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Research Article
Effect of *in ovo* Injection of Selenium in Isa Brown Fertile Eggs on Hatching Process, Chicks Quality and Post Hatch Growth

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Abstract

Background and Objective: Selenium is an essential micro-nutrient for the maintenance of animal and human health. It is well known for its anti-oxidant activities. The aim of the present study was to evaluate the effects of *in ovo* Selenium administration in Isa Brown fertile eggs on the hatching process and post hatch growth. **Materials and Methods:** A total of 750 hatching eggs from 40 weeks old Isa Brown breeders were set and at day 18th of incubation, 600 eggs with live embryos were assigned to 5 treatments of 120 eggs each. These groups were: ($T0^{-}$) eggs without *in ovo* injection (negative control); (T_0^{+}) eggs injected with NaCl 9‰ (positive control); (T_{10}) eggs injected with 10 µg Selenium per egg; (T_{20}) eggs injected with 20 µg Selenium per egg and (T_{30}) eggs injected with 30µg Selenium per egg. Parameters such as pipping time, hatching rate, chick's quality, blood biochemistry parameters, organs and chicks weight were assessed at hatch. During the rearing period, weight of chickens was recorded weekly. **Results:** Results showed that T_0^{-} had significantly ($p<0.05$) the shortest hatching duration compared to others. No significant ($p<0.05$) difference was observed for hatchability rate. However, chicks from T_{30} had significantly ($p<0.05$) higher quality and growth after hatch. Chick's quality was higher as the Selenium concentration increased in the injected solution. **Conclusion:** In conclusion, the injection of Selenium in the fertile eggs improved the growth performance of chicks.

Key words: *In ovo* nutrition, Isa brown breeders, hatching eggs, hatching process, egg hatchability, chick's quality, growth performance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Generally, Chicks need some hours or days to have access to feed due to the time required for hatchery treatments and transport to the rearing farm. This delay in feeding impacts negatively on their future performance. Early feeding has more beneficial effect than feeding high-quality diets after a delay in feeding[1-3]. In commercial poultry rearing, getting a good start at the hatchery is an important factor to maximize profits. It is often thought that, feeding the chick during the embryonic stage would provide more nutrients necessary for improved starting weights, better feed utilization and faster growth. Thus, researchers are focusing on a new feeding strategy in order to increase the nutrients at the embryonic stage including *in ovo* feeding. Recent articles have focused on the effect of *in ovo* feeding on hatchability and early growth performance. In an earlier study, Ohta *et al*.[4] showed that amino acids injected into the yolk sac at 7 days of incubation, did not affect hatchability but chick body weight increased relative to egg weight prior to incubation. In another experiment, Uni and Ferket[5] showed that, *in ovo* feeding positively impacted hatchability, hatchling weights and this advantage was observed to be sustained until at least 35 days of age. Besides, the advanced development of the intestinal mucosa was also observed. So far, several studies have been conducted by using nutrients that have been applied for *in ovo* feeding include amino acids[6-8]; carbohydrates[9,10]; modulators[11,12] and minerals[13].

Among the minerals, Selenium (Se) is one of the elements that has many benefits for poultry but its use is very limited. It has been proven that Se deficiency in the poultry diet causes pathological problem that can affect the growth performance of poultry[14]. Many studies have been conducted on Selenium because of its physiological importance in the organism[15,16]. Selenium acts as a modulator of reproductive system, growth performance, immunity function and aging (or anti-aging) process in the organism[17]. Selenium acts as a component of the enzyme glutathione peroxidase (GSH-Px). Thus it plays a central role in maintaining the integrity of cellular membrane. Hariharan and Dharmaraj[18] found that Selenoprotein derivative such as Thioredoxin reductase plays important role during oxidative stress and inflammation in wound healing process. With all these properties, Selenium can be considered as an ideal dietary supplement in poultry industry to enhance growth and the overall production performance. Although preliminary studies are underway in different laboratories to evaluate the effect of Selenium on the poultry performance, many of these studies have only used it as a component or supplement of feed ingredient. There is a scarcity of studies available on the use of Selenium as nutrients for *in ovo* administration. Thus the aim of this study was to investigate the effect of *in ovo* injection of Selenium on hatchability of Isa Brown fertile eggs and on post hatch performance of progenies.

MATERIALS AND METHODS

Experimental site: The experiment was carried out at the laboratory of poultry production of Regional Centre of Excellence in Poultry Science situated at the agronomic experimental station at the University of Lomé in Togo. All study protocols used in this study were approved by the Centre of Excellence in Poultry Science Animal Used and Care review committee, University of Lome in Togo.

Experimental design: A total of 750 hatching eggs from Isa Brown breeders were used for this study. Prior to the incubation, the eggs were numbered and weighed. The eggs were then incubated at 37.8°C, relative humidity of 60% and were turned once every hour at 90° angle. At day 18th of incubation, 600 incubated eggs with evidence of live embryos were divided into five (5) groups that were: (T_0^-) eggs without any injection, (T_0^+) eggs injected with the saline solution, (T_{10}) eggs injected with 10 µgSe, (T_{20}) eggs injected with 20 µgSe, (T_{30}) eggs injected with 30 µgSe. Selenium was injected by drilling two holes with a syringe needle after candling the eggs. The different solutions were injected into the eggs through the holes. The appropriate solution corresponding to each group of eggs was injected at 0.1 mL per egg using an automatic syringe. After the injection, both holes were sealed with adhesive paper tape and the eggs were transferred into the hatching baskets then placed in the hatcher for hatching. From 456-516 h, after every 3 h, the hatching times were recorded. After hatch, the quality of the chicks, the relative organs weight and blood parameters were determined. The chicks were then reared and fed *ad libitum* with the standard feed (Table 1) and chicks weight was recorded weekly during 6 weeks.

Table 1: Percentage gross composition of diet

Ingredients	Rate
Maize	55.2
Wheat offal	5
Soya bean	32
Lysine	0.3
Methionine	0.2
Oyster shell	2
Salt	0.2
Concentrate	5.1
Total	100

ME: 3071.34 kcal, Crude protein: 22, Crude fat: 9,11, Fiber: 4, Lysine: 1.23, Methionine: 0.65, Methionine+Cystéine: 0.88, Calcium: 0.99, Phosphorus: 0.57

Selenium solution administration: Five milligrams of Selenium were dissolved in 10 mL of a physiological saline (NaCl 9‰) and vortex to obtain a solution of 500 µgSe mL^{-1}. Then, the mother solution was diluted successively with NaCl 9‰ to obtain different solutions of 400, 300, 200 and 100 µgSe mL^{-1}.

The test product L-Seleno-methionine was provided by Orffa additive company.

Data collection

Hatching events: Between 456 and 516 h of incubation, the eggs were removed from the hatcher and checked after every three hours to determine the Internal Piping (IP), External Piping (EP) and Hatching time (Ha) for each egg. At the end of the incubation, hatching time for each egg was determined by calculating the difference between the time when the eggs were set in the incubator and the time when the eggs were hatched.

Hatchability and chick quality assessment: Hatched chicks from each treatment were removed, counted to determine the hatching rate and then Tona scoring method[19] was used to assess the chicks quality. For the quality assessment, 10 chicks were removed from each treatment and every chick was assessed on the basis of physical parameters including reflex, down and appearance, eyes, conformation of legs, navel area, yolk sac, remaining membranes and yolk. Non hatched eggs were counted and then opened to determine the time of the embryo mortality (Early mortality, late mortality, mortality at internal or external piping).

Relative organ weight and blood parameters: A total of 6 hatched chicks per treatment were selected and sacrificed through cervical dislocation. Blood samples were collected in Eppendorf tube and centrifuged at 3000 rpm for 15 min for serum collection. Blood parameters (total protein, triglyceride, aspartate transaminase, alanine transferase, albumin and uric acid) were determined per treatment by the spectrophotometric method using EPOCH 2 spectrophotometer and commercial kit manufactured by CYPRESS DIAGNOSTIC. Organs (liver, yolk sac and heart) were removed and weighed to determine the relative weight of the organ.

Bird management and feeding: A total of 60 hatched chicks per treatment except the positive control group were selected. They were divided into 4 replicates of 30 chicks each. Chicks were weighed individually and reared for 6 weeks on deep litter system. Feed (Table 1) and water were provided *ad libitum*. Body weight, weight gain, feed intake and feed conversion ratio was calculated weekly.

Statistical analysis: Data collected were expressed as Mean±standard error (SE) of mean. Statistical Analysis was performed using a statistical software Graph Pad Prism 5. Differences of $p<0.05$ were considered statistically significant. The effects of the treatments were analysed using one way ANOVA followed by Turkey test for Comparisons among means.

RESULTS

Effect of Selenium inoculation on incubation duration: Table 2 shows the effect of *in ovo* Selenium administration on internal, external and hatching duration. The results showed that the external piping (477.5 h) and hatching (468.7 h) time was significantly ($p<0.05$) shortest in the negative control group (T_0^-) compared to other treatments. The highest hatching duration was observed in T_{20} group (eggs injected with 20 µgSe egg^{-1}) while the highest external piping time was observed for eggs injected with the saline solution. However, the incubation duration between internal and external piping; external piping and hatching did not show any significant ($p>0.05$) difference for all the treatments.

Table 2: Effect of *in ovo* Selenium administration on internal piping, external piping and hatching duration

	Treatments				
Parameters	T_0^-	T_0^+	T_{10}	T_{20}	T_{30}
IP	498.00±0.82	500.10±0.79	499.40±0.73	500.30±0.88	499.80±0.97
EP	477.50±14.57^{b}	509.20±0.87^{a}	506.50±0.58^{a}	504.40±0.75^{a}	508.50±0.72^{a}
Ha	468.71±18.20^{b}	513.30±1.32^{ab}	516.30±0.49^{a}	516.50±0.58^{a}	517.40±0.47^{a}
dIP-EP	8.93±0.64	10.62±0.82	8.67±0.62	9.08±0.89	10.00±1.26
dEP-Ha	10.29±0.61	8.25±1.04	9.67±0.57	10.14±0.63	7.00±1.00
dIP-Ha	20.50±1.12	17.50±1.37	18.85±0.61	18.69±0.65	20.00±0.43

^{ab}Means in the same row with different superscripts are significantly different ($p<0.05$), IP: Internal piping, EP: External piping, Ha: Hatch, dIP: Duration between IP and EP, dEP: Duration between EP and Hatch., dH: Hatch duration. T_0^-: Chicks from no injected eggs, T_0^+: Chicks from eggs injected with NaCl 9‰ T_{10}: Chicks from eggs injected 100 µg of Selenium, T_{20}: Chicks from eggs injected 200 µg of Selenium, T_{30}: Chicks from eggs injected 300 µg of Selenium

Effect of *in ovo* inoculation of Selenium on eggs hatchability, embryonic deaths and chick's quality: Figure 1 shows the effect of *in ovo* injection of Selenium on egg hatchability. No significant (p>0.05) difference was observed across treatments.

The effect of Selenium inoculation on embryonic mortality rate is presented in Fig. 2. The mortality rate was significantly (p<0.05) higher for the eggs that received the saline injection compared to others.

Figure 3 shows the quality of the hatched chicks assessed by Tona score. The higher concentration of Selenium in the injected solution significantly (p<0.05) increased the quality of chicks. Chicks quality in treatment T_{20} and T_{30} were significantly (p<0.05) higher compared to the negative control group (T_0^-). Whereas the chicks quality in T_0^+ (positive control) and T_{10} were similar (p<0.05).

Effect of Selenium inoculation on day old chick's organ relative weight and serum biochemistry profile: Table 3 shows the effect of *in ovo* inoculation of Selenium on relative organ weights of day old chicks. There were no significant differences (p>0.05) in the different treatments with respect to the relative yolk sac and liver weights. In contrast, the relative weight of the heart was significantly (p<0.05) lower for the chicks in the treatment T_0^+ compared to others.

The serum biochemistry profile is shown in Table 4. No significant (p>0.05) difference was observed across treatments.

Effect of Selenium inoculation on the evolution of chick's body weight: Table 5 shows the weight of the chicks per week. Only T_0^-, T_{10}, T_{20} and T_{30} were involved in this part of the experiment. The weights of the chicks were similar (p>0.05) from the first to the fourth week. But a significant (p<0.05) difference was observed in the fifth and sixth week of age. The highest body weight was observed for the chicks of T_{20} group compared to others. The lowest body weight was observed for the chicks of the negative control group (T_0).

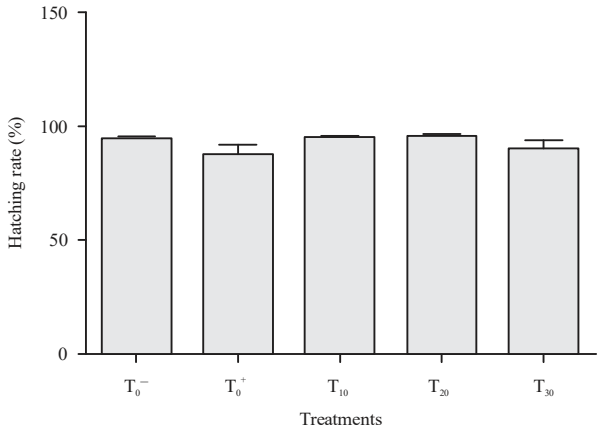

Fig. 1: Effect of *in ovo* inoculation of the Se on the hatching rate of chick

T_0^-: Chicks from no injected eggs, T_0^+: Chicks from eggs injected with NaCl 9‰ T_{10}: Chicks from eggs injected 100 µg of Selenium, T_{20}: Chicks from eggs injected 200 µg of Selenium, T_{30}: Chicks from eggs injected 300 µg of Selenium

Table 3: Effect of *in ovo* inoculation of se on relative organ weight at hatch (g)

	Treatments				
Parameters	T_0^-	T_0^+	T_{10}	T_{20}	T_{30}
Yolk	12.08±0.33	11.68±0.82	9.35±0.63	12.30±0.89	12.35±1.17
Chick's weight	41.49±0.33	39.11±0.83	40.83±0.93	41.48±0.85	40.46±0.89
Heart	0.74±0.05^{ab}	0.68±0.04^{b}	0.72±0.04^{ab}	0.82±0.04^{ab}	0.86±0.02^{a}
Liver	2.18±0.10	2.27±0.25	2.14±0.22	2.29±0.22	2.47±0.12

^{ab}Means in the same row with different superscripts are significantly different (p<0.05). T1: Chicks from no injected eggs, T2: Chicks from NaCl, T3: Chicks from 100 µg of Selenium, T4: Chicks from 200 µg of Selenium, T5: Chicks from 300 µg of Selenium

Table 4: Biochemistry parameters at hatch

	Treatments				
Parameters	T_0^-	T_0^+	T_{10}	T_{20}	T_{30}
Albumine (g dL^{-1})	1.91±0.13	1.88±0.07	1.96±0.07	2.03±0.04	1.83±0.06
Urique acid (mg dL^{-1})	3.47±1.08	4.42±0.93	3.25±0.58	3.52±0.58	3.34±0.45
AST	213.60±33.13	187.50±70.28	247.50±95.1	153.50±32.95	264.40±84.52
ALT	65.73±6.82	45.18±13.62	86.55±19.85	53.56±7.94	33.46±14.02
Triglyceride (mg dL^{-1})	141.80±11.07	138.30±10.0	120.80±31.48	134.20±10.28	133.90±15.32
Total protein (g dL^{-1})	3.47±0.26	3.99±0.47	4.28±0.31	4.11±0.26	3.94±0.51

T_0^-: Chicks from no injected eggs, T_0^+: Chicks from eggs injected with NaCl 9‰ T_{10}: Chicks from eggs injected 100 µg of Selenium, T_{20}: Chicks from eggs injected 200 µg of Selenium, T_{30}: Chicks from eggs injected 300 µg of Selenium

Table 5: Effect of *in ovo* inoculation of Se on chick's weight (g)

	Treatments			
Weeks	T_0^-	T_{10}	T_{20}	T_{30}
1	35.78±0.96	36.70±0.66	36.35±0.52	36.61±0.62
2	60.13±1.14	59.15±1.09	60.26±2.13	58.00±1.16
3	143.80±3.23[a]	136.80±5.90[a]	141.20±3.30	151.80±4.19
4	201.70±4.36	206.80±4.90	197.50±4.04	209.20±4.01
5	218.20±13.96[c]	255.40±6.63[ab]	271.40±6.99[ab]	297.20±8.39[a]
6	300.30±16.82[b]	338.50±16.06[a]	328.70±7.38[a]	352.50±8.40[a]

[ab]Means in the same row with different superscripts are significantly different (p<0.05). T_0^-: Chicks from no injected eggs, T_0^+: Chicks from eggs injected with NaCl 9‰ T_{10}: Chicks from eggs injected 100 µg of Selenium, T_{20}: Chicks from eggs injected 200 µg of Selenium, T_{30}: Chicks from eggs injected 300 µg of Selenium

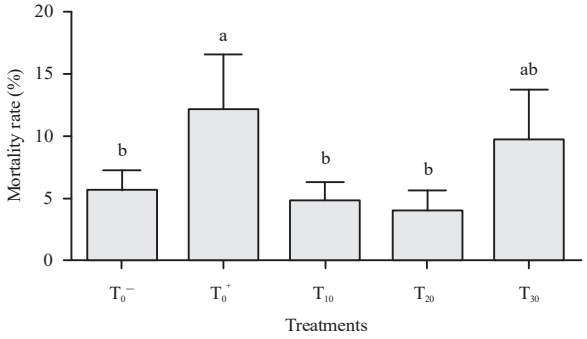

Fig. 2: Effect of *in ovo* inoculation of Selenium on embryo mortality

[ab]Means in the same row with different superscripts are significantly different (p<0.05). T_0^-: Chicks from no injected eggs, T_0^+: Chicks from eggs injected with NaCl 9‰ T_{10}: Chicks from eggs injected 100 µg of Selenium, T_{20}: Chicks from eggs injected 200 µg of Selenium, T_{30}: Chicks from eggs injected 300 µg of Selenium

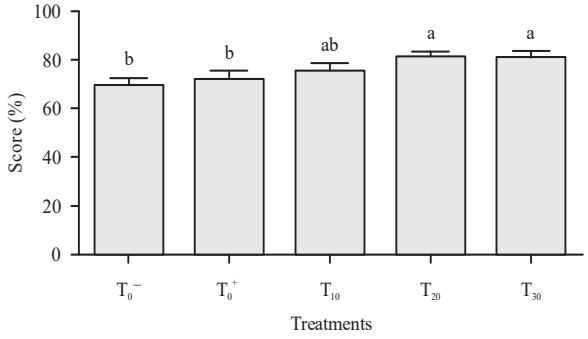

Fig. 3: Chick's quality

[ab]Means in the same row with different superscripts are significantly different (p<0.05). T_0^-: Chicks from no injected eggs, T_0^+: Chicks from eggs injected with NaCl 9‰ T_{10}: Chicks from eggs injected 100 µg of Selenium, T_{20}: Chicks from eggs injected 200 µg of Selenium, T_{30}: Chicks from eggs injected 300 µg of Selenium

DISCUSSION

In ovo feeding is a newly developed technology that has been used during the incubation process in the poultry field. Recent articles have focused on the effect of early nutrition on the hatchability, early growth, gut health and immune response. In the current study, the shortest durations observed in the negative control for external piping (Ep) and the hatch can be explained by the fact that embryos were not disturbed during the hatching process. In fact, the batch of eggs in the negative control group was not injected with the test solutions so, the embryos developed normally without disturbance. In consequence, this group hatched earlier than others. According to Kucharska-Gaca *et al.*[20] the use of *in ovo* feeding technology affected the physiological state of broiler embryos before and after hatching. The perturbation in the physiological state of the embryo during their development in treated eggs, implied an increase in length of hatching events. For that reason, a significant (p<0.05) difference was observed in the hatching time and external piping. This result agrees with a previous study conducted by Kucharska *et al.*[20] who also observed a longer hatching time while the eggs were inoculated with L-carnitine.

The highest mortality rate in treatment T_{30} (30 µgSe egg^{-1}) might be due to the highest concentration of Selenium in the injected solution. In fact, Selenium becomes toxic at high concentrations. Akulov *et al.*[21] observed a decrease in egg production from 64-8.7% at the highest inclusion level of Selenium, meaning that highest inclusion level of Selenium in the diet of laying hen has a negative effect on the animals. Although, no significant (p<0.05) difference was observed neither in the relative internal organ weight (especially liver) nor in the blood biochemistry parameters. Furthermore, the chicks from the highest selenium inoculation were the best in term of chick's quality. Despite toxic effect of Selenium, it plays important roles in the organism. Surai[17] reported that the antioxidant defense system in the organism depends on the glutathione peroxidase activity which in turn depends on adequacy of selenium in the cell. Delezie[15] also found a significant increase in the level of glutathione peroxidase concentration in the blood as Seleno-methionine dosage increased in the diet of laying hen. The Selenium injection at the highest concentration (300 µg) improved the birds immunity, in consequence, they were more active than others. The chicks from the eggs injected with 300 µg of Selenium showed the highest weight compared to others in

the sixth week of age. This result agrees with a previous study conducted by Kucharska *et al.*[20] who stipulated that proper injection improved not only hatching but also the nutritional status of chicks and thus improved the growth performance.

CONCLUSION

In ovo injection of Selenium at different concentrations in fertile eggs at 18 days of incubation showed that eggs receiving 200 µg of Selenium has the highest hatching rate (76.32%). Chicks from eggs injected with 300 µg of Selenium had the highest chick's quality. The quality of the chicks was better as the concentration of selenium increased. Consequently, chick's post hatch weight was better for chicks from eggs injected with Selenium and the highest concentration of Selenium had the highest chick's weight.

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