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# Effects of *in ovo* electrolyte feeding and delayed placement on post-hatch chick quality, yolk sac utilization, and gut organ weight in neonatal broiler chicks

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ABSTRACT

This study investigated the effects of *in ovo* electrolyte feeding and delayed placement on chick quality, yolk sac utilization, and gut organ weight in broiler chicks. It followed a 3×3 factorial arrangement in a completely randomized design with the two factors being *in ovo* treatment (non-injected [NI], 0.5mL normal saline [NS], and 0.5mL oral rehydration solution [ORS]) and delayed placement (0, 24, and 48 hours). 420 fertile eggs from Arbor Acres breeders were randomly distributed into the *in ovo* treatment groups on incubation day 18. On day 21, 50 chicks per treatment were held and fasted for 48 hours. 14 chicks per treatment were evaluated, eviscerated, and organs weighed. Chick length (CL), relative yolk-free body mass (RYFBM), and Tona scores (TS) were higher ($p < 0.05$) in the ORS and NS groups than in NI. With increasing delayed placement, chick weight, RYFBM, TS, and relative yolk sac weight decreased ($p < 0.05$) while CL and relative gizzard and intestine weight increased ($p < 0.05$). The NI and ORS groups showed the highest relative yolk sac (9.88%) and gizzard (7.85%) weights, respectively. It was concluded that IOEF reduced hatch rate but improved broiler chicks' quality, yolk utilization, and gut organ weight.

**Keywords**: *in ovo* feeding, delayed placement, electrolytes, broiler chicks, chick quality

## INTRODUCTION

Broiler chickens have the genetic potential for rapid growth and maturity. Apart from proper management, they require adequate and high-quality feed for optimal performance. While early access to feed and water is vital to chickens' growth and welfare (Boyner et al., 2023), post-hatch delayed access to feed remains a common challenge in the poultry industry. Delayed placement refers to the time gap between a chick hatching and being moved to an environment where it has access to feed, water, warmth, and appropriate care (Vieira and Moran, 1999). It occurs due to reasons such as wide hatch windows, long distances between hatchery and brooding facilities, transportation delays, and emergencies. Delayed placement for up to 48 hours has been shown to negatively impact chick quality, such as reduced body weight, impaired gut development (de Jong et al., 2017; Moon, 2019); dehydration, and impaired immune system function (Tanimowo and Longe 2020; Modej et al., 2024), all of which contribute to economic losses. Therefore, early feeding and proper management within the first 48 hours after hatching are important to post-hatch quality of chicks and performance.

Feeding nutrients to developing chicken embryos could help overcome some problems associated with placement delay in chickens. The simplest method or technique to achieve this is called *in ovo* feeding (IOF) or *in ovo* injection (IOI). *In ovo* feeding, a technique invented and patented by Uni and Ferket (2003), involves injecting bioactive compounds such as vaccines and nutrients into the amniotic fluid of eggs during incubation. Feeding chicken embryos with nutrients *in ovo* before hatching stimulates early development of the gut, resulting in efficient digestion of feed and enhanced nutrient utilization (Givisiez et al., 2020). Some bioactive compounds have been successfully supplemented *in ovo* to enhance the post-hatch performance of broilers. They include amino acids (Elnesr et al., 2019), vitamins (Soltani et al., 2019), carbohydrates (Ding et al., 2020), electrolytes (Pawłowska et al., 2022), fruit extracts (Kuka et al., 2023; Kpossou et al., 2024), and other metabolites (Tanimowo and Longe 2020; Saleh et al., 2021).

Electrolytes, including sodium, chloride, potassium, magnesium, and calcium, are naturally occurring minerals important for maintaining fluid balance within the body of chickens (Mushtaq and Pasha, 2013). They ensure that the hydration level within body cells is optimal for normal body functions by retaining or drawing more water into the cells during extreme dehydration. Moreover, electrolytic balance significantly affects young chicks, influencing weight gain, feed intake, and feed conversion ratio (Borges et al., 2002). Delayed placement has been linked with weight loss and dehydration due to lack of prompt access to feed and water after hatching (Abed et al., 2011), and feeding electrolytes *in ovo* to broiler chicks could be one of the solutions to the problem. Therefore, this study investigated the impacts of *in ovo* electrolyte feeding

(IOEF) and delayed placement on chick quality, yolk utilization, and gut organ weight in neonatal broiler chicks.

## MATERIALS AND METHODS

### *Egg incubation and in ovo feeding procedures*

This experiment was conducted at Obasanjo Farms Hatchery, Ibadan, and the Department of Animal Science, University of Ibadan, Nigeria, and it followed a 3 × 3 factorial arrangement in a completely randomized design. A total of 500 hatchable eggs, obtained from 35-week-old Arbor Acres parent stock, were used. The eggs were placed in a Petersime NV incubator and incubated at 99.5°F with 55% relative humidity (RH) for 18 days. On the 18th day, the eggs were candled, and all non-viable ones were discarded. After candling, A total of 420 fertile eggs were randomly assigned to three groups (NS, ORS, and NI; n=420) and evenly distributed across two replicate trays per group (70 eggs per tray). 9 g of sodium chloride (NaCl) was dissolved in 1 liter of distilled water to produce normal saline while 20.5 g of oral rehydration salt was dissolved in a liter of distilled water to produce an oral rehydration solution (ORS). The composition of ORS is presented in Table 1.

**Table 1.** Composition of oral rehydration solution per liter

| Ingredient | Concentration (g/L) |
|---|---|
| Glucose, anhydrous | 13.5 |
| Trisodium citrate, dihydrate | 2.9 |
| Sodium chloride | 2.6 |
| Potassium chloride | 1.5 |

Uni and Ferket's (2003) *in ovo* feeding procedures were followed for the current experiment. Before injecting the electrolytes into the eggs, the electrolyte solutions were warmed to about 95°F, and the point of injection (egg's broad end) was sterilized by swabbing with sterile cotton wool soaked in 70% ethanol. A sterile 21G 25mm hypodermic needle was used to drill through the air sac, inserted to about 1 cm, and 0.5 mL of the solution was injected into the amniotic fluid with a syringe. Eggs in the NI group were drilled through, but no substance was injected. After injection, the injection hole on the eggshell was sealed with paper adhesive tape, and the injected and non-injected eggs were transferred into the hatcher in six labeled hatch baskets.

*Data collection*

On the 21st day of incubation, the baskets were pulled out of the hatcher. All hatched chicks in each treatment group were weighed on a CAMRY® digital scale and counted to determine hatchability per treatment using the formula below.

$$\text{Hatchability (\%)} = \frac{\text{Number of chicks hatched}}{\text{Number of eggs set per treatment}} \times 100$$

Fifty (50) chicks were randomly picked from NI, NS, and ORS treatment groups (n=150) and put in three labeled perforated chick boxes. After that, fourteen (14) chicks were randomly picked in each box (n=42), weighed, and evaluated according to Tona et al. (2023) quality scoring method. This method is based on the physical condition of the chick's eyes, legs, navel area, activity, down and appearance, retracted yolk, remaining membrane on the chick's body, and remaining yolk (Tona et al., 2003). One human evaluator carried out Chick quality assessment and scoring. The Tona score was calculated by adding the scores of various features. Chick length was measured with a ruler from the beak's tip to the end of the middle toe. The chicks were then euthanized and dissected, and the residual yolk sac (RYS) was removed to calculate the relative yolk-free body mass using the formula below:

$$\text{Relative YFBM (\%)} = \frac{\text{Body weight (g)} - \text{Yolk sac (g)}}{\text{Total body weight (g)}} \times 100$$

After removing the RYS, the heart, gizzard, liver, and small intestine were also removed and weighed to determine the relative organ weight. The eviscerated carcass was also weighed. The relative weight of each organ was calculated using the following formula.

$$\text{Relative organ weight (\%)} = \frac{\text{Weight of organ (g)}}{\text{Total body weight (g)}} \times 100$$

All organs, including the eviscerated carcass, were dried in a laboratory oven at 70°C for 72 hours to determine their moisture content. The moisture content was calculated using the following formula.

$$\text{Moisture content (\%)} = \frac{\text{Organ wet weight (g)} - \text{organ dry weight (g)}}{\text{Organ wet weight (g)}} \times 100$$

The remaining chicks, while in their respective labeled chick boxes, were put in a chick room under similar ambient conditions (25-30°C and 40-50% RH) for 24 hours and 48 hours without providing feed or water. This approach simulates common conditions and delays that most chicks experience before being transported to their destination farms. After 24 hours, another 14 chicks were randomly picked from the remaining chicks in each box (n=42). Their weight, Tona score, and chick length were determined before they were euthanized and eviscerated. Their RYS and other organs were also weighed and oven-dried. The same process was repeated after 48 hours.

*Statistical analysis*

SPSS v20.0 software was used to analyze all statistical data. Normality of the data was checked using the Shapiro–Wilk test on SPSS before proceeding with data analysis. The hatchability data were analyzed using one-way ANOVA, while others were analyzed using two-way ANOVA with the mathematical model below:

$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$

where:

- $Y_{ijk}$ = observation for the k$^{th}$ replicate in the i$^{th}$ *in ovo* treatment (i = NI, NS, ORS) and j$^{th}$ placement delay (j = 0, 24, 48 hours),
- $\mu$ = overall mean,
- $\alpha_i$ and $\beta_j$ are the main effects of *in ovo* treatment and placement delay, respectively,
- $(\alpha\beta)_{ij}$ = interaction effect,
- $\varepsilon_{ijk}$ = random error term

Mean differences were evaluated using Duncan's multiple range test at a significance level of $p < 0.05$.

## RESULTS

*Hatchability of fertile eggs*

The present study investigated the effects of IOEF on the hatchability of fertile broiler chicken eggs. As shown in Table 2, *in ovo* feeding (IOF) of electrolytes on the 18th day of incubation significantly affected the hatchability of fertile broiler chicken eggs ($p = 0.048$). The hatchability of eggs that were injected with 0.5 mL of ORS was significantly lower (64.3%) compared to eggs that received 0.5 mL of normal saline (79.1%) and non-injected (82.0%). However, the hatchability between the NI and NS treatment groups was not significantly different. The total hatchability of eggs was 75.13%.

**Table 2.** Effects of IOEF on the hatchability of fertile eggs

| Treatment | Hatchability (%) |
|---|---|
| NI | 82.0$^{a}$ |
| NS | 79.1$^{a}$ |
| ORS | 64.3$^{b}$ |
| Total | 75.13 |
| SEM | 1.700 |
| p-value | 0.048 |

$^{ab}$ Means within a column differ significantly ($p < 0.05$)
NI = non-injected; NS = injected with 0.5 mL of normal saline; ORS = injected with 0.5 mL of oral rehydration solution; SEM = Standard error of the mean

*Body weight, chick length, and chick quality*

In the current study, chick quality was assessed using chick weight, chick length, RYFBM, and Tona score. Table 3 shows the effects of IOEF and delayed placement on the quality of neonatal broiler chicks. A significant interaction effect ($p < 0.05$) was found between IOEF and delayed placement for RYFBM and Tona score. Relative YFBM and Tona scores were significantly higher at 0 hours, 24 hours, and 48 hours of delayed placement in the ORS treatment group compared to the NS and NI groups. Moreover, broiler chicks in the NS and ORS treatment groups exhibited significantly higher RYFBM at both 24-hour and 48-hour delayed placement. While there were significant reductions (9.2-11.2%) in the Tona scores of broiler chicks with increasing delayed placement time across treatment groups, the reduction rate was lower (9.2-9.5%) in the NS and ORS treatment groups compared to the NI group.

The main effect of IOEF on chick weight was not significant ($p > 0.05$). Chick length, RYFBM, and Tona score were significantly affected by IOEF ($p < 0.05$). Broiler chicks that received ORS *in ovo* exhibited significantly higher ($p < 0.001$) chick length (17.57 cm) compared to the NI (16.95 cm) and NS (17.22 cm) treatment groups. The RYFBM and Tona scores of broiler chicks in the NS and ORS groups were significantly higher ($p < 0.05$) than those in NI. The main effect of delayed placement was also significant ($p < 0.001$) on chick weight, chick length, RYFBM, and Tona score. Chick weight and Tona score significantly decreased ($p < 0.001$) with increasing placement delay. Chick weight decreased from 35.47 g at 0 hours (pull time) to 32.02 g and 29.48 g at 24 hours and 48 hours respectively. The Tona score decreased from 96.97/100 at pull time to 91.71/100 and 86.98/100 at 24 hours and 48 hours respectively. Conversely, chick length and RYFBM significantly ($p < 0.001$) increased with increasing placement holding time where chick length increased from 16.77 cm at 0 hours to 17.47 cm and 17.50 cm at 24 hours and 48 hours. Relative yolk-free body mass also increased from 90.65% at pull time to 93.61% at 48 hours.

**Table 3.** Effects of IOEF and delayed placement on broiler chick quality

| **Treatment** | | **Chick quality parameters** | | | |
|---|---|---|---|---|---|
| | | **CW (g)** | **CL (cm)** | **RYFBM (%)** | **TS** |
| **IOEF** | | | | | |
| NI | | 31.48 | 16.95[b] | 90.13[b] | 89.88[b] |
| NS | | 32.71 | 17.22[b] | 94.12[a] | 92.42[a] |
| ORS | | 32.77 | 17.57[a] | 93.98[a] | 93.36[a] |
| SEM | | 0.536 | 0.118 | 0.687 | 0.535 |
| **Delayed placement (hours)** | | | | | |
| 0 | | 35.47[a] | 16.77[b] | 90.65[b] | 96.97[a] |
| 24 | | 32.02[b] | 17.47[a] | 93.97[a] | 91.71[b] |
| 48 | | 29.48[c] | 17.50[a] | 93.61[a] | 86.98[c] |
| SEM | | 0.503 | 0.111 | 0.645 | 0.502 |
| **IOEF × Delayed placement (hours)** | | | | | |
| NI | 0 | 36.30 | 16.26 | 86.52[d] | 95.20[c] |
| | 24 | 31.00 | 17.29 | 94.14[a] | 90.43[de] |
| | 48 | 27.14 | 17.30 | 89.73[c] | 84.00[f] |
| NS | 0 | 34.50 | 17.07 | 93.79[a] | 97.40[ab] |
| | 24 | 32.93 | 17.40 | 92.86[b] | 92.00[d] |
| | 48 | 30.71 | 17.20 | 95.72[a] | 87.86[e] |
| ORS | 0 | 35.60 | 16.98 | 91.64[b] | 98.30[a] |
| | 24 | 32.14 | 17.71 | 94.92[a] | 92.71[cd] |
| | 48 | 30.57 | 18.01 | 95.38[a] | 89.07[e] |
| SEM | | 0.872 | 0.192 | 1.118 | 0.870 |
| | | | | **p-value** | |
| IOEF | | 0.162 | 0.001 | 0.003 | <0.001 |
| Delayed placement | | <0.001 | <0.001 | <0.001 | <0.001 |
| IOEF × Delayed placement | | 0.063 | 0.129 | 0.004 | <0.001 |

[abcde], means within a column differ significantly ($p < 0.05$)
IOEF = *in ovo* electrolyte feeding; DP = delayed placement; CW = chick weight; CL = chick length; RYFBM = relative yolk-free body mass; TS = Tona score; NI = non-injected; NS = injected with 0.5mL of normal saline; ORS = injected with 0.5mL of oral rehydration solution; SEM = Standard error of the mean

*Relative weight of residual yolk, gut organs, and eviscerated carcass*

Table 4 presents the effects of IOEF and delayed placement on the relative weight of RYS, liver, gizzard, small intestine, and eviscerated carcass of neonatal broiler chicks. A significant interaction ($p < 0.05$) between IOEF and delayed placement was observed for the relative weight of RYS, gizzard, and eviscerated carcass. A higher relative RYS weight was observed at 0 hours, 24 hours, and 48 hours in the NI group compared to NS and ORS, with the highest RYS weight observed at 0 hours (13.49%). In contrast, relative RYS weight decreased significantly ($p < 0.05$) across NI, NS, and ORS groups with increased placement delay, with the greatest reduction observed in the NS group at 48 hours (4.28%). Relative gizzard weight was highest in the NS treatment group at 0 hours (8.41%) and exhibited an increase with placement delay for ORS, reaching 8.41% at 48 hours, whereas NI exhibited a comparatively lower relative gizzard weight in 0 hours, 24 hours and 48 hours. The relative eviscerated carcass weight was also influenced by both IOEF and placement delay, with the NI group exhibiting a significantly reduced relative eviscerated carcass weight at 24 hours while the NS group at 48 hours. However, the relative eviscerated carcass weight in the ORS treatment group was statistically the same from 0 hours to 48 hours of delayed placement. The main effects of IOEF on relative liver, small intestine, and eviscerated carcass weight were not significant ($p > 0.05$) but significant for gizzard and RYS ($p < 0.05$). A higher relative gizzard weight (7.84%) was observed in the ORS treatment group compared to the NI and NS treatment groups while the relative RYS weight was higher in the NI group. Moreover, delayed placement had significant effects ($p < 0.05$) on the relative weight of RYS, liver, gizzard, small intestine, and eviscerated carcass of the neonatal broiler chicks. The relative weight of RYS, liver, small intestine, and eviscerated carcass were observed to decrease with an increase in placement delay while the gizzard exhibited an increased relative weight with an increase in placement delay.

**Table 4.** Effects of IOEF and delayed placement on the relative residual yolk sac, gut organs and carcass weight of neonatal broiler chicks

| Treatment | | Relative weight (%) | | | | |
|---|---|---|---|---|---|---|
| | | RYS | LV | GZ | SI | EC |
| **IOEF** | | | | | | |
| NI | | 9.88[a] | 3.37 | 7.08[b] | 5.53 | 66.72 |
| NS | | 5.88[b] | 3.50 | 7.20[b] | 5.10 | 66.34 |
| ORS | | 6.02[b] | 3.47 | 7.85[a] | 5.42 | 68.36 |
| SEM | | 0.687 | 0.081 | 0.201 | 0.196 | 0.617 |
| **Delayed placement (hour)** | | | | | | |
| 0 | | 9.66[a] | 3.64[a] | 6.56[b] | 4.10[b] | 69.26[a] |
| 24 | | 7.19[b] | 3.46[a] | 7.62[a] | 5.90[a] | 67.45[b] |
| 48 | | 4.92[c] | 3.25[b] | 7.95[a] | 6.05[a] | 64.71[c] |
| SEM | | 0.645 | 0.079 | 0.189 | 0.184 | 0.580 |
| **IOEF × Delayed placement (hour)** | | | | | | |
| NI | 0 | 13.49[a] | 3.72 | 6.72[c] | 4.60 | 68.58[a] |
| | 24 | 10.28[ab] | 3.30 | 7.49[ab] | 5.98 | 65.80[b] |
| | 48 | 5.86[bc] | 3.08 | 7.04[bc] | 6.01 | 65.78[b] |
| NS | 0 | 7.14[bc] | 3.63 | 5.75[c] | 3.25 | 69.84[a] |
| | 24 | 6.22[bc] | 3.50 | 7.44[ab] | 5.84 | 67.92[a] |
| | 48 | 4.28[c] | 3.38 | 8.41[a] | 6.22 | 61.27[b] |
| ORS | 0 | 8.36[abc] | 3.57 | 7.20[bc] | 4.45 | 69.36[a] |
| | 24 | 5.08[bc] | 3.57 | 7.93[ab] | 5.88 | 68.64[a] |
| | 48 | 4.62[c] | 3.28 | 8.41[a] | 5.92 | 67.09[a] |
| SEM | | 1.118 | 0.131 | 0.327 | 0.318 | 1.004 |
| | | | **p-value** | | | |
| IOEF | | <0.001 | 0.456 | 0.018 | 0.288 | 0.053 |
| Delayed placement | | 0.003 | 0.003 | <0.001 | <0.001 | <0.001 |
| IOEF × Delayed placement | | 0.004 | 0.442 | 0.024 | 0.160 | 0.002 |

[abc] Means within a column differ significantly ($p < 0.05$)
IOEF = *in ovo* electrolyte feeding; EC = eviscerated carcass; LV = liver; GZ = gizzard; SI = small intestine; RYS= residual yolk sac; NI = non-injected; NS = injected with 0.5mL of normal saline; ORS = injected with 0.5mL of oral rehydration solution; SEM = Standard error of the mean

*Moisture content of organs and eviscerated carcass*

The effects of IOEF and delayed placement on the moisture content of organs and eviscerated carcasses of neonatal broiler chicks were presented in Table 5. No significant interaction ($p > 0.05$) was observed between IOES and delayed placement on the moisture content of liver, gizzard, small intestine and eviscerated carcass. A significant main effect ($p < 0.001$) of IOEF was observed on the moisture content of the liver, gizzard, small intestine, and eviscerated carcass. Significantly higher moisture content ($p < 0.05$) was observed for the liver, gizzard, and small intestine in the NS and ORS groups compared to those in the NI treatment group. However, chicks from the ORS group had the highest moisture content for all organs. The main effect of delayed placement was significant ($p < 0.001$) on the moisture content of the gizzard, small intestine, and eviscerated carcass except for the liver. A decrease in moisture content with increasing placement delay was observed for gizzard, small intestine, and eviscerated carcass. Moreover, no significant interaction effect ($p > 0.05$) of IOEF and delayed placement was observed on the liver, gizzard, small intestine, and eviscerated carcass.

**Table 5.** Effects of IOEF and delayed placement on the moisture content of gut organs and eviscerated carcass of neonatal broiler chicks

| Treatment | | Moisture content (%) | | | |
|---|---|---|---|---|---|
| | | LV | GZ | SI | EC |
| **IOEF** | | | | | |
| NI | | 61.80$^{b}$ | 65.46$^{c}$ | 61.83$^{c}$ | 63.93$^{b}$ |
| NS | | 67.27$^{a}$ | 70.55$^{b}$ | 65.58$^{b}$ | 69.32$^{a}$ |
| ORS | | 68.21$^{a}$ | 72.10$^{a}$ | 69.90$^{a}$ | 69.43$^{a}$ |
| SEM | | 0.639 | 0.352 | 0.770 | 0.225 |
| **Delayed placement (hours)** | | | | | |
| 0 | | 66.68 | 72.51$^{a}$ | 69.26$^{a}$ | 72.25$^{a}$ |
| 24 | | 65.77 | 69.69$^{b}$ | 65.99$^{b}$ | 67.59$^{b}$ |
| 48 | | 64.82 | 65.91$^{c}$ | 62.05$^{b}$ | 62.84$^{c}$ |
| SEM | | 0.600 | 0.331 | 0.723 | 0.239 |
| **IOEF × Delayed placement (hours)** | | | | | |
| NI | 0 | 62.72 | 68.99 | 65.34 | 69.13 |
| | 24 | 61.89 | 65.84 | 62.37 | 64.05 |
| | 48 | 60.78 | 61.54 | 57.77 | 58.61 |
| NS | 0 | 68.32 | 73.54 | 69.26 | 73.73 |
| | 24 | 67.19 | 70.70 | 65.53 | 69.29 |
| | 48 | 66.29 | 67.41 | 61.96 | 64.94 |
| ORS | 0 | 69.01 | 75.01 | 73.18 | 73.89 |
| | 24 | 68.23 | 72.52 | 70.08 | 69.43 |
| | 48 | 67.40 | 68.78 | 66.43 | 64.98 |
| SEM | | 1.040 | 0.573 | 1.253 | 0.414 |
| | | **p-value** | | | |
| IOEF | | <0.001 | <0.001 | <0.001 | <0.001 |
| Delayed placement | | 0.137 | <0.001 | <0.001 | <0.001 |
| IOEF × Delayed placement | | 0.998 | 0.806 | 0.992 | 0.311 |

[abc] Means within a column differ significantly ($p < 0.05$)
IOEF = *in ovo* electrolyte feeding; EC = eviscerated carcass; LV = liver; GZ = gizzard; SI = small intestine; NI = non-injected; NI = non-injected; NS = injected with 0.5mL of normal saline; ORS = injected with 0.5mL of oral rehydration solution; SEM = Standard error of the mean

## Discussions

According to Oliveira et al. (2023), the impact of *in ovo* injection on the hatchability of poultry eggs varies depending on the injected substance and its concentration. In the present study, a lower hatchability of 64.3% was observed for broiler chicken eggs injected with 0.5 mL of ORS on the 18th day of incubation, while the hatchability between normal saline (79.1%) and non-injected (82.0%) groups were not significantly different. Cönerr and Saçaklı (2023) had similar hatchability results in which IOF of 0.5 mL glucose solution (0.25g/mL) significantly reduced the hatchability of broiler eggs. They reported that the possible cause of decreased hatchability is egg dehydration linked to the high osmolarity (1696 mOsm/L). Similarly, Salmanzadeh et al. (2011) observed that IOF of 0.5 mL glucose solution (20% glucose in deionized water) resulted in a significant reduction in the broiler eggs' hatchability compared to the non-injected eggs, likely due to the effects of glucose concentration. In the current study, the glucose concentration in the ORS injected was 13.5 g/L, which may have similarly contributed to the lower hatchability observed in the ORS treatment group by increasing embryonic mortality before the hatch day. However, further research is necessary to determine if *in ovo* injection of lower dosages of ORS would improve hatchability. Additionally, the total hatchability of 75.13% observed in the current study was considerably lower compared to the average estimated hatchability of 86% expected from 31- to 36-week-old broiler breeder flock (Yassin et al, 2008). The *in ovo* injection procedures were followed and the incubation conditions were appropriately set in the present study, but stress-related factors may have contributed to the low hatchability observed across all treatments, including the non-injected eggs. Specifically, the injection of electrolytes began immediately after egg candling on the 18th day of incubation, and the embryos may have experienced stress due to disturbance and temperature changes, as the eggs remained outside the hatcher for more than an hour during the allotment and injection process. Hence, automating the IOI procedure may help reduce such stress and improve hatchability. It can also enhance production efficiency in hatcheries by enabling precise delivery of nutrients into embryos for improved post-hatch performance or productivity.

While high hatchability is important to commercial hatcheries, producing high-quality chicks is paramount to both hatcheries and poultry farmers (Pawłowska et al., 2022). Chick quality was assessed using chick weight, chick length, RYFBM, and Tona score in the current study. Pawłowska et al. (2022) observed that the IOI of electrolyte solution had no significant effect on chick weight, but the length and Tona score of chicks that received saline *in ovo* were higher than those that were not injected. Salahi et al. (2011) also observed higher chick lengths in broiler chicks from eggs injected with normal saline at day 18.5 of incubation compared to the control group. The findings of Pawłowska et al. (2022) and Salahi et al. (2011) agreed with the current study as IOF of normal saline and ORS did not influence chick weight but resulted in increased chick length, RYFBM, and Tona score. Moreover, increased chick length has been linked to yolk utilization (Wolanski et al., 2003). This relationship is evident in the current study as RYFBM was higher in chicks that received electrolytes *in ovo*, which also indicated faster utilization of residual yolk compared to the non-injected. Additionally, neonatal broiler chicks subjected to delayed placement exhibited reduced body weight and Tona score with increasing placement delay. These results agree with the findings of de Jong et al. (2017) and Abed et al. (2011), who observed that lack of access to water and feed for more than 24 hours significantly reduced chick body weight compared to those fed early. However, normal saline and ORS injected were effective in mitigating the effects of delayed placement on the Tona score from pull time to 48 hours. On the other hand, chick length and RYFBM increased with increasing placement delay in the present study. This showed that the residual yolk or yolk reserve was efficiently utilized by the chicks for development despite having no access to feed and water, with broiler chicks that received normal saline and ORS showing higher chick lengths during the 48-hour placement delay.

In the current study, the IOF of electrolytes had no significant impact on the relative liver, small intestine, and eviscerated carcass weight of neonatal broiler chicks, except for the gizzard and residual yolk sac. Relative RYS weight was lower with both normal saline and ORS injections while the relative gizzard weight was higher with ORS injections. Relative RYS weight also decreased as delayed placement increased, indicating a significant utilization of the yolk by the neonatal broiler chicks. Özlü et al. (2023) similarly reported that broiler chicks' relative residual yolk weight decreased as preplacement delay increased from 0 to 72 hours. Residual yolk, a vital post-hatch nutrient reserve, is utilized by chicks for gut and organ development in preparation for the digestion of exogenous feed (Noy and Sklan, 1999). However, when exogenous feed is unavailable, chicks are compelled to rely on the protein-rich residual yolk as an energy source (Lamot et al., 2014). This reliance can hinder the development of their digestive system and organs, as observed in this study. Previous research has indicated that residual yolk utilization is more efficient in chicks fed immediately after hatching compared to those deprived of feed (Noy et al., 1996; Bhanja et al., 2009; Bhuiyan et al., 2011). In the current study, chicks injected with electrolytes *in ovo* demonstrated greater yolk absorption and utilization after hatching compared to non-injected chicks. This indicates that injecting electrolytes especially ORS may promote early utilization of the retained yolk and its nutrients, which is beneficial for chicks that do not get feed and water as soon as they leave the hatcher. These nutrients, including the amino acids, are utilized for tissue growth and development in the neonatal chicks. Additionally, it is evident in the current study that delayed placement has a significant effect on liver and eviscerated carcass development as the relative weights of both were negatively impacted by delayed placement for 48 hours. Surprisingly, the small intestine and gizzard continued to increase in weight despite the post-hatch feed and water deprivation. This might be due to the preferential intestinal growth demonstrated by neonatal chicks whether exogenous feed is available or not (Noy and Sklan, 1999; Sklan, 2001). The gizzard increasing in size despite feed deprivation as observed in the present study is likely due to a compensatory mechanism in which the gizzard prepares for anticipated feed intake, adapting in size to optimize digestion once feed is available as postulated by Boyner et al. (2023). It is also noteworthy that broiler chicks fed ORS and normal saline *in ovo* exhibited higher gizzard development than the non-injected. Conversely, the eviscerated carcass weight of broiler chicks that were fed ORS *in ovo* was statistically the same as placement delay increased while the normal saline-injected and the non-injected exhibited significant weight loss.

The present study showed that feeding normal saline or ORS *in ovo* reduces the rate of moisture loss in neonatal chicks. Chicks that received 0.5 mL normal saline or ORS *in ovo* exhibited higher moisture content in the liver, gizzard, small intestine, and eviscerated carcass than non-injected chicks. This may be due to the osmoregulatory effects of electrolytes, allowing moisture to be residual in the organs (Baloš et al., 2016; Goldstein, 2022). However, comparing the two electrolytes, ORS injection was more effective in moisture retention than normal saline. Moreover, post-hatch holding for long periods has been linked with dehydration or moisture loss in chicks (Warris et al., 1992; Xin and Lee, 1997), which was evident in this study. There was a reduction in moisture content of the gizzard, small intestine, and eviscerated carcass of neonatal broiler chicks with increasing placement delay, indicating a significant moisture loss due to deprivation of water during the placement delay. This suggests that irrespective of the normal saline and ORS injected pre-hatch, delayed placement negatively affects moisture content in critical tissues and organs of neonatal chicks. More importantly, it highlights the vulnerability of neonatal chicks to post-hatch dehydration when they have no access to water, a situation that has been linked with weight loss (Warris et al., 1992; Abed et al., 2011), reduced weight of organs (Maiorka et al., 2003), impaired gastrointestinal development (Uni et al., 1998; Geyra et al., 2001). Nevertheless, *in ovo* supplementation or administration of an electrolyte such as oral rehydration solution or normal saline may help mitigate early dehydration risks in neonatal chicks, but will not entirely stop the effects of prolonged water deprivation after hatch.

## CONCLUSION

The findings of this study suggest that *in ovo* feeding of oral rehydration solution or normal saline at 0.5 mL per egg can reduce hatch rate but enhance the post-hatch quality of neonatal broiler chicks as well as residual yolk utilization and gut organ development. In addition, it can mitigate the effects of delayed placement for up to 48 hours on neonatal broiler chicks.

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