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# Influence of *In-ovo* Administration of Electrolyte on Eggs of Broiler Breeder During Perinatal Period and its Impact on Subsequent Broiler Performance

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## Abstract

**Background:** The perinatal period (final some days of pre-hatch to initial some days of post-hatch) is mainly critical moment during the growth of hatchling because it is a evolutionary time during which the hatchling undertakes catabolic, anabolic and physiological transfers from the consumption of egg components to external diet. Though, with the present endeavor of viable hatcheries as well as in view of instance to shift and release of hatchling to poultry farms, the hatchlings are unavoidably displayed to delay rationing from 48 to72 hrs. Consequently, tardy dieting, hatchling undergo deprivation as well as allocate the restricted deposits of nutrition food substances for maintenance of temperature modulation as well as anabolism & catabolism that hampers growth performance. *In-ovo* injection of nutrient (like, electrolytes) during last period of incubation can be applied as an approach to overcome above mentioned constraints.

**Methods:** A total 240 broiler breeder (Ross-308) fertile eggs were set in incubator trays representing 60 eggs for each treatment. A 200-µL electrolyte mixture solution (comprising NaCl 3.5g, KCl1.5g, $Na_3C_6H_5O_7$ 2.9g and dextrose 20g) with volumes of 100, 500 or 1000ml insertion treatment as well as non-injected control were incorporated during this trial. The mixtures of electrolytes solutions with different concentration is used in all treatments with dose of 200-µL into amniotic fluid of hatching eggs at day 18 in hatchery, and subsequent hatchability, blood profile and post-hatch performance were examined.

**Results:** The results showed that none of the injections exhibited substantial ($p > 0.05$) influences on hatch rate or body weight (BW) at hatch, 3d as well as10d post hatch. Likewise, plasma refractive index (PRI), plasma triglyceride as well as glucose contents at d 3 & 10 were not influenced ($p > 0.05$) through any insertion treatments. Body weight gain (BWG), feed consumption and feed conversion ratio (FCR) throughout period at 0–32 weeks were also not influenced ($p > 0.05$) by *in-ovo* administration of the upgraded electrolyte mixture.

**Conclusion:** This study envisaged that tested electrolyte mixture fluids were showed safe and sound for the incipient and hatchlings. It may be proposed that electrolyte mixtures possess ability intended for usage in blend with other electrolytes, nutrition food substances as well as encourages to the viable insertion of broiler hatching embryos for the advancement of incipient and initial post-hatch chick growth as well as advancement.

## Introduction

Latest considerable studies committed to investigate the employ of *in-ovo* administration as resources of supplying additive nutrients to incipient. These additives contain amino acids [1], carbohydrates [2], L-carnitine [3], vitamins [4], egg white [5], nucleotides [6], hormones [7], plant extract [8] and mannan oligosaccharides [9]. Such trials were carried out derived from the postulation that the incipient growth of marketable roaster strain might be accommodated through restricted accessibility of indispensable nutrition food substances in the egg consequently vigorous assortment for fast growing development. *In-ovo* insertion skill evolved as well as licensed by Uni and coworker [10] offers a technique to securely initiate extraneous nutrients toward progressing incipient. By this method, liquid nutrients are inoculated into amniotic fluid of hatching eggs [11]. This mechanism is important regarding supply of nutrients to young chicks from egg yolk during last days of incubation [12]. Numerous aspects affect the fruitful employ of *in-ovo* administered nutrients as additives that is the particular location, day of administration [13], the variety of nutrient as well as constitution & quantity of the conveyor mixture which is inserted [14]. Mineral deposits in yellow middle part of the egg reduce substantially from the day of eggs setting; it left the incipient with less mineral deposits during the final stage of hatch as well as most likely guides to a mineral shortage position of the incipient [15]. *In-ovo* nourishing of minerals also acquired significance like the high-metabolic rate, rapid-rearing roaster incipient can approach status of mineral shortage which may direct to abnormal chemical reactions in body [16]. Electrolytes are essential to regulate the physiological function of birds particularly under heat stress [17]. Potassium chloride (KCl) is a common physiological salt. Though, heat stress may enhance potassium excretion and thereby reduce plasma potassium in birds [18]. Therefore, KCI supplementation may be beneficial to embryos during the last few days of incubation, as their level of heat production increases significantly during pipping. Saltwater (Sodium Chloride) has been accustomed as a conventional transporter for inserted nutrients [19, 20]. Though, an earlier trial has exhibited that 5.5 mM potassium chloride can truly provide like electrolyte which is better than sodium chloride in favor of the *in-ovo* administration of mixtures because of potassium chloride is more efficient in cellular pumping activity of cells in order to retain more potassium inside the body and expel sodium outside. An increased potassium intake has beneficial role in developing cardiovascular system of human [21]. Overall, very restricted information is available on the effect of electrolyte mixture on pre-or post-hatch parameters in broiler breeder.

Thus, the aim of this research trial is to check an effect of *in-ovo* inoculation with different concentrations of electrolytes to broiler breeder eggs and their subsequent effect on hatchability, blood profile and growth performance of broiler chicks.

## Methods

### Fertile eggs, electrolytes mixtures and hatchling

Meat type breeder fertile eggs (Ross-308) were attained from M/s K.K Breeding farms Mansehra, Khyber Pakhtunkhwa province, Pakistan. Entire eggs were collected from birds' group at age of 35 weeks. These eggs were apprehended for 3-4 days prior placing in incubator. Individual egg was weighed as well as placed arbitrarily at every of 4 incubator trays during experiment. Entire experimental groups were symbolized on every tray as well as sixty eggs were placed at every experimental group. Eggs were incubated in a chick master multistage incubator for this trial. The dry (37.6°C) and wet (29°C) bulb temperatures of fore-mentioned incubator were adjusted. The incubating eggs turning in the incubator were done by angle 45° on an hour basis until eggs transferring to hatcher trays. The entire inserted mixtures were made ready from 2 to 5 days prior insertion employing de-ionized $H_2O$ as thinner. Electrolyte's mixtures sachet (20g packing) of company Searle (comprising NaCl 3.5g, KCl1.5g, $Na_3C_6H_5O_7$ 2.9g and dextrose 20g) was purchased from W. Wilson Chemist and dissolved it into deionized water at room temperature (25°C) for making three dilutions i.e., 100, 500 and1000 ml. The mixtures of electrolytes solutions with different concentration are used in all treatments with dose of 200-µL into amniotic fluid of hatching eggs at day 18 in hatchery. Insertions were executed by single ovum inserter. Eggs were inserted by the air cell with the help of dulled tip inserter pointer into mark the amnion. The pointer allowed about 2.49cm insertion profundity from the apex of the big end of ovum [22]. To make sure to facilitate the mixture was being distributed in the amnion; confirmation experiment was carried out employing color (dissolvable in $H_2O$) which was inserted at 18th day of incubation. This trial verified that mixture was being inserted inside the amnion. At 18th day of incubation, eggs were weighed as well as allocated to an insertion experimental group at random prior administration. To keep away from consequent adulteration as well as experimental mixture overlap, the inserter was set along with automatic washing rotation following the insertion of every single egg as well as at every tray, total eggs concerning for specific treatment were administered with their respective mixture prior

shifting to other treatment. Mixtures were early drawn (60 mL) by hypodermic needle, after that this needle was fixed with the appliance. Every mixture (3 mL) was siphoned in the machine prior insertion into main scheme. In this study, 200 µL insertion quantities were employed. The standard error relating to insertion amount was only 0.1%. These eggs were putdown again in the incubator into their relevant tray following inserting entire the experimental groups. Whole eggs were apprehended out of the incubator < 5minutes whilst inserting. During this experiment, the quantity of hatchlings which hatched was observed each 12hours accompanied by 19.5 and 21days of incubation for measurement of hatchability. At day 0 post hatch or day 21 of incubation, whole hatchlings were labeled with everlasting tinted ink and marked with coded card instead of every replicate tray of the incubator as well as allocated to related floor pen. Total number of pens was four; within every pen consisting of hatchlings from whole experimental groups intermixed. Wood shavings were used as litter on cemented pen. Brooders were employed to keep maintain the recommended temperatures in every pen. The room temperature of whole four pens was noticed two times daily during the rearing phase. The birds were offered freely approach for ration and clean drinking water as well as experimental rations was prepared keeping in view of NRC [23] standard during the trial (Table 1).

### Parameters studied

In this trial, set egg weight (SEW), rate of hatch, hatchling body weight (BW) as well as bird's body weight at days 3 & 10 after hatch was noted. Also, at days 3 & 10, blood samples of 5 chicks from every experimental group were collected for determination of plasma refractive index (PRI), triglycerides & glucose levels. These samples of blood were taken into glass tubes as well as centrifuged for removal of plasma. The PRI was carried out by plasma refractometer (Model 10406 TS) following the method explained by Morgan and coworkers [24]. For measurements of triglyceride and glucose, An Ek- tachem-Vitros system DT 6011 was applied as described by Peebles and coworkers [25].

For measuring of growth performance traits and feed intake was noticed on weekly basis till last week at the age of 32 days. Body weight of chicks was recorded on weekly basis. The mean value of body weight was then applied for the arithmetical assessment of experimental groups. The feed conversion ratio (FCR) was calculated on days 7, 14, 21, 28 & 32 by dividing feed consumption by body weight gain (BWG). Mortality, if any, was also recorded.

### Statistical evaluation

Entire information of trial was analyzed through applying SPSS version 9.5 statistical evaluation scheme. A p-value of <0.05 was measured substantial variation among experimental groups as well as contrast of average values was done by applying the Duncan's Multiple Range Test [26].

## Results

### Effect of electrolyte on hatchability and body weight

In the present trial, the impact of *in-ovo* insertion of electrolytes mixture with different quantities on the performance of birds after hatching was given in Table 2. No one quantity of insertion exhibited substantial (p>0.05) influences on both hatch rate as well as body weight at hatch, 3d & 10d post hatch. Similarly, non-significant variations were observed in SEW amongst all experimental groups, that might eradicate the influences of SEW difference on the factors explored. The *in-ovo* injection of electrolytes mixtures did not display any considerable variation (p>0.252) in hatch rate of *in-ovo* inserted groups (79.5to 90.7 %) compared to control group (83.7%). Similar trend was recorded in chick weight.

| Ingredients | Composition |
|---|---|
| Corn | 500.00 |
| Rice broken | 50.00 |
| Corn gluten meal (60%) | 20.00 |
| Canola meal | 80.00 |
| Soyabean meal (47.5%) | 300.00 |
| Vegetable oil | - |
| Molasses | 30.00 |
| Marble chips | 5.00 |
| Dicalcium Phosphate | 10.00 |
| Vitamin premix[1] | 2.00 |
| Trace mineral mix[2] | 1.00 |
| Choline Cl (60%)[3] | 1.00 |
| L-Lys HCl (98%) | 1.00 |
| Total | 1,000.00 |
| ME, kcal/kg | 2896.60 |
| CP, % | 22.85 |
| CF, % | 3.77 |
| Ash, % | 7.15 |
| Available Phosphorus (%) | 0.40 |
| Lysine, % | 1.27 |
| Methionine, % | 0.50 |
| Met + Cys, % | 0.84 |
| Sodium, % | 0.21 |
| Chloride, % | 0.28 |
| Lino , % | 1.16 |

**Table 1:** Composition (g/kg) of basal diets

### Effect of electrolyte on some blood traits

Plasma refractive index, plasma triglyceride as well as glucose contents at days 3 & 10 did not affect (p>0.05) through all insertion treatments (Table 3). The PRI, plasma triglyceride and glucose levels at days 3 & 10 might be influenced merely through chick age however not via insertion treatments. Moreover, a higher PRI content of birds was recorded at day 3 than that of day 10.

### Broiler's performance

Body weight gain, feed intake and FCR during 0-32 wk were not influenced (p> 0.05) because of *in-ovo* injection of electrolyte mixture (Table 4). Numerically, without *in-ovo* injection group showed better growth parameters.

| Treatments | Set EW (g) | Hatch rate (%)[1] | d 0 BW (g)[2] | d3 BW (g)[3] | d10BW (g)[5] |
|---|---|---|---|---|---|
| Control | 60.8 | 83.7 | 45.6 | 62.4 | 118.9 |
| Electrolytes mixture with 100ml volume | 58.2 | 79.5 | 44.5 | 61.3 | 114.6 |
| Electrolytes mixture with 500ml volume | 58.8 | 88.6 | 45.5 | 62.5 | 118.2 |
| Electrolytes mixture with 1000ml volume | 60.9 | 90.7 | 46.4 | 62.8 | 119.1 |
| SEM | 1.02 | 4.64 | 0.77 | 1.16 | 3.40 |
| p-value | 0.06 | 0.252 | 0.430 | 0.710 | 0.701 |

**Table 2:** Set egg weight (EW), hatch rate, body weight (BW) on hatch day, d 3 BW and d 10 BW in treatment groups

| Treatments | Age (d) | PRI* | Triglyceride (mg/dL)* | Glucose (mg/dL)* |
|---|---|---|---|---|
| Control | 3 | 69 | 85 | 239.8 |
| | 10 | 68.5 | 81.8 | 249.0 |
| Electrolytes mixture with 100ml volume | 3 | 85.5 | 82.0 | 238.0 |
| | 10 | 71.0 | 83.3 | 235.5 |
| Electrolytes mixture with 500ml volume | 3 | 76.8 | 79.9 | 241 |
| | 10 | 71.5 | 77.0 | 255.8 |
| Electrolytes mixture with 1000ml volume | 3 | 79.3 | 70.3 | 239.0 |
| | 10 | 76.8 | 88.5 | 241.0 |
| SEM | - | 2.67 | 9.43 | 9.00 |
| p-value | - | 0.51 | 0.943 | 0.66 |

**Table 3:** Plasma refractive index (PRI), triglycerides and glucose concentrations in treatment groups

| Treatments | FI (g) | BWG (g) | FCR |
|---|---|---|---|
| Control | 2905 | 1954 | 1.49 |
| Electrolytes mixture with 100ml volume | 2770 | 1886 | 1.52 |
| Electrolytes mixture with 500ml volume | 2856 | 1912 | 1.50 |
| Electrolytes mixture with 1000ml volume | 2895 | 1909 | 1.51 |
| SEM | 29.07 | 15.55 | 0.0095 |
| p-value | 0.12 | 0.10 | 1.00 |

**Table 4:** Effect of *in ovo* feeding of broiler eggs with different concentration of electrolyte mixture on overall feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of broilers (0-32d)

## Discussion

Electrolytes may be employed for creating electro-chemical as well as osmotic gradients that are necessary for the motion of water crossover the layers [27]. The minerals equilibrium at embryonic stage may be recognized through harmonized events of the amnion, chorioallantois, yolk sac, embryonic movement as well as embryonic tissues [28]. Embryo has capability to adjust its growth progress in accordance with the nutrient provisions made accessible to him [14]. The amnion provides to defend the egg embryo from dehydration & inflow of water to the amnion as well as embryo can take place from another section in the egg by overturn of osmolarity or water capacity between the embryo as well as remainder of the egg [29]. There is very limited information available on this subject. The findings of current trial are concurred with the results of Zhai and coworkers [30], who found that hatch rate was not influenced as the amount of the insertion mixture ranged (0-2,320 mOsm) at capacity of 1.2 milliliter. Likewise, most recent study [31] exhibited that *in-ovo* addition of minor mineral upgraded mixtures were not found considerable variation (p>0.05) in both parameters of hatchability and chick weight of injected group (87.3% and 40.1g, respectively) than that of control group (without addition: 96.9 % and 41.3g, respectively).

Plasma Refractive Index is susceptible process for measuring protein content as well as while raised, is a precise marker of tissue dryness [32], this could suppose that the chicks can have practiced some extent of lack of moisture at day 3.

Lack of substantial influences of *in-ovo* electrolyte administration on growth performance in this study because of best concentration of nutrient found in egg attained from hatchery at commercial level. Such clarification is conceded through the results of Kop-Bozbay and Ocak [33], who reported no considerable impact of *in-ovo* addition of building blocks of protein employing eggs with best feed contents. Healthy birds cannot react to *in-ovo* additions [34]. There are many studies which did not display origin of fertile eggs particularly strain of layers employed. Moreover, another study showed that well-grown birds did not react to *in-ovo* additions [31, 34]. These researchers explained that impact of *in-ovo* addition is very much reliant on the motherly based ration because some shortage is conquered through further addition.

It may be concluded that though the electrolyte with different dilutions tested in the present study did not substantial effect on hatchability, chick weight, PRI, plasma triglyceride, plasma glucose and growth performance parameters. The results exhibited that nothing of electrolyte mixture was harmful for hatch rate or after-hatch development. Thus, under trial electrolyte mixtures were indicated to be shield to incipient as well as hatchlings, it may be recommended that they have capability for utilize in blend with other electrolytes, stimulant and nutrients for the viable insertion in broiler hatching embryos in favor of the enhancement of embryo as well as primary post-hatch chick performances.

From this study, we concluded that parasitism is a major health problem for camels because parasites get food and shelter from the host and cause disease. This research shows the high prevalence rate of nematyhelminthes of different species. In this research area, people use camels for meat and draught purpose. This study shows the high attack of gastrointestinal

nematyhelminthes on camels and their production is used as food by people. For this purpose, it is suggested that deworming should be done properly after regular intervals with safe hand low cost effective anthelmintic drugs (Albendazole, levamizole and Ivermectin). Farmers should be educated through the trained team of the Camel Center to assess the aspects of camel health, management and breeding. Due to the high cost of anthelmintic drugs and checkup fees of veterinarians poor owners do not connect with them and destroy the health of animals which causes a huge earning loss.

## Competing Interests

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

## Authors' Contribution

Nasir Mukhtar: Designed the study, Khawaja Yasir Imtiaz: Conducted the research, Arfan Yousaf: Supervised the study and statistical analysis. Javid Iqbal: Wrote first draft. Tanveer Ahmad: Edited the manuscript.

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