《Research Note》

Physiological Relationships of the Middle and Late Post-Hatch Performance of Broilers to their Embryo and Eggshell Characteristics^{1,2}

Radhakrishna Pulikanti¹, Edgar D. Peebles¹, Lloyd W. Bennett², Wei Zhai¹ and Patrick D. Gerard³

¹ Department of Poultry Science, Mississippi State University, Mississippi State, Mississippi, 39762, USA

² College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi, 39762, USA

³ Department of Mathematical Sciences, Clemson University, Clemson, SC 29634, USA

Relationships between physiological variables of middle and late post-hatch broilers with their corresponding egg and embryo variables were examined. Sixty Ross×Ross 708 broiler hatching eggs were randomly set on each of 8 replicate tray levels of an incubator. On 10.5 d of incubation, transponders were implanted in the air cells of 4 embryonated eggs per replicate for determination of internal egg temperature (T_{emb}) through d 18.5. On d 18.5, the implanted embryonated eggs were transferred to a hatcher unit where they were monitored for hatch every 12 h. Incubation length, eggshell water vapor conductance (G_{H_2O}), specific G_{H_2O} (g_{H_2O} ; G_{H_2O} adjusted to a 100 g set egg weight basis), and G_{H_2O} constants (K_{H_2O}) of the embryonated eggs were determined. Chicks were placed in corresponding replicate floor pens and were grown out from 0 (21.5 d of incubation) to 48 d of age. On 28 and 48 d post-hatch, approximately 2 birds per pen were necropsied for determination of BW, and the relative weights and moisture concentrations of their liver, breast muscle, and left gastrocnemius muscle. Breast muscle moisture concentration on d 28 was negatively correlated with G_{H_2O} , g_{H_2O} , and K_{H_2O} , and breast muscle moisture concentration on d 48 was negatively correlated with incubation length. However, relative body and breast muscle weights on d 48 were positively correlated with g_{H_2O} and K_{H_2O} , and relative BW on d 48 was positively correlated with incubation length. More importantly, relative breast muscle weight on d 48 post-hatch was negatively correlated with T_{emb} . The variables T_{emb} , G_{H_2O} , g_{H_2O} , and K_{H_2O} may differentially influence the physiological variables in birds during the middle and late post-hatch grow out periods, and bird metabolic and hydration statuses may be adjusted later during the post-hatch period, so that bird BW will eventually reach its genetically predetermined level.

Key words: broiler, eggshell conductance, embryo temperature, post-hatch, transponder

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Introduction

Approximately one third of a broiler's life is spent inside the egg as an embryo, and the factors that influence embryonic life may have an impact on overall broiler processing yield. Therefore, an examination of relationships between pre- and post-hatch physiological variables may be critical

for the optimization of broiler productivity. The existence of relationships between incubation temperature (Lourens *et al.*, 2005; Yalcin *et al.*, 2005; Hulet *et al.*, 2007; Leksrisompong *et al.*, 2007; Molenaar *et al.*, 2011a; Willemsen *et al.*, 2011) and relative humidity (Bruzual *et al.*, 2000a, b) on embryonic and post-hatch broiler development have been reported in earlier research work. Similarly, several researchers have previously suggested that set egg weight (Pinchasov, 1991; Vieira and Moran, 1999; Moran, 2007; Wolanski *et al.*, 2007; Abiola *et al.*, 2008), egg nutritional composition (Moran, 2007), yolk sac nutrient utilization by embryo (Murakami, 1992), eggshell temperature (Lourens *et al.*, 2005; Molenaar *et al.*, 2011b), incubational oxygen (Onagbesan *et al.*, 2007; Celen *et al.*, 2009) and CO₂ concentrations (Everaet *et al.*, 2007; Onagbesan *et al.*, 2007; Willemsen *et al.*, 2008), incubation length (van de Ven *et al.*, 2011), and incubational egg weight loss (Peebles *et al.*, 2005) may also have an effect

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Correspondence: E.D. Peebles, Department of Poultry Science, Mississippi State University, Mississippi State, Mississippi, 39762, United States of America. (E-mail: dpeebles@poultry.msstate.edu)

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on broiler embryogenesis and post-hatch growth and development.

In many of the studies discussed earlier, an examination of the influence of various treatments imposed during incubation on overall broiler post-hatch performance was limited to within 48 h post-hatch. In a companion article, effects of pre-hatch physiological variables such as egg internal temperature and eggshell water vapor conductance on the post-hatch broiler growth and performance of corresponding chicks through 3 d of age were presented by Pulikanti *et al.* (2012b). Except for the aforementioned report by Pulikanti *et al.* (2012b), no studies have been previously conducted to individually examine the relationships of the characteristics of eggs and the embryos they contain with the post-hatch characteristics of the respective birds that hatched from those specific eggs. Although there have been previous reports indicating that eggshell porosity (Tullett and Burton, 1982) or eggshell water vapor conductance (G_{H_2O} ; $mg\ H_2O/d/Torr$; Hocking, 2009) may influence broiler embryo metabolism, growth, and development, information is lacking regarding the influence of G_{H_2O} on the middle and late post-hatch performance of broilers that hatched from those specific eggs.

An in-depth understanding of the relationships between pre- and post-hatch physiological variables is critical for the improvement of broiler incubational and post-hatch grow out management practices so as to achieve optimal broiler processing yield (Bamelis *et al.*, 2005; Peebles *et al.*, 2005). Therefore, the current experiment was conducted on progeny from a Ross 708 broiler breeder flock, to examine specific relationships of egg and embryonic variables, such as embryonated egg internal (air cell) temperature (T_{emb}), incubation length (IL), G_{H_2O} , specific G_{H_2O} (g_{H_2O} ; G_{H_2O} adjusted to a 100 g set egg weight basis) and G_{H_2O} constant (K_{H_2O}), with broiler growth and developmental characteristics through the middle and late terms of the post-hatch grow out period.

Materials and Methods

General

The current experimental protocol was approved by the Institutional Animal Care and Use Committee of Mississippi State University. Ross $\times$ Ross 708 broiler hatching eggs were collected from a 30-wk-old breeder flock, and were held under standard storage conditions for 3 d before setting.

Incubation

On d 0 of incubation, the eggs that weighed within $\pm 10\%$ of the mean weight of all eggs collected and those that were not contaminated or visibly abnormal were incubated. Sixty eggs were randomly labeled and weighed to record their set egg weights, and were then set on each of 8 replicate tray levels (480 total eggs) of a Jamesway Model 500 single stage incubator (Jamesway Incubator Company, Inc., Cambridge, ON, Canada). The eggs were evenly distributed among the 8 central trays to ensure even air flow among all eggs (Pulikanti *et al.*, 2012b) and they were incubated for 18 d under standard commercial conditions at 37.5°C dry bulb and 28.8°C wet bulb temperatures.

Transponder Implantation

On d 10.5 of incubation, the eggs were weighed and candled, and those containing slanted air cells were discarded. Subsequently, on each tray level, 4 embryonated eggs were randomly selected and the air cells of those eggs were implanted with a pre-calibrated temperature transponder (IPTT-300; Bio Medic Data Systems, Inc., Seaford, DE) for determination of T_{emb} . Transponders were implanted in the air cells of 2 unfertilized eggs and these were positioned within 5 cm from each implanted egg on each tray level for determination of unfertilized egg internal (air cell) temperature. Furthermore, 2 sealed water filled plastic vials (10 mL capacity), each containing a transponder, were positioned within 5 cm from each implanted embryonated and unfertilized egg for determination of external microenvironment egg temperature (Pulikanti *et al.*, 2011b; Pulikanti *et al.*, 2012a, b). The materials and procedures used for transponder implantation and temperature data recording in this experiment were as previously described by Pulikanti *et al.* (2011a, b; 2012a, b).

Hatch Monitor

On d 18.5 of incubation, each implanted embryonated egg was candled, and the eggs that contained live embryos were placed in individual hatching baskets that were specifically labeled according to egg numbers. The hatching baskets were subsequently transferred to their corresponding tray levels in a Jamesway Model 500 single stage hatcher unit (Jamesway Incubator Company, Inc., Cambridge, ON, Canada). The eggs in the hatcher were maintained at approximately 36.1°C dry bulb and 27.6°C wet bulb temperatures, and were individually monitored for hatch (hatch rate) every 12 h through d 21.5 of incubation. Hatchability on d 21.5 of incubation, as a percentage of total eggs set and as a percentage of fertilized eggs, was also determined.

Data Collection

Embryo survivability was determined by candling eggs on d 18.5 of incubation, and was further confirmed based on hatch success through d 21.5 of incubation (Pulikanti *et al.*, 2011b). Between 0 and 18 d of incubation, incubator dry and wet bulb temperature readings, and the ambient atmospheric pressure values were recorded every 12 h. Moreover, between 0 and 18 d of incubation, incubator data logger temperature readings were recorded every 5 min using pre-calibrated wireless data loggers (Model TX7U; La Crosse Technology, La Crescent, MN; Pulikanti *et al.*, 2011b; Pulikanti *et al.*, 2012a, b). Furthermore, between 10.5 and 18.0 d of incubation, temperature readings were recorded every 12 h from transponders contained within water vials, unfertilized eggs, and embryonated eggs for the determination of external microenvironment egg temperature, unfertilized egg internal temperature, and T_{emb} , respectively. Moreover, between 0 and 21.5 d of incubation, incubator temperature and relative humidity readings were recorded twice daily, and incubator data logger temperature and relative humidity readings (from 3 data loggers placed equidistantly inside the incubator; Pulikanti *et al.*, 2011a; Pulikanti *et al.*, 2012a, b) were recorded every 5 min.

Eggshell Water Vapor Conductance

Average daily incubational weight (moisture) loss values of embryonated and unfertilized eggs between 10.5 and 18.0 d of incubation were determined for the subsequent calculation of the respective average daily percentage incubational egg weight loss (%EWL) values. Mean values of G_{H_2O} and g_{H_2O} of embryonated and unfertilized eggs were calculated using the procedures and equations described by Ar *et al.* (1974) and Ar and Rahn (1978) and modified by Pulikanti *et al.* (2011b). Based on the hatch monitor data every 12 h between 18.5 and 21.5 d of incubation, IL (d) of individual embryonated eggs was determined. Furthermore, the K_{H_2O} of the embryonated eggs were calculated by using a formula described by Ar *et al.* (1974) and Ar and Rahn (1978).

Post-hatch Grow Out

On d 0 post-hatch (d 21.5 of incubation), the live chicks (at least 2 chicks per tray level) that hatched from the implanted eggs were specifically labeled according to their eggs of origin, and were subsequently used for post-hatch grow out. The chicks were grown out between 0 and 48 d post-hatch on corresponding climate controlled floor pens (8 replicate floor pens), where they were provided ad libitum feed and water and heated brooding, and were maintained under standard industry temperature and relative humidity settings. The chicks were provided starter, grower, and finisher broiler diets according to standard industry feeding regimens.

Necropsy, and Sample Collection and Analyses

On each of d 28 and 48 post-hatch, approximately 2 live birds per pen that were hatched from implanted eggs were used for determination of live bird BW and rectal temperature. Subsequently, those birds were euthanized by cervical dislocation and necropsied for determination of sex and for organ collection. Upon necropsy of each bird, the liver, whole breast muscle, and left gastrocnemius muscle were extracted. Subsequently, relative BW (% of set egg weight), and relative weights (% of live bird BW) of the liver, and of the breast and gastrocnemius muscles were determined (Keralapurath *et al.*, 2010). In addition, the moisture concentrations (%) of the liver and of the breast and gastrocnemius muscles were determined by the procedures used by Peebles *et al.* (1998) and Pulikanti *et al.* (2010).

Analyses of Tissue Nutrient Profiles

Immediately after necropsy, a 0.25 g sample was taken from the liver, breast muscle and gastrocnemius muscle of each bird, and was stored and preserved in a vial containing 10% perchloric acid. Subsequently, the glucose, glycogen, fat, and protein concentrations of the individual liver, breast muscle and left gastrocnemius muscle samples were analyzed. Tissue glucose and glycogen concentrations were determined using the Phenol-Sulfuric acid method as described by Bennett *et al.* (2007). Colorimetric protein estimation was accomplished according to Lowry *et al.* (1951) and colorimetric fat concentration was determined using the methodology of Van Handel (1985). Concentrations of the above tissue variables were expressed as a percentage of fresh sample weight.

Statistical Analysis

Using the GLM procedure of SAS (2003), partial correlations between pre- and post-hatch variables were generated with data for each individual egg and its corresponding bird. The pre-hatch variables included in the correlation analyses were set egg weight, % EWL, IL, T_{emb} , embryonated egg external microenvironment temperature, unfertilized egg internal temperature, incubator dry bulb temperature, incubator data logger temperature, G_{H_2O} , g_{H_2O} , and K_{H_2O} . The post-hatch variables included in the correlation analyses were relative BW (% of set egg weight), and relative weights (% of live bird BW) and moisture concentrations of the liver, breast muscle, and left gastrocnemius muscle of 28- and 48-d-old birds. Partial correlations of the pre-hatch variables with post-hatch variables on d 28 were determined separately from those on d 48. Each individual egg and its corresponding bird were considered as replicate units. The GLM procedure was used to compute the partial correlations in a model that simultaneously fit the variables to be correlated with tray level or floor pen as a fixed effect. The MANOVA statement with the printe option produced the appropriate partial correlations. Partial correlation coefficients were considered significant at $P \leq 0.05$.

Results and Discussion

Significant partial correlations of pre-hatch physiological variables with various post-hatch growth and tissue variables of the corresponding 28- and 48-d-old birds in the current study are presented in Table 1. Breast muscle moisture concentration on d 28 (BRM28) was negatively correlated with G_{H_2O} , g_{H_2O} , and K_{H_2O} . However, breast muscle moisture concentration on d 48 (BRM48) was not significantly correlated with G_{H_2O} , g_{H_2O} , or K_{H_2O} . Embryonic metabolism and internal egg temperature have been shown to increase in response to an increased G_{H_2O} (Hocking, 2009; French, 1997), and changes in the K_{H_2O} of an egg affect its rate of water loss during incubation as do changes in incubational relative humidity (Ar *et al.*, 1974). Moreover, an increased embryonic metabolism subsequently increases tissue metabolic water production (Ar and Rahn, 1980), and evaporative moisture loss from an incubating egg is increased by increases in G_{H_2O} (Ar *et al.*, 1974; Ar and Rahn, 1978). Further, the distribution of water among the various egg and embryonic compartments may be largely influenced by embryo metabolism (Simkiss, 1980).

Based on the information provided by the previous researchers indicated above, it is suggested that an increased rate of water loss during incubation, as reflected by an increased G_{H_2O} , g_{H_2O} , or K_{H_2O} , may result in a lower BRM28. This would imply that the water budgeting properties of broilers during embryonic life may be carried over into the middle post-hatch period. Although breast muscle moisture concentration was inversely related to G_{H_2O} , g_{H_2O} , and K_{H_2O} through d 28, these relationships did not exist through d 48 post-hatch. This suggests that environmental (i.e. water consumption) and physiological (i.e. tissue water retention) factors during the late post-hatch period (d 28 to 48) may

Table 1. Coefficients and *P*-values for statistically significant ($P \leq 0.05$) partial correlations of pre-hatch physiological variables of broilers with respective post-hatch physiological variables on d 28 and 48 of age

Item	BRM28 (%)	BW48 (%)	BRW48 (%)	BRM48 (%)	GW48 (%)	GM48 (%)
T_{emb} (°C)	—	¹ −0.88 (² 0.05)	−0.97 (0.01)	—	—	—
G_{H_2O} (mg H ₂ O/d × Torr)	−0.93 (0.003)	—	0.89 (0.04)	—	—	—
g_{H_2O} (mg H ₂ O/d × Torr × 100 g)	−0.89 (0.01)	0.90 (0.04)	0.96 (0.01)	—	—	—
K_{H_2O}	−0.90 (0.01)	0.92 (0.03)	0.96 (0.01)	—	—	—
IL (d)	—	0.95 (0.01)	—	−0.93 (0.02)	0.88 (0.05)	−0.93 (0.02)

¹ Correlation coefficient.

² *P*-value.

T_{emb} : Embryonated egg air cell temperature.

G_{H_2O} : Eggshell water vapor conductance.

g_{H_2O} : Specific G_{H_2O} .

K_{H_2O} : G_{H_2O} constant.

IL: Incubation length.

BRM28: Breast muscle moisture concentration of 28-d-old bird.

BW48: Relative BW (% of set egg weight) of 48-d-old bird.

BRW48: Relative breast muscle weight (% of live bird BW) of 48-d-old bird.

BRM48: Breast muscle moisture concentration of 48-d-old bird.

GW48: Relative left gastrocnemius muscle weight (% of live bird BW) of 48-d-old bird.

GM48: Left gastrocnemius muscle moisture concentration of 48-d-old bird.

influence the relationship between incubational egg water loss and breast muscle moisture. This also suggests that the post-hatch physiological responses of broilers to the pre-hatch properties of the eggs from which they hatch do not remain the same throughout the post-hatch period, but are highly related to post-hatch age. Therefore, it may not be advisable to make predictions concerning late post-hatch broiler performance in response to various physiological variables during incubation that are based on previous early and middle post-hatch bird performance data.

Mean T_{emb} for the 10.5 to 18.5 d incubation period in the current study was $38.1 \pm 0.05^\circ\text{C}$, which indicates that incubation was conducted under normal temperature conditions (Pulikanti *et al.*, 2012a). This also minimized the possibility of abnormal breast muscle development that may result from extremely low or high incubation temperatures. Relative BW (BW48) and breast muscle weight (BRW48) of birds on d 48 post-hatch were negatively correlated with T_{emb} , suggesting that at a fixed eggshell porosity (i.e., g_{H_2O}), an increased T_{emb} may negatively influence processing yield. However, BW48 and BRW48 were not significantly correlated with incubator dry bulb temperature, the external microenvironment temperature of the embryonated eggs, or with the internal temperature of the unfertilized eggs. This would indicate that it is important to closely and more accurately monitor the internal temperature of embryonated eggs for the estimation of embryonic temperature during

incubation in order to optimize broiler processing yield. These findings support the effective use of temperature transponders to determine egg air cell temperature, as a closer estimation of true broiler embryo temperature, as suggested by Pulikanti *et al.* (2011a, b; 2012a, b) and Peebles *et al.* (2012).

Furthermore, BW48 and BRW48 were positively correlated with g_{H_2O} and K_{H_2O} . Similarly, there existed a positive correlation between G_{H_2O} and BRW48, and a positive correlation between G_{H_2O} and BW48 approached significance ($P=0.06$). These relationships indicate that an improved respiratory functionality of the avian eggshell (i.e., g_{H_2O}) to support an increase in embryonic metabolism (Hocking, 2009) and an associated increase in rate of embryogenesis may have a positive influence on subsequent yield at processing age in broilers. Modern strain broilers, such as the ones used in this study, have resulted from intensive genetic selection to reach maximal processing yields at a faster rate (Berri, 2000). Therefore, it is suggested that when embryos of modern strain broilers exhibiting elevated metabolic and growth rates, are encompassed by eggshells that can accommodate these characteristics, that their genetic potential may be more fully realized. This would be subsequently reflected by an increase in processing yield.

The BRM48 and moisture concentration of the gastrocnemius muscle on d 48 were negatively correlated with IL. However, BW48 and relative weight of the gastrocnemius

muscle of the d 48 bird were positively correlated with IL. This indicates that the birds that hatched after a more prolonged incubation period tended to retain less moisture in their tissues during the late post-hatch period, despite the fact that their relative body and muscle weights were higher compared to the birds that hatched earlier during the incubational period.

The glucose, glycogen, fat, and protein concentrations of the liver, breast muscle, and gastrocnemius muscle on 28 and 48 d post-hatch were not significantly correlated with their corresponding egg and embryo variables. It is speculated that this may be attributable to possible changes in the nutritional status of the broiler through the middle and late terms of the post-hatch grow-out period, which are largely influenced by various external factors including feed consumption rate and feed conversion efficiency rather than by the corresponding pre-hatch physiological variables examined in this study.

In conclusion, the current results further establish that T_{emb} , G_{H_2O} , g_{H_2O} , and K_{H_2O} influence embryogenesis during incubation and subsequent broiler processing BW and breast meat yield. Moreover, these factors may differentially influence various physiological variables in birds during the various phases of the post-hatch grow out period. The initial metabolic and hydration statuses of the birds were negatively influenced by G_{H_2O} , g_{H_2O} , and K_{H_2O} ; however, bird growth, metabolism, and water retention were naturally adjusted later in the post-hatch period to allow bird BW to reach a genetically pre-determined level. Finally, it is apparent that a corresponding increase in g_{H_2O} may be necessary to accommodate an elevated T_{emb} , so as to positively influence processing yield. Nevertheless, the close and accurate monitoring of internal egg temperature during incubation is strongly recommended.

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