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The Effects of In Vivo Glucose Injections On Hatchability and Subsequent Broiler Body Weight

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ABSTRACT

This experiment examined the effect of an injection of glucose into the yolk sac of broiler breeder embryos on the 18th day of incubation. Two trials were run. 0.1 mL (50 mg), 0.2mL (100mg), and 0.3 mL (150 mg) of 500mg/mL glucose solution was injected into three treatment groups in the first trial. A non-injected treatment group and water sham 0.3 mL sterile water) were also used. Thirty birds from the non-injected, water sham and 0.1 mL treatments, 15 male and 15 female, were grown out to 21 days. Individual bird weight was measured on day 0, day 7, and day 21.

The second trial injected three treatments of eggs with .1 mL of varying concentrations of glucose. A low level of 12.5 mg, a medium level of 25 mg, and a high level of 50 mg served as the glucose treatments. A non-injected control and water sham treatment was used also. No birds were grown out from Trial 2.

Hatch data were collected from both trials and analyzed. The mean body weight, percent hatchability, percent pips and percent unhatched eggs were compared between treatments within each trial. Analyses of the data showed significant differences in the percent hatchability in Trial 1. Significant differences were found in all parameters in Trial 2 ($P<0.05$). No significant benefit was derived from injecting the eggs with glucose in either trial.

INTRODUCTION

Glucose is the primary energy source for practically all living things. From whatever source an organism may derive their nutrients, it is ultimately converted into glucose or a similar compound. Within a chicken egg, very little carbohydrate exists, so during incubation, the embryo is only sparingly dependent on glycolysis for energy. In the early stages of incubation, glycolysis is needed for germ development(Romanoff and Romanoff, 1967). If there is a lack of glucose and/or oxygen early germ deaths are suspected to arise (Moran and Reinhart, 1980).

As the embryo develops within the egg, it appears to be partially dependent on the metabolism of glucose for energy. Within the yolk sac, lactic acid, a by-product of glycolysis, does not exist prior to incubation. During the second week of incubation, the level of lactic acid in the yolk sac increases substantially. Shortly thereafter, the lactic acid concentration in the chick's yolk sac sharply decreases as it is metabolized(Romanoff and Romanoff, 1967).

Of the carbohydrates in the embryo, about 80 percent are glucose. The remaining 20 percent is composed mostly of glycogen. Up to the 14th day, free glucose is prevalent in the developing chicken embryo. After the 14th day, it is glycogen which predominates the carbohydrates in the developing embryo. The 14th day of incubation is the turning point for the amount of glucose present because this is when the liver initiates the glycogenic function. During incubation, there is a considerable amount of change in the carbohydrate level of the yolk, albumen and embryo. As the embryo develops, the total amount of carbohydrates present continuously decreases.

In a fresh yolk, only 145 mg of carbohydrates are present. A little over twice that, 313 mg of carbohydrates are found in the albumen. The amount of carbohydrates in the yolk reaches its maximum amount (282 mg) on the fourteenth day of incubation. By hatch, on the 21st day, the level of carbohydrates in the yolk has diminished to 153 mg. The glucose/glycogen ratio in the yolk decreases steadily on days 0, 5, 10, 15, and 18 to values of 5.5, 4.8, 4.6, 4.3, and 2.9 respectively (Romanoff and Romanoff, 1967).

As the embryo grows, its level of carbohydrates increases from 0 mg on day 0 to 125 mg on day 21. In the albumen, 313 mg of carbohydrates is present on day 0. This diminishes to 34 mg on day 15. On the 19th day of incubation, the chick swallows the remaining amnionic fluid, so none of the carbohydrates from the amnionic fluid are discernible at hatch (Table 1).

TABLE 1
Carbohydrate levels in the egg

Age of Embryo	Amount of Carbohydrates (mg.)			
(days)	Embryo	Yolk	Albumen	Total
0	-	145	313	458
5	0.3	158	262	420
10	6	243	177	426
15	40	257	34	331
20	118	178	-	296
21	125	153	-	178

Romanoff and Romanoff 1967, p.190.

During incubation the embryo stores the glucose present in the yolk and albumen as glycogen. The embryo depends on this build up of a glycogen reserves during the prenatal period. This reserve is depleted during the chick's emergence from its shell (Freeman & Manning, 1971; Freeman, 1969). This glycogen comes from the traces of glucose found in the yolk and albumen during the early part of incubation.

Very little research has been done on the effects of glucose supplementation during the incubation period. The demand for energy during hatch is higher than the amount supplied by the glycogen reserve in the liver. Once all the energy has been depleted from this stored glycogen, the chick depends on ketosis until after hatch when it has access to another supply of carbohydrates.

Moran and Reinhart (1980) provided Diamond White broiler breeder turkey eggs glucose via pressure differential antibiotic dipping. The eggs were dipped 4 days after collection and 1 day before setting. The pressure differential process involves placing the eggs in a 4 C solution containing antibiotics and 10 percent glucose. The eggs were then placed under negative pressure then allowed to slowly return to atmospheric pressure. The eggs were dipped in antibiotics which is a usual commercial procedure for turkey eggs. The glucose was provided on the notion that the added glucose would lower the number of early germ deaths and be of benefit in post hatch performance. The addition of the 10 percent glucose solution lead to a 100 mg increase in the egg glucose level.

They found no difference in early deaths, pips and total hatchability between non-dipped and dipped treatments. The quality of poults was not affected, nor was the proportion of cull poults or the sex distribution of saleable poults. However, the eggs which had been dipped in a glucose dip had a significantly higher hatch weight.

Hughes *et al* . (1974) found that as little as 0.3 mg of glucose placed adjacent to the blastoderm during the first 24 hours of incubation induced teratogenesis. Moran and Reinhart (1980) found no proportional differences in malpositions, malformations and early germ deaths between the glucose dipped treatments and their non-glucose dipped controls. This

would suggest that the glucose did not permeate the shell membrane and reach the embryo within the first 24 hours of incubation. It was not until chorioallantoic circulation was active that the glucose passed through the membrane and was made accessible to the embryo.

In the commercial poultry industry, eggs are removed from the incubator on the 18th day of incubation. They are then vaccinated and placed in the hatcher. If a method could be found which would increase the total hatchability even slightly, it could be a great benefit. The embryo requires a great deal of energy during the hatching process. Many run out of energy and do not make it out of the shell. Some of these pip, that is the chick is able to break the shell but unable to hatch out of the shell.

By providing chicken eggs with an added energy supplement on the 18th day of incubation, a significantly larger percentage of eggs could possibly hatch. In this experiment eggs were injected with glucose in hopes that it would increase the number of chicks which would hatch.

MATERIALS AND METHODS

Eggs were collected from Arbor Acres broiler breeders at the LSU Agricultural Center Poultry Science research farm in Baton Rouge, LA. Two trials were run, each consisting of 5 treatments of 150 eggs per treatment. Each treatment consisted of 3 replications of 50 eggs. On the 10th day of incubation, the eggs were candled. All infertile or early dead eggs were removed. On the 18th day of incubation, the eggs were removed from the incubator and separated into treatments.

A sterile dextrose solution was prepared at 500 mg/mL. Dextrose is a specific stereo isomer of glucose. The solution was heated and maintained at a temperature of 20.8 C. In all injected treatments a small hole was drilled over the air cell of the egg using an electric engraver. They were injected with the glucose solution using 1 mL syringes with 20 gauge needles and then the hole was sealed with heated, liquid paraffin wax. All eggs were transferred to a Robbins Hatcher.

In Trial 1, Treatment 1 served as the non-injected control. Treatment 2 received an injection of 0.3 mL of sterile water. The third set of eggs was injected with 0.1 mL of solution (50 mg glucose). In jected into the fourth set of eggs was 0.2 ml (100 mg) of the same glucose solution. The fifth set of eggs was injected with 0.3 mL (150 mg) of the glucose solution.

At hatch, the birds were group weighed by treatment/replication and hatch data were collected. They were sexed and banded. Thirty birds per treatment from Treatments 1-3, 15 males and 15 females, were grown to 21 days of age. The birds that were selected to be grown out were individually weighed on day 1, day 7, and day 21 with a Mettler PE 12

balance.

On day 1, 30 chicks per treatment were placed in a 1.52m x3.05m pen according to their treatment. The pens were equipped with , manual chick waterers for immediate use, automatic water dispensers for the chicks after the first week, chick feeder lids, and gas-burning brooders designed to warm up to 100 chicks. The birds were fed a standard starter ration ad libitum and were maintained on a constant lighting cycle with 60 watt light bulbs(Table 2). Trial 2 consisted of 5 treatments with 150 eggs per treatment. These were divided into three replications of 50 eggs each. On the 18th day of incubation the eggs were removed from the incubator and divided into treatments.

A sterile dextrose solution was prepared. The solution was heated and maintained at 20.8 C. In all injected eggs a small hole was drilled with an electric engraver in the shell. They were injected with the dextrose solution with 1 mL syringes with 20 gauge needles. The hole was then sealed with liquid paraffin wax. All eggs were transferred to a Robbins Hatcher.

Treatment 1 served as the non-injecteded control. Treatment 2 was injected with .1 mL of sterile water. Treatment 3 was injected with 0.1 mL of a low concentration glucose solution (12.5 mg). Of a medium concentration glucose solution 0.1 mL (25 mg) was injected into treatment 4. Treatment 5 was injected with 0.1 mL of a high concentration glucose solution (50 mg). At hatch, the birds were group weighed by Treatment/Replication group. Hatch data were collected. No chicks from Trial 2 were grown out.

After all the data had been collected from both trials, they were analyzed by the Statistical Analysis System (SAS). When significant effects

were found, the means were separated by Duncan's Multiple Range test.

RESULTS

The parameters measured in Trial 1 were: individual mean bird body weight at day 1, day 7, and day 21, body weight for all birds at hatch, percent hatchability, percent pips, and percent unhatched eggs.

When weighed on Day 1, no significant differences were found between the mean weights of the individual birds in the 3 treatments. Weights at Days 7 and 21 found no significant differences between treatments individual mean bird body weight either(Table 3). For the mean body weights of all birds collected at hatch, there were no significant difference. No significant differences were found in the mean number pips between the treatments.

A significant difference ($P<0.0001$) was found in hatchability between treatments. No significant difference was found between the control, the .3 mL sterile water, and 50 mg of glucose treatments . However their hatchability was significantly different from the 100 mg glucose and the 150 mg glucose treatments. A significant difference ($P<0.0001$) was also found in the number of eggs not hatched or pipped. Again there was no significant difference between the number of unhatched eggs in the control, .3 mL of water, and the 50 mg glucose treatments. There was a significantly lower ($P<0.0001$) number of unhatched eggs in these three treatments as compared to the 100 mg glucose and 150 mg glucose treatments (Table 4).

In Trial 2 a different set of parameters were examined. Only hatch data were collected. The chick weights of the treatments were compared as was the hatchability, number of pips, and percent unhatched eggs.

The chick weights between the treatments in each treatment was found to be significantly different ($P<0.07$). The control group was

significantly hevier than the .1 mL sterile water and 12.5 mg glucose treatments. However there were no significant differences in chick weight between the control, the .1 mL sterile water, and the low (12.5 mg) glucose treatments, nor was there a significant difference in the chick weight between the .1 mL sterile water, low (12.5 mg) glucose, medium (25 mg) glucose, and high (50 mg) glucose treatments.

A significant difference ($P<0.01$) was found in the hatchability between treatments. The control treatment had a significantly higher hatchability rate than the low (12.5 mg) glucose and the high (50 mg) glucose treatments. The control, .1 mL sterile water, and medium (25 mg) glucose treatments had no significant difference in hatchability. They were all significantly higher than the low (12.5 mg) glucose treatment, but the hatchability in the .1 mL sterile water and medium (25 mg) glucose treatments were not significantly higher than the high (50 mg) glucose treatment. The low (12.5 mg) glucose and the high (50 mg) glucose treatments were not significantly different.

The number of pips in the treatments were highly significant ($P<0.0003$). The low (12.5 mg) glucose treatment had a significantly higher percentage of pips than the other 4 treatments. The high (50 mg) glucose treatment had a significantly higher percentage of pips than the control treatment. There was no significant difference in the percent of pips in the control, the .1 mL sterile water, and the medium (25 mg) glucose treatments (Table 5).

DISCUSSION AND CONCLUSIONS

Throughout both trials, one pattern emerged: The non-injected control group had the best hatch. By injecting the eggs with either water or glucose solution at least one parameter for which we were testing was significantly worse than the non-injected treatment. In many cases the differences between treatments were significant. Our findings do not support the notion that added glucose will lead to an added boost of energy at hatch and increase total hatchability.

In Trial 1, the percent hatchability of the 100mg glucose and 150 glucose treatments were significantly lower than the control, the 0.3mL sterile water, and the 50 mg glucose treatments. One reason for this may be that we saturated the embryo with glucose. By adding 100mg and 150mg of glucose on the 18th day of incubation,there was a 33 percent and 50 percent increase in the amount of glucose available to the chick in a very short amount of time. These high levels of glucose could have caused an osmotic imbalance in the egg causing water to have been taken from the chicks cells.

The conditions under which the solutions were prepared allowed for the chance of slight contamination. This could account for some of the difference in the results obtained. Also the injections were done by hand, so a difference in needle placement could have caused some variance in the results.

The chicken seems to have evolved an efficient process for which to deliver chicks. They are supplied with enough energy to grow and hatch. An attempt to alter the balance which has been created with glucose injections has only served to hinder the quantity of hatched eggs.

TABLE 2

Broiler Starter Diet

Ingredient	Percent
Corn	54.74
Soybean Meal - 44	34.00
DIKAL - 21	2.00
Oyster Shell Flour	1.50
Microingredients*	0.25
Salt	0.50
Choline	0.13
DL - Methionine	0.13
Tallow	6.75

Calculated Analysis:

Protein (%)	20.00
Metabolizable Energy (Kcal/Kg)	3070
Calcium (%)	1.14
Phosphorus (%)	0.072

* Provided the following per kilogram of diet: Vitamin A, 6000 IU; Vitamin D3, 1000 ICU; Vitimin E, 15 IU; Menadione dimethylpyrimidinol bisulfite, 4.4 mg; thiamin, 2.7 mg; riboflavin, 5.4 mg; pantothenic acid, 15 mg; niacin, 41 mg; pyridoxine, 4.5 mg; biotin, .23 mg; choline, 1450 mg; folacin, .83 mg; copper, 6 mg; iodine, .58 mg; iron, 120 mg; manganese, 83 mg; zinc, 60 mg; and cobalt, 5 mg

Table 3

Body weight of chicks as influenced by egg injections at 18 days of incubation

	Day 0	Day 7	Day 21
Control	46.0	116	534
Water Sham	46.9	112	524
50 mg Glucose	47.7	116	530

TABLE 4.

Body weight and hatchability parameters as influenced by glucose injections on day 18 of incubation (Trial 1).

	Mean Body Weight	Percent Hatchability	Percent Unhatched Eggs	Percent Pipps
Control	46.3	91.2A	9.5B	0.8
Water Sham	49.7	89.3A	7.4B	4
50mg Glucose	45.6	83.7A	16.3B	1.3
100mg Glucose	47	37.3B	60.7A	2
150mg Glucose	47.6	45.3B	54.7A	1.3
P of F	0.3967	0.001	0.0001	0.3812

TABLE 5.

Body weight and hatchability parameters as influenced by glucose injections on day 18 of incubation (Trial 2).

	Mean Body Weight	Percent Hatchability	Percent Unhatched Eggs	Percent Pipps
Control	47.8A	86.7A	11.3B	2.0C
Water Sham	47.4AB	80.7AB	14.7B	4.7BC
12.5mg Glucose	46.7AB	65.3C	17.3B	17.3A
25mg Glucose	46.1B	76.5AB	19.5A	3.3BC
50mg Glucose	46.1B	71.3BC	15.3A	8.0B
P of F	0.0700	0.0100	0.0105	0.0003

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