressive increase in ACD. The observed progressive increases in ACD and ACV were also associated with the loss of water from the egg and a subsequent decrease in EW. These relationships were further established by the significant positive correlation that CPEWL had with ACD and ACV across the entire 3 to 12 doi time interval, and by the significant correlation that CPEWL had with both ACV and ACD at 8 of the 10 time periods within the 3 to 12 doi interval.

The earlier established rectilinear relationship between doi progression and increase in ACV observed by Hasselbalch [16] was likewise observed in the Ross 708 eggs of the current study. Furthermore, when expressed as a percentage of total egg volume, the relative ACV (RACV) of these eggs correspondingly maintained this same relationship with doi. However, the current data contrast with an earlier report by Needham [17], in which it was stated that the greatest increase in ACV occurred between 6 and 13 doi. In the Ross 708 eggs of this study, a 37% increase in ACV occurred between 3 and 6 doi, which corresponded to a 0.44 mL daily increase in ACV within that same period. This was in conjunction with a 12.02% daily increase in RACV between 3 and 6 doi, which equaled a 36.06% total increase for that period. On the other hand, between 6 and 12 doi, the average daily increase in ACV was 0.37 mL, and the average daily increase in RACV was 10.7%. The lower daily increases in ACV and RACV in the 6 to 12 doi period in comparison to those in the 3 to 6 doi period suggest that these eggs from this modern strain of broiler breeder may allow for an earlier or more rapid rate of air cell development, which may be related to differences in embryo metabolism and eggshell permeability.

In concurrence with increases in ACD and ACV with doi, it is suggested that air cell size between 6 and 12 of doi will be adequate for the pragmatic implantation of thermistor probes in the air cells of Ross 708 broiler hatching eggs for more accurately estimating the core body temperature of embryos. Nevertheless, as ACD increases between 3 and 12 doi, the depth of thermistor probes inserted into the air cell should be adjusted to increase by as much as 0.048 cm per d to allow the tip of the probe to remain in close proximity to the inner shell membrane. This will optimize the accuracy of subsequent embryo temperature readings throughout the 3 to 12 doi period.

ACKNOWLEDGMENT

This work was funded by grant No. 58-6406-4-016 from the United States Department of Agriculture (USDA).

REFERENCES

1. Romijn C and Ross J. The air space of the hen's egg and its changes during the period of incubation. J Physiol. 1938;94:365-379.
2. Ar A and Rahn H. Water in the avian egg: overall budget of incubation. Am Zool. 1980;20:373-384.
3. Rahn H and Ar A. Gas exchange of the avian egg: time, structure, and function. Am Zool. 1980;20:477-484.
4. Burton FG, et al. The relationship between eggshell porosity and airspace gas tensions measured before and during the parafoetal period and their effects on the hatching process in the domestic fowl. Resp Physiol. 1989;77:89-100.
5. Rahn H and Paganelli CV. Energy budget and gas exchange of avian eggs. In: Avian Incubation (Tullett SG Edn). Butterworth-Heinemann, London. 1991;pp:175-193.
6. Carey C. Incubation in extreme environments. In: Avian Incubation: Behaviour, Environment, and Evolution (Deeming DC Edn). Oxford University Press, New York. 2002;pp:238-253.
7. Deeming DC. Functional characteristics of eggs. In: Avian Incubation: Behaviour, Environment, and Evolution (Deeming DC Edn). Oxford University Press, New York. 2002;pp:28-42.
8. Deeming DC. Embryonic development and utilization of egg components. In: Avian Incubation: Behaviour, Environment, and Evolution (Deeming DC Edn). Oxford University Press, New York. 2002;pp:43-53.
9. Rahn H and Ar A. The avian egg: incubation time and water loss. Condor 1974;76:147-152.
10. Rahn H, et al. The avian egg: air cell gas tension, metabolism and incubation time. Resp Physiol. 1974;22:297-309.
11. Paganelli CV, et al. The avian egg: In vivo conductances to oxygen, carbon dioxide, and water vapour in late development. In: Respiratory Function in Birds, Adult and Embryonic (Piiper J Edn). Springer-Verlag, Berlin. 1978;pp:212-218.
12. Rahn H, et al. How bird eggs breathe. Sci Amer. 1979;240:46-55.
13. Paganelli CV. The physics of gas exchange across the avian eggshell. Am Zool. 1980;20:329-338.
14. Duncker HR. Development of the avian respiratory and circulating systems. In: Respiratory Function in Birds, Adult and Embryonic (Piiper J Edn). Springer-Verlag, Heidelberg. 1978;pp:260-273.

15. Visschedijk AHJ. The air space and embryonic respiration: 3. The balance between oxygen and carbon dioxide in the air space of the incubating chicken egg and its role in stimulating pipping. Br Poult Sci. 1968;9:197-210.

16. Hasselbalch KA. The production of oxygen in hens' egg. Skand Arch Physiol. 1902;13:170-192.

17. Needham J. Chemical embryology 2. Cambridge University Press. Cambridge, UK. 1931;3:482.

18. Collins KE, et al. Egg characteristics and hatch performance of Athens Canadian Random Bred 1955 meat-type chickens and 2013 Cobb 500 broilers. Poult Sci. 2014;93:2151-2157.

19. Pulikanti R, et al. Physiological responses of broiler embryos to in ovo implantation of temperature transponders. Poult Sci. 2011;90:308-313.

20. Pulikanti R, et al. Use of implantable temperature transponders for the determination of air cell temperature, eggshell water vapor conductance, and their functional relationships in embryonated broiler hatching eggs. Poult Sci. 2011;90:1191-1196.

21. Durojaye OA. The development of a pragmatic methodology for the more accurate determination of the core body temperature of broiler embryos during incubation (M.S. Thesis). Mississippi State University, Mississippi State, 2017.

22. Ragni L, et al. Quality evaluation of shell eggs during storage using a dielectric technique. Trans ASABE. 2007;54:1331-1340.

23. Rush SA, et al. A precise water displacement method for estimating egg volume. J Field Ornithol. 2009;80:193-197.

24. SAS Institute. SAS Proprietary software 9.4. SAS Inst. Inc., Cary, NC, USA, 2012.

25. Microsoft Office. Microsoft Office Professional, 2016. Microsoft, Inc. Redmond, WA, 2016.

26. Ar A and Sidis Y. Nest microclimate during incubation. In: Avian Incubation: Behaviour, Environment, and Evolution (Deeming DC Edn). Oxford University Press, New York. 2002;pp:143-160.

27. Wilson HR. Physiological requirements of the developing embryo: temperature and turning. In: Avian Incubation (Tullett SG Edn). Butterworth-Heinemann, London. 1991;pp:145-156.

28. Calder WA. Characteristics and constraints of incubation in hummingbirds. In: Avian Incubation: Behaviour, Environment, and Evolution (Deeming DC Edn). Oxford University Press, New York. 2002;pp:207-222.

29. French NA. Effect of incubation temperature on the gross pathology of Turkey embryos. Br Poult Sci. 1994;35:363-371.

30. Maatjens CM, et al. Temperature during the last week of incubation. II. Effects on first week broiler development and performance. Poult Sci. 2016;95:2136-2144.

31. Lopez JC, et al. Effect of eggshell temperature on survival rate, development at hatch and 7-day growth. J Appl Poult Res. 2018;27:249-252.

32. Meijerhof R and van Beek G. Mathematical modelling of temperature and moisture loss of hatching eggs. J Theor Biol. 1993;165:27-41.

33. French NA. Modelling incubation temperature: the effect of incubation design, embryonic development, and egg size. Poult Sci. 1997;76:124-133.

34. Sotherland PR, et al. Avian eggs: barriers to the exchange of heat and mass. J Exp Zool Suppl. 1987;1:81-83.

35. Janke O, et al. Comparative investigations of heat production and body temperature in embryos of modern chicken breeds. Avian Poult Biol Rev. 2004;15:191-196.

36. Vleck CM. Allometric scaling in avian embryonic development. In: Avian Incubation (Tullett SG Edn). Butterworth-Heinemann, London. 1991;pp:39-57.

37. Lourens A, et al. Effect of eggshell temperature during incubation on embryo development, hatchability and posthatch development. Poult Sci. 2005;84:914-920.

38. Brand Z, et al. Changes in air cell volume of artificially incubated ostrich eggs. S Afr J Anim Sci. 2013;43:98-104.