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Impact of In-Ovo Injection of Folic Acid and Glucose on Hatchability, and Post-Hatching Performance of Broiler Chicken

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ABSTRACT

The present study was designed to investigate the impact of in-ovo injection of folic acid and glucose on hatching eggs from 55 weeks old broiler breeders. A total number of 900 hatching eggs were collected from Arbor Acres broiler breeders, then, eggs were divided into 6 groups including 1) Negative Control (non-injected, NC), 2) Dry Punch Control (pricked without injecting any solution, DPC), 3) Positive Control (eggs were injected with 0.5 mL normal saline, PC), 4) Folic Acid group (eggs were injected with 0.2 mg/ egg folic acid, FA), 5) Glucose group (eggs were injected with 125 mg/ egg glucose, Glu), and 6) Folic Acid with Glucose group (eggs were injected with 0.2 mg folic acid with 125 mg/ egg glucose, FA+Glu). Each treatment was divided into five replicates of 30 eggs each. Eggs were injected into the albumen under the air sac. After in-ovo injection, the eggs were stored for four days before hatching. After hatching, the chickens were reared in groups according to the treatments. All treatments were divided into 10 replications of 9 chickens in each. In-ovo injection with folic acid decreased the albumen pH significantly to 9.19 after 4 days of injection, while the negative control was 9.43. Hatching quality was severely affected by all in-ovo injection treatments, but no significant differences were found between the treatment groups concerning the hatchability of fertile eggs. Injection treatments had no significant effect on the growth rate or the production number in any of the weeks. Injection of folic acid and (FA+Glu) significantly increased chickens' body weight at two and four weeks of age. Also, the dressing percentage when using folic acid and (FA+Glu) was significantly increased to 72.1% and 72.5%, respectively, compared to the positive control group (68.3%). In conclusion, our data suggested that in-ovo injection with a mixture of folic acid and glucose (0.2 mg folic acid+ 125 mg/ egg glucose) could be used to enhance carcass characteristics. Further studies should be conducted to find the effects of in-ovo injection folic acid and glucose on different incubation days and at different sites of injection.

Keywords: Broilers, Folic Acid, Glucose, Hatchability, In- Ovo injection, Old breeders, Post-hatch

INTRODUCTION

A range of healthy chickens with high growth ability and viability is very important to the poultry industry. The age of broiler breeder is one of the most important factors affecting hatchability and chickens' quality. The eggs of old breeders have a lower fertility and hatchability than those of the young breeder (Elibol et al., 2002; Vieira et al., 2005; Iqbal et al., 2016). Previous studies showed that the low incidence of hatchability in eggs of older breeders was due to many contributing factors, such as a poorer eggshell quality due to the larger surface (Bennett, 1992), and the deterioration in the albumen quality (Tona et al., 2004).

Older breeders' eggs were associated with larger size and thinner shells with higher porosity, which had been associated with a higher percentage of egg moisture loss during incubation. This increased mortality in the early phase of embryogenesis due to dehydration (Peebles et al., 2001), led to poor hatching quality (Narushin and Romanov, 2002). With increasing age of the breed, the albumen pH increased at oviposition, which may be due to the faster release of CO_2 through the eggshell due to a higher eggshell porosity (Meijerhof, 1994). There seems to be an association between albumen pH before incubation and viability of embryo during early phase of embryogenesis, an albumen pH of 8.2 appears to be optimal for embryogenesis (Reijrink et al., 2008). Lapao et al. (1999) found that most of the rise in albumen pH occurred during the first four days of storage.

At hatching, older breeders were associated with an increase in day-old chicken weight and a decrease in chick quality (Koppenol et al., 2015). Also, the chickens from an old breeder were less feed efficient (higher FCR) than the chickens from the young breeder (Ulmer-Franco et al., 2010).

In recent years, much attention has been paid to the field of in-ovo injection. In-ovo technology is a method that can potentially enhance the hatchability and post-hatch performance of broiler chickens (Zhang et al., 2019). With In-ovo technology, various substances were injection into the air chamber or directly into the egg (Kucharska-Gaca et al., 2017). Various factors influenced the effectiveness of in-ovo injection, including the injection site, the stage of development of the embryo, the level of contamination in the hatchery, and the in-ovo injection equipment (Ricks et al.,

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1999). Various nutrients were examined for effectiveness in improving embryonic development, hatchability, and post-hatch performance including carbohydrates, amino acids, peptides, electrolytes and vitamins (Kucharska-Gaca et al., 2017). The effectiveness of in-ovo injection methodology on hatching quality is still under study due to the optimum site and time of injection and the suitable volume and nutrient of injected solution (Tasharofi et al., 2018). Folic acid is a critical vitamin during reproduction and the hatching requirement is higher compared to egg production (Vieira, 2007). Folate was essential for embryonic development, regardless of whether in-ovo feeding of folic acid could influence the growth performance (Li et al., 2016). Parnian et al. (2019) found that in-ovo injection of folic acid improvement the body weight of the chickens.

Embryonic blood plasma glucose concentrations reduced with increasing age of the breeder (Christensen et al., 1996). Glucose or dextrose is a simple sugar that is used by cells as an essential source of energy and a metabolic intermediate, and is the most important source of energy for embryonic development (Starck and Ricklefs, 1998). Therefore, the aim of this work was to investigate if the in-ovo injection of folic acid and glucose into eggs of old broiler breeders could be useful for improving hatchability and post-hatching productivity.

MATERIALS AND METHODS

Ethical approval

The present study has been conducted in accordance with the guidelines of the Ethics Committee of the Faculty of Agriculture of Cairo University. The experimental fieldwork was carried out in the Agriculture Experimental Station of the Faculty of Agriculture- Cairo University in Giza, Egypt.

Structure

A total number of 900 hatching eggs were collected from Arbor Acres broiler parents at 55 weeks of age. The eggs were prepared at El Ahlia hatchery, El Ahlia Poultry Company, Tanta city, Egypt. . The eggs were randomly divided into six treatments group (150 eggs each) four days before incubation; each group was divided into five replicates (30 eggs each). The treatments consisted of (1) Negative Control, in which no eggs were injected (NC), (2) Dry Punch Control, in which shell and shell membranes were pricked without injecting any solution (DPC), (3) Positive Control, in which eggs were injected with 0.5 mL normal saline (PC), (4) Folic Acid group, in which eggs were injected with 0.5 mL normal saline containing 0.2 mg folic acid (FA), (5) Glucose group, in which eggs were injected with 0.5 mL normal saline containing 125 mg glucose (Glu) and (6) Folic acid with Glucose group, in which eggs were injected with 0.5 mL normal saline contain 0.2 mg folic acid and 125 mg glucose (FA + Glu). The eggs were injected in the albumen under the air sac.

In-ovo injection

At the time of injection, the large ends of the freshly laid eggs were cleaned with ethyl alcohol (70%) and were penetrated by a pin, taking care not to injure the outer egg membrane. The solutions were injected into the albumen (0.5 mL/egg) at a depth of 12 mm (Akhlaghi et al., 2013), via a disposable syringe (1 ml syringe). The punched eggs were sealed with melted paraffin wax, then were given an identification number and stored for four days (18°C and 75% relative humidity (RH)) until the end of incubation. Two eggs from each replicate were randomly selected to evaluate the albumen pH before injection and after two to four days of injection. The pH of egg albumen was measured using a pH meter at room temperature. The measured pH of all solutions was 7.23, 3.98, 4.08, and 3.46 for sodium chloride, folic acid, glucose and the combination of folic acid and glucose, respectively.

General Management

All eggs were set in an incubator at average temperature of 37.5°C, and relative humidity of 56.5%. The eggs were turned hourly. At 444 hours of incubation, the eggs were transferred to a hatcher at an average temperature of 36.6°C, and a relative humidity of 61.2%. After hatching, all unhatched eggs were examined to calculate the embryonic mortality, which was classified as unfertile and early mortality (1-12 days) and late embryo mortality (13-21days). Total embryonic mortality was determined as the amount of the all dead embryos. Other calculated parameters consisted of fertility percentage, hatchability of total eggs and hatchability of fertile eggs. Fertility percentage was calculated as (number of fertile eggs divided by number of total eggs) multiply by 100. Hatchability of total eggs calculated as (number of hatched chicks divided by number of total eggs) multiply by 100. Hatchability of fertile eggs calculated as (number of hatched chicks divided by number of fertile eggs) multiply by 100

After hatching, 540 chickens were then transported to the farm of the Faculty of Agriculture Cairo University. Each treatment was divided into ten replicates (Nine chickens each). Each replicate was housed separately in several cages in semi-closed system house.

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Feed and water were available *ad libitum*. Chickens received a commercial broiler starter diet with 3025 kcal of ME/kg and 23% Crude Protein (CP) from first to 14th day. At the age of 14 to 28 days, the chickens were fed a grower diet with 3150 kcal of ME/kg and 21% CP. From 28 days to 35 days of age, the chickens were fed a finisher diet containing 3200 kcal of ME/kg and 19% CP. Starter feed was provided as crumbles, and subsequent feeds were provided as pellets.

Measurements

The live body weights of chickens were recorded individually weekly from day one to five weeks of age. The weekly feed intake per replicate (g) was calculated and then the feed intake for chickens was calculated as the feed intake for each replicate within a certain time interval divided by the number of chickens in the same replicate during the same time period. Then it was divided into seven parts to calculate the daily feed intake. Feed conversion ratio calculated as the average feed consumption (g) for each replicate with a time period divided by the average body weight gain (g) for the same replicate over the same period. Dead chickens were weighed to include their weight in the feed conversion estimates.

Growth rate (GR) was calculated according to the following:

GR = W2 –W1 / [(W1+W2) / 2]

Where, W1 refers to body weight at the beginning of a certain week and W2 to the body weight at the end of the same week.

The mortality rate was recorded daily and calculated as percentage for each replicate.

Production number: (Average kilograms of growth per day x (100 – mortality %) / FCR) x 100

To determine the carcass quality, ten chickens were randomly selected from each treatment at five weeks of age, weighed individually and slaughtered after eight hours fasting. After the blood was drawn, they were defeathered, processed, and eviscerated. Carcass yield (dressing, breast meat, and hind meat) was determined as a percent of the living body weight. Giblets (liver, heart, and gizzard), spleen, thymus, and bursa of fabricius were obtained.

Statistical analysis

The data were subjected to a one-way Analysis of Variance using the general linear model method of XLSTAT (2014) version 2014.5.03. In-ovo injection treatments were the main factor. Percentage data were subjected to arcsine transformation prior to the analysis. The mean values were compared using Duncan's multiple range test (Duncan, 1955) if there was a significant difference ($p \leq 0.05$). The model used was as follows: $Yjk = \mu + Hj + Ejk$

Where: Yjk = individual observation; μ = Overall mean; Hj = Effect of in-ovo injection treatments (j = 1, 2, 3, 4, 5, 6); Ejk = Residual error.

RESULTS AND DISCUSSION

Albumen pH

The effect of in-ovo injection treatments on albumen pH is presented in table 1. No significant differences in egg albumen pH were observed after two days post injection between any treatment groups. However, in all treatments, the albumen pH appeared to be too high when compared to previous researches. Akhlaghi et al. (2013) reported that the non-injected control was 8.67 after two days was 9.07 after four days of treatment. In our experiment, the albumen pH was 8.98 after two days and was 9.43 after four days. This may be due to the transport of eggs from the parents' farm to hatchery conditions was unsuitable. Four days after the injection, only folic acid could cause a significant ($p = 0.037$) decrease in the albumen pH from 9.43 in the negative control group to 9.19. This result may be due to the acidity of folic acid, folate pH < 5 (Combs and McClung, 2016), and the pH of the solution injected into eggs was 3.98. The recent results partly agreed with Ebrahimi et al. (2012), who found no differences in albumen pH due to injection of bicarbonate or phosphate buffer solutions injected into the albumen. Ebrahimi et al. (2012) stated that the volume of the buffer solutions, which had to optimally lower the albumen pH, was too large, which could potentially be detrimental to the embryo.

Embryonic mortality percentage

The effects of in-ovo injection treatments on embryonic mortality and hatchability are presented in table 2. All injection treatments increased early, late, and total embryonic mortality compared to the negative control, but the differences were not significant.

Fertility and Hatchability

There were no significant differences in the hatchability of fertile eggs between any of the injection groups. All treatments decreased either fertility or the hatchability of fertile eggs (Table 2). Hatchability of total eggs was also adversely affected by in-ovo injection, but the differences were only significant between the negative control and folic

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acid, glucose and the combination of folic acid and glucose. Folic acid, glucose and the combination of folic acid and glucose injection decreased the hatchability of fertile eggs to 76.4 %, 75.4 %, and 73.9%, respectively, compared to 91.0 % in the negative control. The results of the present study indicated that the albumen pH plays a minimal role in decreasing the hatchability. This is because the hatchability of the control eggs was high in eggs four days after injection where the albumen pH was high.

Table 1. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on albumen pH (means ± Standard Error)

Item	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
2 days post injection	8.98 ± 0.05	9.01 ± 0.05	8.94 ± 0.05	8.97 ± 0.05	8.94 ± 0.05	8.88 ± 0.05	0.4741
4 days post injection	9.43 ± 0.05[a]	9.35 ± 0.05[a]	9.36 ± 0.05[a]	9.19 ± 0.05[b]	9.36 ± 0.05[a]	9.31 ± 0.05[ab]	0.0367

[a,b;] Means within a row followed by different superscripts differ significantly ($p \leq 0.05$). SE: Standard Error, FA: Folic Acid, Glu: Glucose

Table 2. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on embryonic mortality and hatchability (means ± Standard Error)

Item	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
Non fertile (%)	12.1 ± 2.6	15.7 ± 2.6	18.6 ± 2.6	18.6 ± 2.6	18.6 ± 2.6	22.9 ± 2.6	0.1368
Early mortality (%)	2.9 ± 2.2	2.9 ± 2.2	9.3 ± 2.2	7.9 ± 2.2	10.0 ± 2.2	6.4 ± 2.2	0.1128
Late mortality (%)	5.0 ± 2.6	13.6 ± 2.6	8.6 ± 2.6	11.4 ± 2.6	10.0 ± 2.6	13.6 ± 2.6	0.1867
Total mortality (%)	7.9 ± 3.4	16.4 ± 3.4	17.9 ± 3.4	19.3 ± 3.4	20.0 ± 3.4	20.0 ± 3.4	0.1408
Apparent fertility (%)	87.9 ± 2.6	84.3 ± 2.6	81.4 ± 2.6	81.4 ± 2.6	81.4 ± 2.6	77.1 ± 2.6	0.1368
Hatchability of fertile eggs (%)	91.0 ± 4.1	80.3 ± 4.1	78.0 ± 4.1	76.4 ± 4.1	75.4 ± 4.1	73.9 ± 4.1	0.0811
Hatchability of total eggs (%)	80.0 ± 4.2[a]	67.9 ± 4.2[ab]	63.6 ± 4.2[b]	62.1 ± 4.2[b]	61.4 ± 4.2[b]	57.1 ± 4.2[b]	0.0144

[a,b;] Means within a row followed by different superscripts differ significantly ($p \leq 0.05$). SE: Standard Error, FA: Folic Acid, Glu: Glucose

Ebrahimi et al. (2012), found that in-ovo injection of bicarbonate or phosphate buffer solutions before incubation decreased the hatchability of fertile eggs to 32.0% and 8.3% respectively, while the hatchability of fertile eggs in the controls was 87.5%. In the present results, injection of glucose decreased the hatchability of fertile eggs, but may not be significant due to the organogenesis of important segments of the chicken embryo occur in the first week of embryonic development. Based on this information, in-ovo injection of glucose prior to this critical phase can be an effective stimulator for optimal organ development (Bellairs and Osmond, 2005). Ebrahimi et al. (2012) stated that decreases in hatchability of fertile eggs were due to the injected active ingredients which adversely affect the environment for the embryo. The pH or osmolality of the solutions could adversely affect the surrounding microenvironment of the early embryo. This could rationalize the numerically increased apparent fertility of the negative control in the results of the present study. The results of the present study were in part with Zhai et al. (2011b), who reported that the hatchability of fertile eggs was lower in eggs injected with glucose, fructose, maltose, sucrose, or dextrin, compared to the control group without injected, dry punch, and saline-injected control groups. However, Zhai et al. (2011a) stated that the hatchability of fertile eggs was not impaired by any injection treatment with Glucose, sucrose, maltose, or dextrin. Salmanzadeh et al. (2012) found that in-ovo injection with 75 mg or 100 mg glucose, dissolved in 0.5 mL deionized water after seven days of incubation into the albumen led to less hatchability than the negative control treatment. They stated that the decrease in hatchability could be due to the injection into the albumin under the air sac, which stopped the respiration in the developing embryo. Tasharofi et al. (2018) explained the decrease in hatchability in eggs injected with dextrose due to an overload of the energy metabolism in embryos due to the injection of high carbohydrate levels, which adversely affected hatchability. However, Zhang et al. (2016) found that the hatchability of eggs injected with glucose was very close to that of the negative control group. The results of the present research did not agree with Li et al. (2016) and Liu et al. (2016) found that in-ovo injection of 100 µg or 150 µg of folic acid (the injection volume in each egg was 0.1 ml) into the yolk sac after 11 days at embryonic age increased the hatchability compared to the control treatment (0 µg of folic acid). This may be attributed to the time difference or to the injection site.

On the other hand, Nouri et al. (2018) revealed that there was no significant difference in hatchability for eggs in-ovo, which had been injected with folic acid (40, 80, and 120 µg) in albumen on day seven of incubation, compared to the negative control group and positive group (in-ovo injection of sterile water, 40 µg). Robel (2002) stated that hatchability in turkey eggs injected with folic acid at 25-day of incubation was not significantly affected compared to the

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negative control.

The discrepancy in the results may be due to the timing of the injection. The eggs were injected before incubation, so it was more susceptible to contamination. In-ovo injection, especially in early embryonic life, did not improve hatchability. It seems that in-ovo injection at the beginning of embryonic development could damage the internal environment of the egg and also have negative effects on hatchability (Salmanzadeh et al., 2012). The injection volume can also be too large. Zhai et al. (2011c) stated that the hatchability of fertile eggs was negatively related to the injection volume. Therefore, the in-ovo injection volume should be limited to prevent the embryo from becoming excessive hydrated and a subsequent decreasing hatchability. The effectiveness of in-ovo injection on hatchability is still not clear enough. The optimal site and time of injection as well as the volume of the injected solution and the appropriate nutrient have yet to be determined (Tasharofi et al., 2018).

Post-hatch performance

The in-ovo injection had no significant effect ($p > 0.05$) on the body weight of chicken at hatching at one, three and five weeks of age (Table 3). However, a significant effect of the in-ovo injection treatment on body weight of the chicken at two and four weeks of age was observed. The body weight of chickens in the group injected with the combination of FA and Glu was the highest compared to the other injection treatments. However, the difference was not significant compared to the negative control. In-ovo injection with FA, Glu, and FA + Glu had no significant influence on the body weight of the chickens at hatching.

Table 3. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on body weight (g) (means ± Standard Error) of broiler chickens

Age	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
One day	47.5 ± 0.4	47.7 ± 0.4	47.7 ± 0.4	48.6 ± 0.4	48.5 ± 0.4	48.2 ± 0.4	0.1671
Week 1	173 ± 1.9	173 ± 2.0	170 ± 2.1	168 ± 2.1	168 ± 2.1	171 ± 2.3	0.2760
Week 2	405 ± 7.3^{ab}	387 ± 7.8^{b}	406 ± 8.2^{ab}	394 ± 7.9^{b}	393 ± 8.3^{b}	425 ± 8.7^{a}	0.0233
Week 3	892 ± 13.8	854 ± 14.7	864 ± 15.4	857 ± 14.9	855 ± 15.6	908 ± 16.3	0.0587
Week 4	1527 ± 22.2^{a}	1447 ± 23.6^{b}	1456 ± 25.0^{b}	1461 ± 24.0^{b}	1454 ± 25.1^{b}	1537 ± 26.3^{a}	0.0184
Week 5	2177 ± 29.3	2122 ± 31.5	2100 ± 33.8	2103 ± 31.9	2123 ± 33.4	2157 ± 35.2	0.4381

$^{a,b;}$ Means within a row followed by different superscripts differ significantly ($p \leq 0.05$). SE: Standard Error, FA: Folic Acid, Glu: Glucose

Liu et al. (2016) found that the in-ovo injection of 150 µg FA into the yolk sac after 11 days of embryonic age significantly increased the body weight of the one-day-old chickens. Liu et al. (2016) also reported that folic acid injection might up-regulate IGF2 expression, and they reported the genomic correlation between chickens' body weight and plasma IGF2 levels. The body weight of chickens in the (FA + Glu) group was significantly higher after two and four weeks than in the DPC group. These results partly agreed with the results reported by Nouri et al. (2018). They stated that body weight on day 21 was significantly improved in chickens that had been injected in-ovo with 120 µg folic acid in albumen on the seventh day of incubation. Li et al. (2016) reported that body weight was significantly increased by the age of 42 days. Salmanzadeh (2012), Kanagaraju and Rathnapraba (2019) also indicated that in-ovo injection with 0.5 ml of 25 % glucose (on day-7 of incubation in the albumen and on day-18 of incubation in the amnion, respectively) improved body weight. Zhai et al. (2011c) found that injected chicken embryo in the amnion on day 19 of incubation with 0.1, 0.4, 0.7, or 1.0 mL of various carbohydrates (Glucose, fructose, sucrose, maltose, and dextrin) associated with chicken's body weight. The recent results indicated that the combination of folic acid and glucose played an important role in poultry growth performance. An increases in the body weight of in-ovo-injected broiler embryos with (FA + Glu) could be viewed as a consequence of the improvement in enteric development and a subsequent enhancement in nutrient absorption (Zhai et al. 2011c), or as a good nutrient for better use of the energy by the embryos Uni et al. (2005).

Kanagaraju and Rathnapraba (2019) also stated that the in-ovo injection of 0.5 ml of 25 % glucose into the amnion on day 18 of incubation significantly improved duodenal, jejunal and ileal histomorphology (villi height, width, crypt depth, and villi surface area) of broilers, which led to the enhancement of digestion and absorption of nutrients. On other hand, Zhang et al. (2016) indicated that the individual injection of 0.4 mL glucose (25 mg) on day 18 of the incubation did not affect hatching weight and growth performance of the chickens during the first week of post-hatching. The difference between Zhang et al. (2016) results and previous studies was related to the carbohydrate type, injection dose, genetic strain, and egg size.

The in-ovo injection had no significant effect on the daily feed intake at the age of one, two, three, and five weeks, the average daily feed intake and the total feed intake (Table 4). The only significant difference was observed at four

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weeks of age between DPC treatment and (FA + Glu) treatment, which consumed more food than the first one. The results of the present study were agreed with Li et al. (2016), who stated that there were no significant differences in the average daily feed intake between control and folic acid injected treatment. However, recent results did not agree with Nouri et al. (2018), they found that the feed intake of in-ovo chickens that were injected with folic acid was significantly increased compared to the control group. Salmanzadeh (2012) reported that the in-ovo injection of 0.5 ml of 25 % glucose into the albumen on day seven of incubation did not affect feed intake. Kanagaraju and Rathnapraba (2019) found that the treatment of in-ovo injection of 0.5 ml of 25 % glucose on day 18 of incubation into the amnion significantly increased feed intake compared to the negative control and positive groups. This discrepancy in the results might be due to the differences in injection time. In-ovo injection (shortly before hatching) with carbohydrates enabled the early adaptation of the avian gastrointestinal tract during embryonic development (Kucharska-Gaca et al., 2017) and adapted them to their new diet after hatching (Cardeal et al., 2015) in more feed intake.

Table 5 present that the in-ovo injection had no significant effects on the Feed Conversion Ratio (FCR) in any of the age group examined. In general, in-ovo injection with FA improved FCR insignificantly compared to (Glu) and (FA + Glu) treatments. The present results were partly agreed with Li et al. (2016), who found that injection treatment with 100 and 150 µg folic acid significantly improved FCR. However, Nouri et al. (2018) stated that the FCR in broilers that had been injected with 120 µg folic acid in albumen on day 7 of incubation was significantly improved on 0–42 days compared to the control treatment. On the other hand, Salmanzadeh (2012), Kanagaraju and Rathnapraba (2019) found that chickens that were injected with glucose in-ovo had a higher FCR than chickens hatched from the control group and the positive group.

No significant effect on weekly chickens' mortality was observed (Table 7). Injection treatments had no significant effect ($p>0.05$) on the growth rate in all weeks (Table 6) and on the production number (Table 8). In-ovo injection of folic acid numerically increased the production number compared to the positive control.

Table 4. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on average daily feed intake (g) (means ± Standard Error) of broiler chickens.

Age	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
Week 1	23.3 ± 0.7	24.0 ± 0.87	23.3 ± 0.9	23.0 ± 0.8	22.9 ± 0.8	23.9 ± 0.9	0.9194
Week 2	44.6 ± 2.3	42.5 ± 2.6	45.7 ± 2.6	41.4 ± 2.5	42.6 ± 2.5	44.7 ± 2.6	0.8282
Week 3	94.6 ± 2.3	90.0 ± 2.7	87.7 ± 2.7	87.0 ± 2.5	89.1 ± 2.5	89.8 ± 2.7	0.2927
Week 4	133.9 ± 3.7^{a}	116.5 ± 4.4^{b}	125.5 ± 4.4^{ab}	124.3 ± 4.1^{ab}	128.3 ± 4.1^{ab}	135.2 ± 4.4^{a}	0.0346
Week 5	156.9 ± 4.7	147.8 ± 5.6	139.7 ± 5.6	145.3 ± 5.2	156.1 ± 5.2	151.6 ± 5.6	0.1802
Average daily feed intake	90.7 ± 1.8	84.1 ± 2.2	84.4 ± 2.2	87.8 ± 2.0	84.2 ± 2.0	89.1 ± 2.2	0.0934
Total feed intake	3173 ± 64	2945 ± 76	2954 ± 76	2947 ± 71	3073 ± 71	3117 ± 76	0.0934

$^{a,b;}$ Means within a row followed by different superscripts differ significantly ($p \leq 0.05$). SE: Standard Error, FA: Folic Acid, Glu: Glucose

Table 5. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on feed conversion (means ± Standard Error) of broiler chickens.

Age	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
Week 1	1.31 ± 0.04	1.33 ± 0.04	1.34 ± 0.04	1.34 ± 0.04	1.35 ± 0.04	1.37 ± 0.04	0.9383
Week 2	1.31 ± 0.04	1.32 ± 0.04	1.33 ± 0.04	1.29 ± 0.04	1.35 ± 0.04	1.24 ± 0.04	0.5759
Week 3	1.40 ± 0.02	1.33 ± 0.03	1.33 ± 0.03	1.32 ± 0.03	1.35 ± 0.03	1.31 ± 0.03	0.1547
Week 4	1.46 ± 0.04	1.37 ± 0.05	1.51 ± 0.05	1.45 ± 0.04	1.51 ± 0.04	1.50 ± 0.04	0.3017
Week 5	1.67 ± 0.05	1.56 ± 0.06	1.52 ± 0.06	1.57 ± 0.06	1.62 ± 0.06	1.66 ± 0.06	0.4033
0 – 35 days	1.49 ± 0.03	1.42 ± 0.03	1.50 ± 0.03	1.42 ± 0.03	1.49 ± 0.03	1.51 ± 0.03	0.1419

No significant differences were observed. SE: Standard Error, FA: Folic Acid, Glu: Glucose

Table 6. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on growth rate (means ± Standard Error) of broiler chickens.

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