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Maximizing Broiler Chicken Production through In Ovo Injection of Vitamin E: Effects on Performance and Immune Function

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Abstract

Investigations were done into how different L-ascorbic acid (LA) injection levels affected the embryos' capacity to hatch and how much LA remained in their serum after the injection. Four different treatment groups were created at random from a total of 980 Ross 708 broiler eggs: non-injected saltwater without LA, saline (SL) injected with LA, and SL carrying either 12 or 27 mg of LA. The eggs were given a 100 µL amount of sterile SL (0.90%), either by itself or when combined with a single of the dual LA levels, during the 18-day implantation (doi) stage. Also calculated were the amount of egg weight reduction from doi 0 to doi 12 to doi. To ascertain the stage of embryo mortality, a hatch residue study was carried out after candling. The hatchability of living embryonic eggs (HI) and the weight (BW) of the hatchlings were calculated at around 21 doi. Serum LA levels were measured using blood samples collected 6, and 24 hours after LA injection. No treatment group differed from the others in terms of serum LA concentrations, HI, or hatchling BW. In contrast to the groups receiving 12 mg of LA in SL and SL alone, the non-injected group of chicks exhibited a greater ($p = 0.05$) at hatching fatality of embryos. These findings imply that high doses of LA (12 and 25 mg) administered in ovo have a chance to enhance embryonic viability without having a deleterious impact on HI or serum LA contents. The prolonged duration of LA in the cornea of the eye and other relevant tissues when subjected to various amounts of supplementary LA requires more investigation.

Keywords: Broiler chicken, Ovo injection, L-ascorbic acid (LA), hatching.

Introduction

L-ascorbic acid (LA) can appear in nature as the decreased format, which can then undergo reversible oxidation to become dehydro-L-ascorbate. LA can be minimised to further LA in cells after being transformed to dehydro-L-ascorbate by a number of enzymatic or nonenzymatic processes throughout its metabolism. While the D-isomer is not physiologically active, the L-isomer is. It is possible to further permanently oxidise the oxidised form to create the passive form (diketogulonic acid). As a result, LA is extremely vulnerable to oxidation-based degradation, which is increased by ultraviolet (UV) rays (1).

Ascorbic acid (LA) that has been absorbed quickly reaches equilibrium with the LA already present in the circulatory system. Since LA has no known particular binding proteins, it is assumed that the vitamin binds to subcellular structures to remain in the body. Normal circumstances allow poultry to synthesise LA within its tissues. In LA-dependent animals, such as chicken, the process of absorbing LA is very similar to the process of absorbing carbs

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(monosaccharides). A sodium-dependent active circulation system is assumed to be necessary for the digestion and absorption of LA in these animals (2).

LA has been seen to control the activity of catalysts that fight free radicals, like superoxide anion dismutase, the enzymes catalyst and peroxidase for glutathione both during embryonic development and in recently fledged chicks. This is crucial because chicken embryo tissues have an increased amount of adipose portion of unsaturated fats, which makes them vulnerable to stress caused by oxidation. Maintaining appropriate antibacterial defence is so essential (3). These findings suggested that raising plasma or organ levels of LA may be advantageous for chicken hatchability and post-hatch efficiency. The antioxidant defence mechanisms can be boosted by raising the levels of LA, which may benefit the chickens' general well-being and athletic ability. It's critical to note that these assertions are based solely on the data presented in the section, and more investigation is necessary to fully comprehend the precise mechanisms and outcomes of LA supplemental funding in chicken (4). LA impact on diet administration on the efficiency of broilers under heat stress have been the subject of multiple research efforts. The injection of 14 g/100 L of LA in consuming H2O was exposed to reveal an improvement in oxidative damage in birds, especially among those enduring severe heat stress. This is an important observation. This shows that providing broilers with LA supplements may be able to reduce the negative consequences of heat stress (5).

LA the consumption is also proven to increase grill tolerance and productivity. The immunity-related impacts of LA on broilers may be associated to the function of phagocytes, which are white blood cells that contribute to the reaction of the immune system that absorb and eliminate pathogens, as well as the generation of lymphocytes and cytokines, which are signalling substances engaged in the control of the immune system. These immune system elements have been demonstrated to be modulated by complementary nutritional LA (6). The goal of the present study (7) determined the impact of in ovo copper injection (sulphate, acetate, and nano) BW, resistance, and blood sugar levels, and carcass features of broiler chicks at 35 days of age. A whole of 463 viable it involved eggs, divided into seven groups of 66 eggs each with a total of three replications. The results showed that BW rose in the Cu-injected groups, with the exception of the Cu acetate groups, but that plasma components, carcass, and relative organ weight were unaffected.

The study (8) looked at how L arginine (Arg) consumption during pregnancy affected NOS (nitric oxide synthase) exertion, generation of nitrogen oxide, and jejunal stimulated N.O.S. (iNOS) biological metabolism fostering popularity and phrase the limit, resistance inflammatory substances, and reactions similar to mRNA level in chicken broilers' digestive tracts. The findings demonstrated that in ovo ingestion of the Arg fluid enhanced ($P>0.05$) the level of iNOS activity, the level of IL-2, IL-4, and produce antibodies A (sIgA), as well as the mRNA phrases of toll-like receptor-2 and toll-like receptor-4 in intestinal mucosa and the overall amount of iNOS protein. The study (9) assessed whether bioluminescent Escherichia coli colonisation or emigration occurred following in ovo injection into grill embryos. On day

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18 of incubation, injection utilising 108 CFU/mL nonpathogenic the airway cell and placental regions received E. coli.To validate the existence of bioluminescent E. coli, an in live imaging system was used to see every tissue. For estimating the bacterial loads in all tissues, the homogenization of samples, 10-fold serially diluted, then distributed on a suitable agar. The eggs that were inserted into the amnion had substantially more E than expected, according to the outcomes. The study (10) showed that symbiotic were more advantageous to chicks' development, gastrointestinal histomorphological factors, and intestinal microbiota population than probiotics alone. A healthy gastrointestinal environment was provided by the in ovo injection of synbiotics, which also improved the immune response of the host and assisted preserve a beneficial intestinal microbiota. Furthermore, improved immune organ index and gastrointestinal function boosted the chicks' efficiency and gained weight without having any negative impact. The study (11) exposed that symbiotic were more advantageous to chicks' development, gastrointestinal histomorphological factors, and intestinal microbiota population than probiotics alone. A favourable gastrointestinal environment was provided by the in ovo injection of synbiotics, which also improved the immune response of the host and assisted preserve a beneficial intestinal microbiota. Furthermore, improved immune organ index and gastrointestinal function boosted the chicks' efficiency and gained weight without having any negative impact.

In ovo feeding of NR improved E19 embryo and chick PMM morphometrics (12). Increased In freshly hatched chicks, PMM measurements were connected with a rise in tissue fiber volume but had zero effect on fiber CSA, just like earlier NR in ovo feeding trial. The PMM morphometrics of emerging chicks were unaffected by raising the amount of administered NR, while muscle fiber and orbiting cell density were enhanced. The absence of an increase in PMM weight, dimension, width, or thickness following NR delivery proves that there has been no advantage to infusing more than 250 mmol of NR; yet, the substantial rise in muscle fiber minimum density as a result of raising the level of injection could possess an effect on subsequent development or meat-specific traits. The study (13) examined how in ovo injection of both sucrose and maltose (MS) affected Langde geese's post-hatching developmental outcomes, anatomy of the jejunum, the disaccharidase enzyme, and expression of genes for fructose transporters. In this investigation, 300 fertilised eggs compared to Langde chickens, three years old (weighing 115.75 1.25 g) were employed. The eggs were divided into two groups at random, and the difference between the two groups was whether or not the amnion was injected with 30g/L maltose or 30g/L sucrose (MS) dissolved in 7.6g/L NaCl on embryonic day 24. There were six replicas in each group, with 30 eggs in each copy. Up until day 28, the goslings were nurtured. The outcomes demonstrated a rise in ultimate body weight, average daily gain (ADG), and food effectiveness following the in ovo injection of MS. Additionally, MS injection enhanced early sugar transporter gene expression, disaccharidase activity, and post-hatching jejunal shape. The purpose of the study (14) was to ascertain the impact of Vitamin B12 inoculation on broiler development and blood component values. The findings show that there was no change in the in ovo implanted

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eggs' rate of hatchability (P > 0.05). On the first day after hatching, folic acid-injected chickens showed a dose-dependent rise in glucose and folic acid levels (P >= 0.001).

The factors that were noticed in this investigation (15) were separated into two stages. Hatchability, hatching weight, and hatching weight ratio are the metrics that emerged at the first stage. Feed consumption, gains in weight, transformation of feed, and growth were among the metrics that were measured for the subsequent stage. According to the study's conclusions, the therapy's effects on hatchability, laying amount of weight, the hatching process weight ratio, and native chicken competence of the in ovo nourishment were not particularly noteworthy. The recognisable trend, on the other hand, suggested that the outcome was superior to that of the control treatment.

Materials and Methods

The process was followed to store an over-all of 1450 broiler hatching eggs from 40-week-old Roger 709 grower of chicken’s hens for 3 days before being set. In a single-stage, conventional NMC2000 incubator, 980 eggs were placed at randomised on all of the 8 identical platter levels in each of the 4 regimens (40 treatments total). The four in ovo injection treatments consisted listed as follows: (1) avoidant authority; (2) 100 litres of Saline injected fictitiously; (3) 100 Litres of Saline ingested with 12 mg of LA (LA 12); or (4) 100 µLitres of Saline injected with 25 mg of LA (LA 25). The eight tray levels in the incubator were distributed at random to the treatment groups. A setter and hatcher unit were both functions of the same incubator. According to the steps outlined, the conditions for incubating were followed. Every 15 minutes, HOBO ZW Series wireless data loggers took data of the outside temperature and humidity levels in 4 different areas inside the incubator. To identify and eliminate any eggs lacking viable embryos, every egg were candled at 10 and 16 doi. Additionally, the difference between the weight of eggs at heating and reproduction value before setting was used to calculate the median percentage of egg weight loss (PEWL) between Zero and twelve, between 12 and 18, and one to eighteen doi in each treatment-replicate group. After removing eggs with unviable embryos, the mean PEWL was computed using the technique, and the PEWL between 11 and 17 doi was stable.

Procedures and Injections for Therapy

The defined method has been used in the preparation of therapeutic mixtures. In a nutshell, LA was utilised to make LA solutions immediately before being injected using a 0.2 µm syringe filtration. Various LA concentrations (12 and 25 mg) were added in varying amounts to 86% SL, which was then carefully mixed to disperse the LA. After 6 hours of cooling, the solutions were ready for injection. Embrex Inovoject, a commercial motorised multi-egg injector was used to insert live egg embryos into the amnion at the age of 18 doi. Recombinant Brilliant Blue by Coomassie G-250 stain was inserted into a total of 36 eggs, which were then immediately terminated to examine the growth of the embryos. This was carried out for each of the eighth layers of the incubator's tray for a single egg from each of the four different therapy groups.

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Data collection using Hatch and serum

Average hatchling BW for each duplicate group of chicks in every group receiving action at 22.94 doi (502 h of implantation (hoi)) and the ability to hatch as a proportion of live egg embryos (HI) were computed. Following the in ovo injection, 4 randomly selected eggs from each person in the group had blood drawn from the chorioallantois capillaries of LA at the levels of 20.29 doi (442 hoi), 20.89 doi (476 hoi), and 22.94 doi (502 hoi) for both Men and women. Each treatment-replicate group's four eggs had their serum collected and pooled in accordance with the instructions.

Following to the company's instructions, the serum LA content was measured using the Chicken Vitamin C (VC) Elisa kit from MyBioSource in Harbin, Heilongjiang, China. As instructed, the LA ELISA assay was carried out. The process was recently changed to employ specific LA monoclonal antibodies, precoated plates and biotinylated chicken LA antibody. Following that, the optical density (OD) at 460 nm (OD460) for LA was calculated using a SpectraMax M5 Microplate Analyzer.

Statistical Analysis

A randomised entire block was used in the experimental setup. Each of them of the 9 tiers of identical trays in the isolator contained one of the five therapies, and the tray level was regarded as the limiting variable. On each level of the incubator tray, an unequal representation of each therapy was used. One-way ANOVA was used to analyse each variable in SAS 9.4's GLIMMIX technique. At $p \leq 0.05$, variations were declared scientifically essential, and the data are demonstrated as median SEM. By using Fisher's safeguarded smallest,

$$Yij = \mu + Bi + Ti + Ei \quad (1)$$

Where μ the average for the population, and the limitation was Bi. (i = 1 or 8), Tj was the impact of each in ovo injection treatment (j = 0 to 5), and Eij was the excess mistake, was the equation used to analyse the incubation information.

Results

The entire treatment groups' dye-injected eggs had 92.69% and 9.45% of the infusions take place in this substance and embryo, correspondingly. Particularly, the dye-injected eggs in the non-injected control group acquired injections in the amnion, while 89.9% and 13.3%. Consequently, the amnion and appropriate bodies were the sites of the injections in the dye-injected ova throughout the process of injection sessions. The study in intraembryonic or amniotic injections resulted in the Marek's disease vaccine's best efficacy (92% protective index). Figure 1 lists the hatch factors, including the zero to twelve, twelve to eighteen and zero to eighteen doi PEWL, HI, and hatchling BW. For any of these characteristics, there were no discernible variations in treatment that were substantial ($p > 0.05$).

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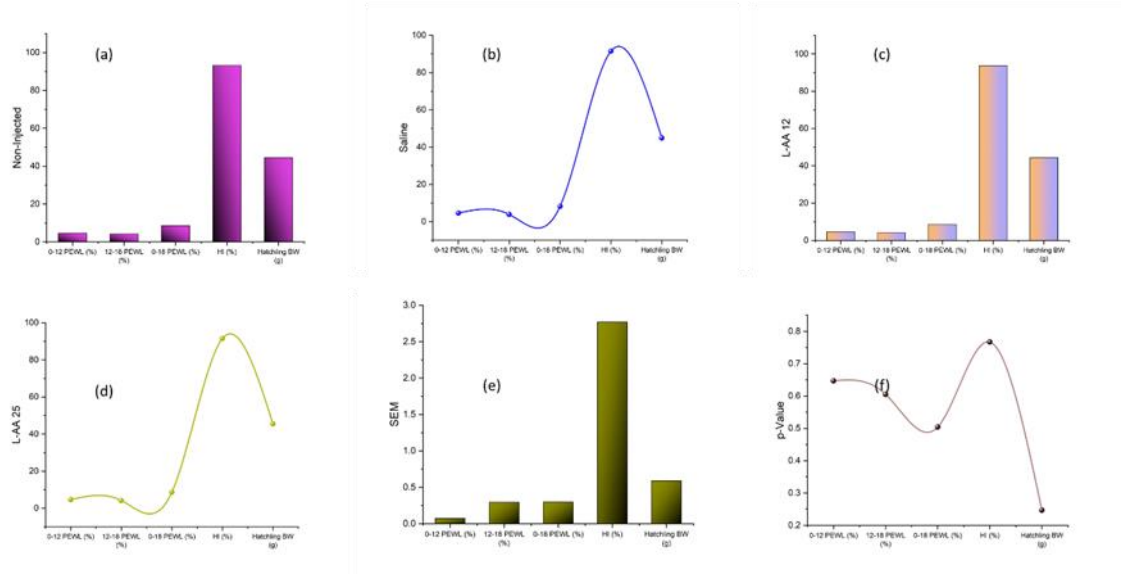

Figure (1): The impact of therapy on percentage egg weight loss (PEWL) between the initial day and twelve, twelve and eighteen, and days 0 and 18 of incubation, on the ability of injected live embryonated eggs to hatch (HI), and on the mean hatchling body weight (BW) at days 21 and 22 of incubation.

Figure 2 contains the hatch residue data. Mortals that happened between eighteen and twenty-one hours before pip, Late, pip, post-pip, and hatchling mortality, respectively, were terms used to describe events that occurred while performing the pips technique, after the pipping method, and promptly after completing hatching from the eggshell. According to Hatch component studies, there were definitely not significant distinctions ($p > 0.05$) between late, pip, and post-pip fatalities throughout therapies.

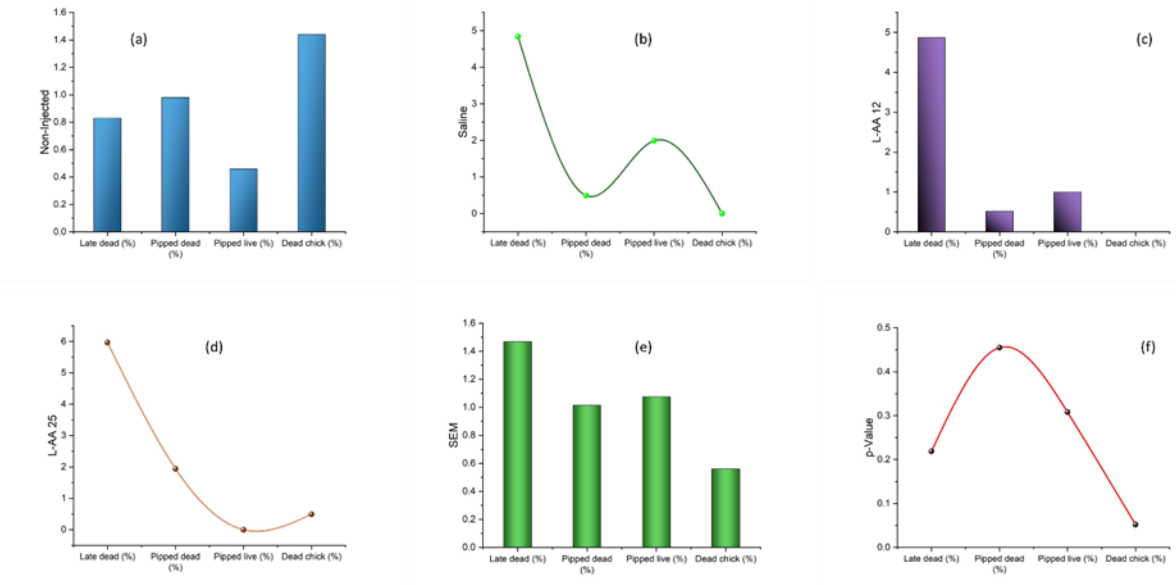

Figure (2): Outcomes of therapy on hatch remains analytical indicators (late, pip, post-pip, and hatchling mortalities) during 3 weeks of incubation (no handling), SA injection (SA), SA containing 12 mg of Lascorbic acid (L-A 12), or 25 mg of (L-A 25)

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For hatchling mortality, however, there was an important intervention variation (p = 0.05) (figure 2). Related to the non-injected control group, hatchling mortality decreased substantially in the solvent and LA 12 therapies, with the LA 26 treatment being in the middle. Table 1 displays the LA serum information on concentrations compared to the various LA in ovo injection amounts.

Table 1: The outcomes on the handling on serum L-A attention at 18 and 19 d, as well as men (L-A-21-F d) and women (L-A-21-F d) at 21 d, whether SA-injected (saline), SA with 12 mg of L-ascorbic acid (L-A 12), or 25 mg of L-A 25.

Handling	Non-injected	SA	L-A 12	L-A 25	SEM	p-Value
L-A-18 d (μM)	8.19	7.69	8.43	8.05	0.569	0.632
L-A-19 d (μM)	12.45	13.06	12.79	12.19	3.249	0.984
L-A-21-F d (μM)	5.66	6.35	5.15	5.19	0.747	0.355
L-AA-21 M d (μM)	9.64	9.65	11.84	7.88	0.327	0.309

The mean value of LA blood levels were roughly twice as high in men. Greater than in women, indicating a statistical distinction involving men and women serum LA levels in hatchlings. But serum levels of LA did not vary within therapies (p > 0.05), and no variations were found between the in ovo administration therapies for LA levels in the blood (p > 0.05) in either Men or women. Table 1 displays the effects of therapy on the percentage of egg weight loss (PEWL) between zero and twelve, between twelve and eighteen and zero to eighteen days following development, the ability to hatch of inserted live embryonated eggs (HI), and the typical hatchling body weight (BW) using saline injection, saline injection with 12 mg of (LA 12), or SL containing 25 mg of LA 25.

Conclusion

The situation was discovered that a large amount of LA (25 mg) had no negative impact on the hatch parameters that were investigated after evaluating the impact of excessive expanses of LA given intravenously to the amniotic sac on several hatch parameters and blood LA levels. The programmed in ovo injection of substantial quantities of LA might encourage embryonic viability and not be negative to hatchling quality, given the greater embryonic viability seen in the SL and 12 mg LA in ovo the infusion therapies compared to the non-injected controls. The different LA dosages given by in ovo injection also had no effect on the serum LA levels. In order to evaluate the persistence levels of LA in the connective tissue of broilers throughout the grow-out period whether LA is administered in ovo or in the diet, more research is required.

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