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Albumen glucose utilization during embryogenesis and the effects of in ovo glucose supplementation in broiler hatching eggs

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Research Article

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ABSTRACT

Egg albumen glucose (AG) utilization during embryogenesis in broiler hatching eggs was investigated. The AG concentration of fertile eggs was measured daily from 0 to 4 days during storage and at 0, 4, 8, 10, 12, and 14 days of incubation (DOI). Furthermore, effects of in ovo injection of exogenous glucose at 0 DOI on embryogenesis was evaluated. Injected eggs received albumen with or without supplemental glucose. Non-injected (C) and sham-injected (S) eggs served as controls. Eggs in the S treatment group received a 100 µL volume of albumen, and those in a G2 treatment group also received a 100 µL volume of albumen but was supplemented with a high level (0.90 mg) of added exogenous glucose. Eggs that received a low level (0.45 mg) of supplemental exogenous glucose (G1) received 50 µL of the supplemented G2 albumen. Each egg in the S, G1, and G2 treatments received 0.30, 0.45, and 0.90 mg of glucose, respectively. At 8, 12, and 18 DOI, absolute whole egg weight, relative internal egg and wet and dry embryo weights, and embryo moisture content were determined. At 21.5 DOI,

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absolute chick weight; and relative yolk sac and liver weights were determined. Percentage egg weight loss (PEWL) between 0 and 14 DOI, and hatchability of fertile eggs (HF) at 21.5 DOI were determined for each treatment group. The AG concentration of eggs did not change between 0 and 4 days of storage, but decreased significantly between 0 and 4, and 4 and 8 DOI. Egg injection at 0 DOI decreased HF but had no significant effect on 0 to 14 DOI PEWL. The G2 treatment also counteracted a decrease in relative wet and dry embryo weights at 18 DOI that occurred in response to the S and G1 treatments. Although in ovo injection at 0 DOI was detrimental to HF, injection of the G2 level of exogenous glucose alleviated the adverse effects of the injection of a 100 µL volume of albumen on embryogenesis.

INTRODUCTION

The albumen content of an egg plays a crucial role during avian embryogenesis. It has been reported that partial albumen removal from eggs negatively affects hatchling weight [1], and reduces amniotic and allantoic fluid volumes in embryonated eggs [2]. [3] further noted that a decrease in the hatching success of chicks following the removal of 3 mL of albumen from their eggs at 2 days of embryonic development. This was associated with catabolic activity reductions in their skeletal muscle and increases in their livers, as well as an overall reduction in their metabolic rates in the last fifth of their embryonic life. Glucose is a vital nutrient for the developing avian embryo. Without glucose, embryonic development would cease [4]. Needham [5] [6] emphasized that glucose is the chief constituent of the egg that is utilized by the embryo during the first few days of incubation (DOI). However, only 6 % of the solids in egg albumen are composed of carbohydrates [7]. Furthermore, chemical analysis of the albumen of incubated live-embryonated Single Comb White Leghorn hatching eggs by Cunningham [8] revealed that glucose levels dropped rapidly after 3 DOI.

Although several other laboratories have sought to better understand the relationship of glucose utilization from albumen during embryogenesis [9] [10] [11], little attention has been given to embryonic glucose utilization in broiler breeder eggs and the efficacy of exogenously administered glucose on development. Ricks [12] stated that the benefits of in ovo injection, which is normally conducted during the transfer of eggs from setter to hatcher between 17.5 and 18.5 DOI, includes increased chick health as a result of a reduction in physiological stress associated with the elimination of the manual handling and the sub-cutaneous injection of hatchlings. [13] provided an exogenous carbohydrate mixture containing maltose, sucrose, and dextrin to broiler embryos by manual in ovo injection into the amnion at 17.5 DOI. A 1.0 mL volume of the solution led to an increase in hatchling and day 10 posthatch (POH) chick body weight, as well as an increase in sucrase-isomaltase activity 48 hours after injection. Moreover, [14] later reported that these carbohydrates improved POH hepatic glycogen concentrations, and [15] confirmed that in comparison to non-injected controls, the amniotic injection of glucose solutions at 18.5 DOI increased absolute broiler body weight at hatch and body weight as a percentage of set egg weight at 19.5 DOI and again on day of hatch. [16] further concluded that at 18.5 DOI, volumes of solutions containing glucose should not exceed 700 µL in order to realize a 90 % hatchability of fertile (HF) broiler hatching eggs.

In contrast to the aforementioned studies, [17] injected 75 or 100 mg of glucose in 0.5 mL of deionized water into the albumen of broiler hatching eggs at 7 DOI, and showed that it increased hatchling body weight, body weight gain through 42 days POH, and blood glucose concentration through 21 days POH.

Furthermore, [18] similarly injected 0.5 mL of deionized water containing 20 or 25 % glucose into the albumen of broiler hatching eggs at 7 DOI. Although the hatchability of live embryonated eggs was significantly reduced by the injection procedure, the in ovo supplemental glucose significantly increased the blood glucose concentrations of newly hatched, 21-day old, and 42-day old broilers, which was associated with increased hatchling body weight. Therefore, several trials were conducted in this current study to determine egg albumen glucose (AG) utilization and the effects of different levels of exogenous glucose administration via in ovo injection at the onset of incubation

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and embryonic development on subsequent embryogenesis, percentage incubational egg weight loss (PEWL), yolk sac absorption, and hatchability in broiler hatching eggs.

MATERIALS AND METHODS

General

The protocols for the current study were approved by the Institutional Animal Care and Use Committee of Mississippi State University.

Broiler breeder use and management were as described by [19]. Briefly, male and female broiler breeder chicks that originated from a primary breeder company (Arbor Acres Farm Inc., Glastonbury, CT, 1990) were obtained from a local company and were raised by standard husbandry practices in a conventional curtain-sided pullet rearing house on the Mississippi State University Poultry Research Center (MSU-PRC). Males were grown separately from females during the first 3 weeks. Blackout techniques were used that conformed to those recommended by the primary breeder company. Chicks were provided continuous lighting through 3 days of age. On day 4 and extending through week 18, birds received an 8L:16D light/dark cycle, and between 18 and 22 weeks, birds received a 10L:14D cycle. Basal corn-soy mash diets were fed to meet body weight recommendations suggested by the primary breeder company. These diets included various levels and types of added dietary fat; however, eggs were randomly selected from the various treatment groups before use to prevent a maternal dietary effect. All breeder dietary treatments were isocaloric.

During week 22, hens were weighed, sorted by size, and moved to one of 24 breeder pens in a curtain-sided breeder house on the MSU-PRC. Each pen (measuring 2.9 m × 3.0 m) provided 0.435 m2 of floor space per breeder hen and were equipped with elevated plastic slats covering 2/3 of the pen area. Pine shavings covered the remaining 1/3 of the pen floor. Twenty hens having a similar body weight and 4 males were assigned to each pen. Ten 2-tier nest boxes were provided in each pen. The photoperiod was changed to a 15L:9D cycle to initiate lay.

Albumen Glucose Concentration During Egg Storage and Incubation

Thirty eggs were collected from hens at 50 weeks of age that were housed in the breeder house on the MSU-PRC. The eggs were stored for a 4-day period at dry and wet bulb temperatures of 17.2 and 15.6 °C, respectively, in a walk-in cooling unit. Storage temperatures were recorded daily. A 1.0 mL sample of albumen was taken from 6 replicate eggs at 0, 1, 2, 3, and 4 days of storage for determination of AG concentration.

Forty-eight eggs were collected from hens at 50 weeks of age and were set in a Petersime Model 5 incubator (Petersime Incubator Co., Gettysburg, OH 45328) in the hatchery of the MSU-PRC. Eggs were incubated for 18 days at dry and wet bulb temperatures of 37.5 and 30.0 °C, respectively. Incubation temperatures were recorded daily. A 1.0 mL sample of albumen was taken from 8 replicate eggs at 0, 4, 8, 10, 12, and 14 DOI for determination of AG concentration.

The AG concentrations (mg/dL) during egg storage and incubation were measured using a One Touch Basic Blood Glucose Meter (Lifescan Inc., Milpitas, CA 95035).

In Ovo Injection of Exogenous Glucose and Embryogenesis

Five hundred and sixty eggs were collected from hens at 53 weeks of age and were randomly assigned to one of 4 treatment groups (140 eggs per treatment). The treatment groups were non-injected controls (C), sham-injected controls (S), eggs injected with a low level of exogenous glucose (G1), and eggs injected with a high level of exogenous glucose (G2). The supplemental glucose used was D-(+)-Glucose (Sigma-Aldrich, St. Louis, MO, 63110). Relative to the amount of glucose present in the S treatment, eggs in the G1 treatment received 1.5 × and those in the G2 treatment received 3 × that amount. Eggs in the S and G2 treatments received 100 uL volume injections. The approximate glucose concentration of the fresh albumen injected into the S treatment eggs was 300 mg/dL.

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Therefore, each egg in the S treatment received approximately 0.30 mg of glucose. Supplemental exogenous glucose was used to increase the glucose concentration of the fresh albumen used for the G2 treatment. The glucose concentration of the supplemented albumen injected into the eggs belonging to the G2 treatment was approximately 900 mg/dL. Therefore, each egg in the G2 treatment received approximately 0.90 mg of glucose. The amount of glucose injected into each egg belonging to the G1 treatment was 0.45 mg, which was achieved by using 50 uL of the supplemented albumen used in the G2 treatment.

In in ovo technology, a syringe that is capable of being used to manually inject eggs has been routinely employed [20]. In this study, eggs were injected prior to set (0 DOI) using a 21-gauge needle and a tuberculin syringe. Injections were given on the side of the egg, approximately 1/3 of the distance from the tip of the small end towards the equator of the egg, in order to target the albumen. The injection site was swabbed with Betadine solution prior to injection, and afterwards, the holes were sealed with paraffin wax. Eggs were incubated from 0 to 18 DOI, as described in the previous section, and on 18 DOI, eggs were transferred to a Jamesway Model 252A incubator (Butler Manufacturing Co., Fort Atkinson, WI 53538) for hatching. The dry and wet bulb temperatures of the hatcher were maintained at 37. 5 and 30.0 C, respectively.

Individual weights of 13 eggs per treatment were determined at 0 and 14 DOI, and the PEWL of the eggs between 0 and 14 DOI was calculated. All eggs were candled at 10 DOI to remove those that were non-embryonated. Therefore, PEWL was only determined for those eggs that were embryonated through 14 DOI. Whole egg weight, wet and dry embryo weights, embryo moisture content, and egg internal content (whole egg weight minus the weights of the shell and shell membrane) weights were determined for 6 replicate eggs in each treatment group at 8, 12, and 18 DOI. Egg internal content, and wet and dry embryo weights, were calculated as percentages of whole egg weight. Embryo moisture content was calculated as a percentage of total wet embryo weight. Chick, yolk sac, and 1iver weights were recorded for 10 replicate eggs in each treatment group at 21.5 DOI. Yolk sac and liver weights were calculated as percentages of total chick weight. Percentage HF was determined for all remaining unsampled eggs in each treatment group at 21.5 DOI.

Statistical Analyses

Data in Trials 1 and 2 were analyzed using one-way analysis of variance (ANOVA) in a completely randomized design, and data in Trial 3 was analyzed using ANOVA in a split-plot design with treatment as the whole-plot factor and day of sampling during incubation as the sub-plot factor. Day means in Trials 1 and 2 were partitioned by Fisher's Protected Least Significant Difference [21]. When day by treatment interactions were significant in Trials 3 and 4, subclass means were compared by the PROC MIXED procedure of SAS [22]. Hatchability of fertile eggs in Trial 3 was analyzed by Chi-square analysis. Comparisons between proportions were analyzed by the method of [23]. No SEM was reported for each HF treatment mean because percentage results were based on one tray of eggs per treatment group. Statements of significance were based on $P \leq 0.05$ unless otherwise noted.

Results and Discussion

The AG concentration of eggs were not significantly ($P > 0.05$) affected due to storage (data not shown). This implies that properly storing eggs over a 4-day period under the conditions specified in this report will not lead to decreases in AG concentrations that are essential to the chick during early embryonic development. However, during incubation, egg AG concentration decreased significantly ($P < 0.0002$) between 0 and 4 DOI, and again between 4 and 8 DOI (Table 1). Levels decreased numerically, but not significantly ($P > 0.05$) between 8 and 14 DOI. These results generally coincide with work by [8], who found that AG levels during incubation dropped dramatically after 3 DOI. Since AG may be a vital nutrient that is readily utilized during the first few DOI, glucose added to albumen was injected into eggs prior to 7 DOI in an attempt to maintain higher available AG concentrations for the developing embryo. Egg PEWL between 0 and 14 DOI was not significantly affected by treatment ($P = 0.7017$). Mean PEWL (mean ± SEM) between 0 and 14 DOI in C, S, G1, and G2 eggs were 9.06 ± 0.585, 8.10 ± 0.610, 8.54 ± 0.585, and 8.83 ± 0.673, respectively. These results indicate that the sealant applied to the shell injection site hole effectively prevented an increase in water loss from the injected eggs.

Table1. Egg albumen glucose concentration (mean ± SEM[1]) at 0, 4, 8, 10, 12, and 14 days of incubation in broiler hatching eggs

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Day of Incubation	Egg albumen glucose (mg/dL)[2]
0	311.13[a] ± 17.07
4	210.63[b] ± 17.07
8	58.86[c] ± 18.25
10	47.50[c] ± 17.07
12	18.75[c] ± 17.07
14	11.00[c] ± 17.07

[a-c]Means for different time periods with no common superscript letter differ significantly (P < 0.0002).
[1]SEM based on pooled estimate of variance.
[2]N = 8 replicate eggs sampled at each time period (day of incubation).

There was a significant (P = 0.01) DOI by treatment interaction for relative egg internal content weight (Table 2). Injection of a 100 µL volume of albumen at 0 DOI caused relative internal contents weight of the eggs in the S treatment to significantly increase at 8 DOI in comparison to those that were not injected in the C treatment group, and to those in the high glucose level-injected group. Conversely, at 8 DOI, the relative internal contents weight of eggs belonging to the G2 treatment was not significantly different from that of the C eggs, and that of the G1 eggs was intermediate to that in the S, C and G2 eggs. By 12 DOI, relative internal contents weight of the S eggs returned to a value that was not significantly different from that of the C and G2 eggs, but by 18 DOI was significantly lower than that of the eggs in the C and G1 treatment groups. On the other hand, the relative internal contents weights of the eggs in the G1 and G2 treatments were not significantly different from those in the C treatment group. The decrease at 18 DOI in the S treatment was not attributable to a change in egg weight loss, because PEWL between 0 and 14 DOI was not different among the treatment groups. However, changes in embryo metabolism, nutrient utilization, and metabolic water and gas production during late incubation (14 to 18 DOI) may have been contributing factors.

Table 2. Relative weights (percentages of total egg weight) of egg internal contents (RINTCON), and wet (RWEW) and dry (RDEW) embryo weights (mean ± SEM1) in non-injected (C) and sham-injected (S) controls, and low (G1) and high (G2) glucose level-injected broiler hatching eggs at 0, 4, 8, 10, 12, and 14 days of incubation (DOI)

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Treatment	C	S	G1	G2
		RINTCON[2] (%)		
8 DOI	87.76[b] ± 0.42	89.67[a] ± 0.73	88.71[ab] ± 0.73	86.63[b] ± 0.73
12 DOI	90.11 ± 0.60	90.12 ± 0.60	90.31 ± 0.73	89.20 ± 0.73
18 DOI	89.03[ab] ± 0.60	85.44[c] ± 0.73	89.95[a] ± 0.73	87.15[bc] ± 0.73
		RWEW[2] (%)		
8 DOI	1.93 ± 0.95	1.94 ± 1.65	2.28 ± 1.65	2.11 ± 1.65
12 DOI	15.27 ± 1.35	13.69 ± 1.35	14.99 ± 1.65	13.40 ± 1.65
18 DOI	57.12[a] ± 1.35	50.54[bc] ± 1.65	46.57[c] ± 1.65	54.45[ab] ± 1.65
		RDEW[2] (%)		
8 DOI	0.13± 0.20	0.13 ± 0.34	0.16 ± 0.34	0.14 ± 0.34
12 DOI	1.50 ± 0.28	1.43 ± 0.28	1.51 ± 0.34	1.28 ± 0.34
18 DOI	10.09[a] ± 0.28	8.70[b] ± 0.34	7.71[c] ± 0.34	9.66[ab] ± 0.34

[a-c]For each variable, means within a time period (day of incubation) with no common superscript letter differ significantly ($P \leq 0.05$).
[1]SEM based on pooled estimate of variance.
[2]N = 6 replicate eggs sampled in each treatment group at each time period (day of incubation)

There were also significant DOI by treatment interactions for relative wet ($P = 0.02$) and dry embryo ($P = 0.01$) weights (Table 2). There were no significant treatment effects on wet or dry embryo weights until 18 DOI. In comparison to embryos in the C treatment group at 18 DOI, wet and dry embryo weights were significantly decreased by the in ovo injection of 100 µL of albumen (S treatment) or by the in ovo injection of albumen containing the low level of supplemental glucose (G1 treatment). However, the relative wet and dry weights of embryos in the G2 treatment were intermediate and not significantly different from those in the C and S treatments. The relative wet weights of the embryos in the G1 treatment were not significantly different from those in the S treatment, whereas the relative dry weights of the embryos in the G1 treatment were significantly lower than those in the S treatment. The current results of relative embryo weight (wet and dry) at 18 DOI are in partial contrast to those of [18], who observed an increase in hatchling body weight in comparison to non-injected and sham-injected

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controls. Nevertheless, in partial agreement with [18], the higher level of glucose currently administered in the G2 treatment group reversed the detrimental effects of the sham injection procedure. The effect of the G2 treatment on relative embryo weight at 18 DOI may have been mediated by an increase in embryonic metabolism in response to an increase in circulating glucose concentrations. This is supported by the reports of [18], who injected glucose into the albumen of broiler hatching eggs at 7 DOI. In those reports, it was shown that blood glucose levels were elevated at 0, 21, and 42 days POH in response to 20 and 25 % glucose [18], or at 0 and 21 days POH in response to approximately 15 and 20 % glucose [18].

Embryo moisture significantly decreased between 8 and 18 DOI. Mean percentage embryo moisture concentrations at 8, 12, and 18 DOI were 93.18 % ± 0.27, 90.03 % ± 0.27, and 82.67 % ± 0.29, respectively. However, there were no significant treatment effects on embryo moisture content at any time during incubation (data not shown). This above result in combination with the lack of a treatment effect on PEWL between 0 and 14 DOI, indicate that the treatment effects at 18 DOI on relative wet and dry embryo weights were not associated with egg water loss during incubation. These results further suggest that the difference in injection volume between the G1 (50 µL) and G2 (100 µL) treatments did not have a significant confounding effect on the treatment effects observed for the various physiological variables examined. Conversely, the decrease in embryo growth in response to the S and G1 treatments implicate the invasiveness of a 0 DOI in ovo injection procedure and show that it can negatively alter embryonic metabolism and development. Nevertheless, the G2 treatment apparently was able to alleviate and reverse these adverse effects.

There were no significant treatment effects for absolute chick body weight (P = 0.564), relative yolk sac weight (P = 0.183), and relative liver weight (P = 0.177) at 21.5 DOI (Table 3). During the last week of incubation, most embryonic body tissues develop with a rapid increase in dry matter. This is associated with a decrease in embryonic moisture content and an increase in yolk sac absorption. Egg HF in the C, S, G1, and G2 treatment groups was 83.57, 25.38, 18.71, and 17.69 %, respectively. The decrease in HF in the eggs injected very early in incubation was primarily a result of early and late dead embryonic mortalities associated with decreased embryo viability. It has likewise been observed that the process of in ovo injection into the albumen of broiler hatching eggs at 7 DOI can significantly reduce hatchability. The hatchability of broiler hatching eggs that were sham-injected in the albumen at 7 DOI has been observed to be decreased by 15 % [18] and by 16 % [18]. The greater decline in HF observed in this study may have been due to the earlier time of injection at 0 DOI. However, injection into the air cell would likely decrease embryonic mortality. The amniotic injection of a carbohydrate solution containing 6.25 % glucose at 18 DOI has been shown to have no detrimental effect on hatchability while increasing embryo body weight relative to set egg weight at 19 DOI [15].

Table 3. Absolute chick body weight (BW), and relative (percentages of chick BW) yolk sac (RYSW) and liver (RLW) weights (mean ± SEM1) in non-injected (C) and sham-injected (S) controls, and low (G1) and high (G2) glucose level-injected broiler hatching eggs at 21.5 days of incubation

Variable[2]	BW	RYSW	RLW
Treatment		——————————(%)——————————	
C	46.0 ± 1.17	8.24 ± 1.025	3.26 ± 0.109
S	46.8 ± 1.17	7.71 ± 1.025	2.94 ± 0.109
G1	48.3 ± 1.17	10.65 ± 1.025	3.20 ± 0.109
G2	47.3 ± 1.17	9.64 ± 1.025	3.20 ± 0.109

[1]SEM based on pooled estimate of variance.
[2]N = 10 replicate eggs sampled in each treatment group at 21.5 days of incubation.

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In conclusion, AG concentrations during incubation decreased significantly between 0 and 4, and 4 and 8 DOI in the broiler hatching eggs. Efforts were made to maintain higher available AG concentrations in the broiler hatching eggs using 900 mg/dL of supplemental exogenous glucose in the G2 treatment. By the methods described, this may have increased embryonic metabolism and thereby counteracted the adverse effects of the in ovo injection of a 100 µL volume of albumen at 0 DOI. This was reflected by the increases in relative wet and dry embryo weights in response to the G2 treatment. However, the experimental procedures utilized in this study did not improve hatchability. Improvements in the administration methodologies used to supplement available AG for developing embryos may be needed before beneficial results are realized.

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