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# Influences of *In-Ovo* Injection of L-Carnitine on the Hatchability and Immunity of Hatched Chicks of Two Breeds of Broiler Chickens

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## ABSTRACT

The research aimed to investigate the impact of injecting various doses of L-carnitine (LC) into hatching eggs on reproductive characteristics, mortality, and Hatched Chicks' immunity post-hatching in two distinct broiler chicken strains on the 13th day of the incubation period. The study utilized 300 fertilized eggs collected from commercial Indian River and Ross 308 broiler breeder parent stocks aged 40-42 weeks. Eggs were randomly distributed to five treatments: T0 (Negative control), T1 (injected with 0.5 ml/egg sterilized distilled water, positive control), T2 (injected with LC 2%), T3 (injected with LC 4%) and T4 (injected with LC 6%). The results demonstrated that, for both chicken strains and their interaction, there were no statistically significant differences ($p > 0.05$) observed in the weight of incubated eggs and the chick: egg weight ratio among the various treatment groups. Nevertheless, the study revealed significant enhancements ($p \leq 0.01$) in various parameters, including chick weight, mortality, Set egg hatchability, fertile egg hatchability, fertility, and immune response against Newcastle disease (ND) and Infectious Bronchitis (IB). The enhancements were linked to the administration of L-carnitine through ovo injection in both strains of broiler chickens. Also, the injection with 2% of LC positively influenced the percentages of fertility and hatchability, while decreasing the percentage of perished embryos. Furthermore, it was noted that the Ross 308 strain closely followed the Indian River strain in most study traits however Indian River strains exhibited significantly better survival ($P \leq 0.01$) with the lowest mortality rate. In conclusion, the research found that the ovo injection of L-carnitine exhibited positive effects on hatchability traits on the 13th day of incubation for both Indian River and Ross 308 strains.



*Keywords: Broiler Breeder, L- Carnitine, In-Ovo Injection, Hatching Traits, Maternal Immunity.*

## 1. Introduction

In commercial poultry rearing, the in-ovo feeding method is essential for optimizing profits. This approach involves administering nutrients directly to developing embryos within eggs through injection into the amniotic fluid. The embryos subsequently absorb this nutrient-enriched fluid, supplementing their needs for growth, development of vital tissues, and supporting the hatching process[11]. This approach ensures that essential nutrients are supplied at a critical stage, potentially resulting in enhanced overall health and productivity in the commercial poultry production cycle[42]. Research emphasizes the significance of ovo injection during the later stages of embryonic development, specifically highlighting its role in enhancing the growth of essential organs like the gastrointestinal system, skeletal system, and immune-related system. Building on this[41], proposes that utilizing this injection technique contributes to increased productivity in chicks. According to[45], hatchability, chick weight, and subsequent production performance improve during the final stages of broiler embryo development by in-ovo injection with a solution of glucose and L-carnitine. Various factors, including genetic traits, incubation conditions, and egg quality, influence hatchability during embryogenesis and post-hatch performance[7]. Access to essential egg nutrients, such as minerals, vitamins, carbohydrate, protein, and lipids, influences the health of avian species both pre- and post-hatching[31]. Achieving optimal embryonic growth necessitates a delicate balance between the nutritional content of the egg and the relatively short period of embryonic development. Ensuring the effective transport of nutrients to the embryo during this short interval was crucial for proper embryonic development. Insufficient nutrition during embryonic formation, potentially linked to genetic factors, has the potential to impede the normal and viable progression of development[29]. Consequently, researchers and producers have shown interest in exploring new feeding methods, such as the use of L-carnitine, which has demonstrated benefits for hatchability and post-hatch performance in recent studies. This approach has gained considerable interest in recent years due to its potential benefits. L-carnitine, chemically defined as β-OH-γ-N-tri methyl amino-butyrate, is a water-soluble quaternary amine. Its primary, principle function is to function as an essential acyl transporter in

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mitochondrial beta-oxidation processes that occur naturally in animals, plants, and microorganisms, specifically in the breakdown of long-chain fatty acids. This beta-oxidation is a crucial mechanism for energy production within cells[20]. Additionally it also assists in the elimination of short- and medium-chain fatty acids from mitochondria, preventing their accumulation as a result of both standard and pathological metabolism[14]. L-carnitine is synthesized through biosynthesis from essential amino acids such as lysine and methionine, a process that necessitates ferrous ions (Fe++) as well as three vitamins (niacin, ascorbate, and pyridoxine). These vitamins serve as cofactors for the enzymes engaged in the carnitine metabolic pathway[9]. The research indicates that the inclusion of L-carnitine in the fertile egg could potentially have a beneficial effect by reducing embryonic mortality. This reduction attributed to the ability of L-carnitine to minimize oxidative stress during the hatching process, ultimately resulting in an increased hatch rate[33, 44] suggests that due to the fast growth, high requirements of energy, and constrained natural production of L-carnitine in chicken embryos, supplementation of L-carnitine could be advantageous, as indicated by[20]. Chick embryos rely heavily on fatty acid oxidation of yolk lipids for about 90% of their total energy requirements, and they exhibit a substantial need for L-carnitine[32]. As a consequence, the ovo injection of L-carnitine might be necessary for optimal β-oxidation. The present investigation aimed to explore how in-ovo injection of L-carnitine, breeds and their interaction affect hatching performance and immunological response of newly hatched chicks.

## 2. Material and Methods

### 2.1 Methods employed in the experimental study.

The study was conducted at a commercial hatchery in Erbil city, starting from November 4$^{th}$ to the hatching day on November 25$^{th}$, 2023, a total of 300 fertilized broiler breeder eggs were obtained from parent stocks of commercial Indian River and Ross 308 broiler breeders, aged 40-42 weeks. The breeder flocks fed a broiler breeder diet complying with the National Research Council (NRC, 1994) guidelines, consisting of 3013.40 kcal ME/kg and 18.48% CP. Both flocks were under identical management conditions as per the recommendations of the breeding company.

### 2.2 Performances of in-ovo injection and hatchability

All eggs in this investigation were put in an automated incubator, on the thirteenth day of the incubation period. A manual candling process was employed to eliminate infertile eggs and embryos that had halted development prematurely. Subsequently, the viable eggs were randomly distributed to five various treatment groups. Every treatment, within each strain, consisted of 30 fertilized eggs distributed into three equal replicates, with each replicate containing 10 eggs. Each of the injected eggs received a 0.5 ml extract in the air space, following the recommendations of both[15, 38], for eggs of local chickens. L-carnitine, obtained in the form prepared by the American company AMS, was used in the experiment. The eggs underwent five different treatments: the first, which served as the negative control with no injection; the second, which served as the positive control, was injected with 0.5 ml/egg of sterilized distilled water; the third, which received an injection of a 2%L-carnitine solution; the fourth, which received an injection of a 4% L-carnitine solution; and the fifth, which received an injection of a 6% L-carnitine solution. Ethanol alcohol with a 70% concentration was utilized to cleanse the injection sites on the egg prior to the injection process. L-carnitine was meticulously injected into the air cell at the larger end of the eggs, using an insulin syringe on the 13th day of incubation. The injection aimed to reach the chorioallantois membrane, located approximately 1 cm away from the eggshell. The hatchery maintained during the incubation period at 37.6°c and 52% relative humidity from 1 to 18 days, but through the last 3 days of incubation, the temperature was reduced to 36.9°c, accompanied by fluctuating relative humidity values: 60% on the 19th day, 75% on the 20th day, and 80% on the 21st day. From the first day to the 18th day of incubation, the hatchery consistently upheld incubator conditions with a temperature of 37.6°c and a relative humidity of 52%. However, in the final three days of incubation, there were specific adjustments. The average temperature was reduced to 36.9°c, and the level of relative humidity varied: 60% on the 19th day, 75% on the 20th day, and 80% on the 21st day. These modifications were implemented to facilitate optimal conditions for the concluding stages of embryonic development and the impending hatching process. All chicks that successfully hatched were utilized to evaluate the hatchability of fertile eggs. The deposition or application of nutrients, as illustrated in Figure 1 by[31], primarily occurred at the amnion. This particular process had a significant impact on embryonic physiology, and its effects were evident in the hatchability rates, as noted in the study by[26].

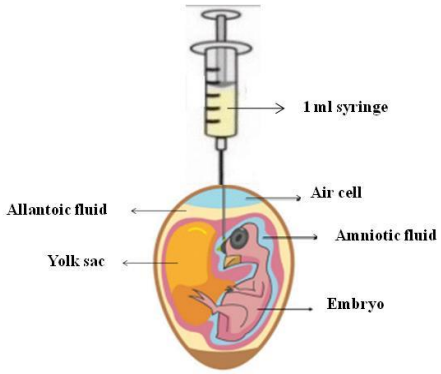


**Figure 1:** Substance delivery into the amniotic fluid through embryonic development[31].

### 2.3 Collecting data and samples.

The following formula can be used to calculate the percentage hatchability:

$$\text{Real hatchability (\%)} = \frac{\text{Hatched chick number}}{\text{Fertilized egg number}} \times 100$$

This formula involves dividing the total number of chicks hatched by the total number of fertilized eggs, and then multiplying the result by 100 to express the hatchability rate as a percentage[10].

The evaluation of embryonic mortality involved the examination of unhatched eggs by breaking them to confirm the presence of either deceased embryos or spoiled eggs. The stage of embryonic mortality was determined through the following calculation:

$$\text{Mortality rate (\%)} = \frac{\text{Number of deceased chicks}}{\text{Number of dead eggs}} \times 100$$

The hatching results were analyzed based on the method outlined in the study by[27]. Subsequently, the weights of Indian River and Ross 308 chicks after hatching in each treatment were determined by weighing all chicks from each replicate of each treatment within each strain. A sensitive digital scale with an accuracy of 0.01 grams was employed for this purpose, and the average weight of each chick was calculated.

### 2.4 Serum Data Collection

On the third day of the chicks' lives, blood samples, were directly obtained from the hearts of three chicks for each replicate within each treatment for both strains. About 2 ml of blood from each chick was collected and placed into test tubes containing the anticoagulant EDTA. Subsequently, these tubes were centrifuged at 3000 rpm for 15 minutes to assist in blood plasma separation. The obtained plasma samples were then meticulously transferred into Eppendorf tubes and kept frozen until immunological tests were performed.

### 2.5 Immunological examinations

In the evaluation of maternal immunity against Newcastle disease (ND) and infectious bronchitis (IB), the standard wavelength for assessing antibody titers in blood serum was determined. The Enzyme-Linked Immunosorbent Assay (ELISA), as outlined in the methodology by[37], was employed for this purpose. The ELISA method was utilized to measure the size standard for immunological antibodies, commonly referred to as antibody titers. This involved utilizing a standard wavelength to gauge the concentration of antibodies in the serum, providing valuable information about the immune response against Newcastle disease and infectious bronchitis.

### 2.6 Statistical analysis

A factorial complete randomized design (CRD) employed a two-way analysis of variance (ANOVA) to statistically analyze the research results. The goal of this analysis was to detect the influence of different strains and in-ovo injection treatments, as well as their interaction; General Linear Model (GLM) within the statistical program SAS [34] was utilized for these analyses. Subsequently, the Duncan multiple range test method[18] was utilized to examine the differences between the means (0.05).

## 3. Flexural Strength of RC of FRP-strengthened RC beams

Egg weight (g), chick weight (g), and the chick-to-egg weight ratio (%) for each treatment group in two broiler breeder strains (Indian River and Ross 308) on the 13th day of incubation are provided in (Table1). The analysis demonstrated a significant impact ($P \leq 0.01$) on the weight of the hatched chicks, with higher weights observed for the T2, T3, and T4 LC injection treatments, with mean values of 49.06, 48.26, and 47.08, respectively, compared to both negative and positive controls. The improvement observed could be linked to enhanced antioxidant levels in the embryos. Nonetheless, mitigating oxidative stress related to hatching might contribute to increased hatch weight and improved post-hatch performance [16]. However, egg weight (g) and chick: egg weight ratios (%) were not influenced by strains, experimental treatments, or the interaction between strains and ovo injection. The Ross 308 strain exhibited higher values for both egg weight and chick weight compared to the Indian River strain. The findings align with those of [25], who observed enhanced hatch weights in Ross 308 broiler chicks when injected with L-carnitine at a dose of 30 mg on the 17th day of egg incubation. The results aligned with those previously documented by[43] for Fayoumi chickens, showing significant improvement in hatch weight with LC injections at doses ranging from 8-16 mg/egg[35] noted a cubic response in chick weight at hatch with increasing L-carnitine levels in-ovo injection, suggesting that higher L-carnitine levels may improve hatch weight by enhancing fatty acid oxidation efficiency, dietary nitrogen utilization, glucose oxidation in the liver, and glucose utilization in chick tissues[44]. On the 13th day of egg incubation, [8] indicated that the weight traits of hatched chicks were not influenced by in-ovo injection with L-carnitine (LC) in the Ross 308 breed.

**Table 1:** The mean ± standard error (SE) impact of in-ovo injection with L-carnitine, on the weight of incubated eggs, chick weight, and the chick-to-egg weight ratio in two broiler strains.

| Treatments | Weight of incubated eggs (g) | Chick weight (g) | Chick: egg weight ratio (%) |
|---|---|---|---|
| L-carnitine dose (LC) | | | |
| Negative control | 65.60 ± 1.06$^{a}$ | 45.63 ± 0.34$^{c}$ | 69.56 ± 0.68$^{a}$ |
| Positive control | 65.43 ± 1.02$^{a}$ | 46.02 ± 0.29$^{c}$ | 69.90 ± 0.71$^{a}$ |
| 2% L-Carnitine | 69.10 ± 1.29$^{a}$ | 49.06 ± 0.27$^{a}$ | 71.26 ± 0.68$^{a}$ |
| 4% L-Carnitine | 67.80 ± 1.31$^{a}$ | 48.26 + 0.35$^{a}$ | 70.92 ± 0.56$^{a}$ |
| 6% L-Carnitine | 68.10 ± 1.30$^{a}$ | 47.08 ± 0.42$^{b}$ | 69.14 ± 0.58$^{a}$ |
| *P-Value* | 0.2089 | <.0001 | 0.1472 |
| Strains (S) | | | |
| Indian River (I) | 66.88 ± 0.89$^{a}$ | 46.70 ± 0.28$^{b}$ | 70.39 ± 0.43$^{a}$ |
| Ross 308 (R) | 67.60 ± 0.67$^{a}$ | 47.72 ± 0.35$^{a}$ | 69.92 ± 0.42$^{a}$ |
| *P-Value* | 0.5288 | 0.0006 | 0.4433 |
| Interaction (LC*S) | | | |
| I×TO | 64.80±0.80$^{a}$ | 45.25±0.37$^{d}$ | 69.28±1.17$^{a}$ |
| I×T1 | 65.40±2.06$^{a}$ | 45.54±0.16$^{d}$ | 70.20±0.86$^{a}$ |
| I×T2 | 68.80±2.35$^{a}$ | 48.31±0.21$^{b}$ | 71.78±0.87$^{a}$ |

| | | | |
|---|---|---|---|
| I×T3 | 67.80±1.91[a] | 47.85±0.58[b] | 71.26±0.76[a] |
| I×T4 | 67.60±2.58[a] | 46.54±0.14c[d] | 69.42±0.93[a] |
| R×TO | 66.40±2.01[a] | 46.00±0.55[d] | 69.84±0.84[a] |
| R×T1 | 65.80±0.66[a] | 46.50±0.50c[d] | 69.60±1.21[a] |
| R×T2 | 69.40±1.36[a] | 49.81±0.09[a] | 70.74±1.09[a] |
| R×T3 | 67.80±2.03[a] | 48.67±0.35[ab] | 70.57±0.89[a] |
| R×T4 | 68.60±0.93[a] | 47.63±0.80[bc] | 68.86±0.77[a] |
| *P-Value* | 0.9935 | 0.0177 | 0.9358 |

[a-f] there are significant differences between means in columns with different superscripts (P≤0.05).

Table 2 demonstrates a significant influence (p≤ 0.01) resulting from the in-ovo injection of L-carnitine (LC) on the 13th day of incubation, impacting various hatching traits in two strains of broiler breeder eggs. These traits include the hatchability of fertile eggs, and hatchability of set eggs. Furthermore, the dosage of L-carnitine delivered by in-ovo injection, strains, and their interactions all exhibited a significantly higher effect (p ≤ 0.0001) on hatching parameters. The highest hatching rate of fertile eggs was observed in the T2 group (98.30%), while the lowest hatching rate was in the T0 group (negative control) at 83.33%. Eggs injected with a 2% L-carnitine solution demonstrated significantly higher hatchability for both set and fertile eggs in comparison to eggs injected with higher concentrations (4% or 6%) of L-carnitine, as well as the control group. This improvement might be attributed the increased energy production available to the embryo through the transfer of fatty acids from the yolk sac, facilitated by L-carnitine, across the mitochondrial membrane, where they undergo oxidation[44].

Furthermore, the antioxidant properties of L-carnitine may contribute to the removal of free radicals produced during the breakdown of fatty acids, thereby aiding the hatching process, as suggested by[44]. Consistent with earlier studies by[12, 19], the in-ovo injection of various nutrients has been shown to enhance the hatchability percentage of broiler breeder eggs. Other studies, such as[40] on Cobb breed chicken eggs and[32] on Turkey eggs, reported improved hatching rates with L-carnitine injection.[22] found that injecting eggs on day 14 of incubation significantly enhanced hatching traits compared to injections on days 16 or 18.[36] noted a significant (P≤0.01) impact of L-carnitine, injection on the fertile hatchability of broiler chicks[28, 24]. observed that the influence of specific genetic lines, the inclusion of LC food additives, and their interaction led to an enhancement in the productive and reproductive performance of quail chicks. These consistent findings underscore the positive influence of L-carnitine on hatching outcomes in various poultry breeds.

**Table 2:** The mean ± standard error (SE) impact of in-ovo injection with L-carnitine, on some of hatchability traits in two broiler strains.

| Treatments | Hatchability of fertile eggs (%) | Hatchability of set eggs (%) |
|---|---|---|
| L-carnitine dose (LC) | | |
| Negative control | 83.33 ± 0.86[e] | 74.96 ± 1.01[e] |
| Positive control | 87.42 ± 0.86[d] | 80.03 ± 1.56[d] |
| 2% L-Carnitine | 98.30 ± 0.80[a] | 96.60 ± 0.75[a] |
| 4% L-Carnitine | 94.89 ± 0.97[b] | 91.67 ± 1.16[b] |
| 6% L-Carnitine | 91.47 ± 1.83[c] | 86.77 ± 1.39[c] |
| *P-Value* | <.0001 | <.0001 |
| Strains (S) | | |
| Indian River | 88.61 ± 1.20[b] | 83.98 ± 1.84[b] |
| Ross 308 | 93.56 ± 1.17[a] | 88.03 ± 1.54[a] |
| *P-Value* | <.0001 | <.0001 |
| Interaction (LC*S) | | |
| I×TO | 81.48±0.98[f] | 73.32±1.16[d] |
| I×T1 | 85.56±0.92[e] | 76.66±1.96[d] |
| I×T2 | 96.60±1.21[b] | 96.60±0.93[a] |
| I×T3 | 93.12±1.11[c] | 90.00±1.70[b] |
| I×T4 | 86.28±1.01[e] | 83.34±1.44[c] |
| R×TO | 85.18±0.79[e] | 76.60±1.36[d] |
| R×T1 | 89.28±0.88[d] | 83.40±1.21[c] |
| R×T2 | 100.00±0.00[a] | 96.60±0.93[a] |
| R×T3 | 96.66±1.20[b] | 93.34±1.32a[b] |
| R×T4 | 96.66±0.80[b] | 90.20±0.86[b] |
| *P-Value* | 0.0020 | 0.0349 |

[a-f] there are significant differences between means in columns with different superscripts (P≤0.05).

The summarized data in Table 3 revealed the impact of in-ovo injection with L-carnitine on overall mortality, early mortality, and late mortality in two strains of broiler breeders, as well as their interaction between factors. The percentage of embryo mortality upon hatching significantly differs (p ≤ 0.01) based on treatment, strains, and their interaction. Notably, the second

treatment (T2), involving eggs injected with 2ml/egg of L-carnitine, exhibited lower embryo mortality percentages compared to other treatments (T0, T1, T3, and T4). The reduction in embryo mortality in the second treatment, across total, early, and late death, might be due to the additional energy support provided to the embryos through the transfer of fatty acids from the yolk sac into the mitochondria and their oxidation due to L-carnitine injection. This energy facilitates the embryo's ability to perforate and break the eggshell, thereby reducing fetal mortality[16]. The mortality rates, both early and late during embryonic development, significantly reduced ($p \leq 0.01$) by all injected treatments compared to both negative and positive control groups.

**Table 3:** The mean ± standard error (SE) impact of in-ovo injection with L-carnitine, on mortality traits in two broiler strains.

| Treatments | Total mortality (%) | Early mortality (%) | Late mortality (%) |
|---|---|---|---|
| L-carnitine dose (LC) | | | |
| Negative control | 0.093 ± 0.02$^{b}$ | 0.054 ± 0.01$^{b}$ | 0.096 ± 0.01$^{a}$ |
| Positive control | 0.092 ± 0.01$^{c}$ | 0.074 ± 0.01$^{a}$ | 0.096 ± 0.02$^{a}$ |
| 2% L-Carnitine | 0.077 ± 0.03$^{d}$ | 0.000 ± 0.00$^{e}$ | 0.017 ± 0.01$^{d}$ |
| 4% L-Carnitine | 0.094 ± 0.02$^{b}$ | 0.023 ± 0.01$^{d}$ | 0.035 ± 0.01$^{c}$ |
| 6% L-Carnitine | 0.114 ± 0.03$^{a}$ | 0.036 ± 0.01$^{c}$ | 0.056 ± 0.02$^{b}$ |
| *P-Value* | <.0001 | <.0001 | <.0001 |
| Strains (S) | | | |
| Indian River | 0.066 ± 0.01$^{b}$ | 0.022 ± 0.01$^{b}$ | 0.044 ± 0.01$^{b}$ |
| Ross 308 | 0.121 ± 0.01$^{a}$ | 0.056 ± 0.01$^{a}$ | 0.076 ± 0.01$^{a}$ |
| *P-Value* | <.0001 | <.0001 | <.0001 |
| Interaction (LC*S) | | | |
| I×TO | 0.036±0.01$^{c}$ | 0.071±0.02$^{d}$ | 0.151±0.02$^{b}$ |
| I×T1 | 0.074±0.01$^{a}$ | 0.077±0.001$^{c}$ | 0.107±0.02$^{c}$ |
| I×T2 | 0.000±0.00$^{d}$ | 0.000±0.00$^{f}$ | 0.000±0.00$^{f}$ |
| I×T3 | 0.000±0.00$^{d}$ | 0.034±0.00$^{e}$ | 0.034±0.01$^{e}$ |
| I×T4 | 0.000±0.00$^{d}$ | 0.036±0.01$^{e}$ | 0.036±0.01$^{e}$ |
| R×TO | 0.071±0.01$^{b}$ | 0.120±0.02$^{a}$ | 0.034±0.00$^{e}$ |
| R×T1 | 0.074±0.01$^{a}$ | 0.115±0.01$^{b}$ | 0.070±0.001$^{d}$ |
| R×T2 | 0.000±0.00$^{d}$ | 0.033±0.01$^{e}$ | 0.154±0.01$^{b}$ |
| R×T3 | 0.034±0.01$^{c}$ | 0.036±0.01$^{e}$ | 0.154±0.02$^{b}$ |
| R×T4 | 0.069±0.01$^{b}$ | 0.076±0.001$^{c}$ | 0.191±0.01$^{a}$ |
| *P-Value* | <.0001 | <.0001 | <.0001 |

**a-f** there are significant differences between means in columns with different superscripts (P≤0.05).

Strains also had a significant impact on mortality, with the Indian River strain showing lower total, early, and late embryonic death compared to the Ross 308 strain. The interaction between injection materials and strains significantly influenced mortality traits. Our findings align closely with those documented by[3,5], as well as [22]. Occurrences of embryo mortality resulting from in-ovo injection identified, with potential causes including contamination with microorganisms[15], embryo sac damage[13], and changes in the concentration of egg fluid[30]. Our study found a higher rate of embryo mortality in the later stages as opposed to the initial stages. Building on the research of[45] by emphasizing that elevated temperature, and humidity, microbial infection, and insufficient egg nutrition are critical factors associated with embryonic mortality during the middle and late stages. Furthermore, our study also indicated that non-injection treatments exhibited high embryo mortality, a result linked to the constrained supply of essential nutrients crucial for the development of embryos. L-carnitine injections demonstrated an elevation in the maternal immune response to Newcastle disease and Infectious Bronchitis in two strains of broiler breeder chicks when contrasted with both control treatments (T0 and T1), as illustrated in Table 4. Specifically, the antibody titers against both diseases significantly increased in the L-carnitine injection treatments when contrasted with both the negative control (T0) and positive control (T1). This observation implies that the in-ovo injection of L-carnitine has a positive impact on the development of maternally acquired antibodies against these diseases in the chicks. The higher antibody titers observed in the LC injection treatments indicate an improved immune response, potentially leading to better disease resistance or protection in the offspring after hatching. According to[9], this effect may be linked to L-carnitine's role in increasing the levels of total immune globulins and IgG, consequently improving the immune system. Additionally, L-carnitine could function as an immune modulator, as suggested by its influence on increasing the relative weights of lymph nodes such as the spleen, thymus, tonsils, and cecal glands[6]. The critical role of increased B lymphocytes in enhancing antibody production in the blood is supported by evidence of heightened growth, and development of lymphatic tissues, and organs. Numerous studies, including those conducted by[4, 2, 39], have underscored this relationship.

The study results indicate that both the chicken strains and the process of injecting L-carnitine (LC) into eggs significantly influenced the enhancement of the hatched chick's immunity. Specifically, Table 4 shows that the antibody titers against Newcastle Disease were higher in the Indian River strain compared to the Ross 308 strain. Conversely, the antibody titers

against Infectious Bronchitis were higher in the Ross 308 strain compared to the Indian River strain. This indicates that the immune responses of the two chicken strains vary when exposed to in-ovo injection of L-carnitine, resulting in variations in antibody titers against specific diseases. The variations in immune responses among hatched chicks can be linked to both the inherent genetic characteristics of the strains and potential interactions between the injected substance (LC) and the immune system. The repeated measures analysis revealed a non-significant ($p > 0.05$) interaction effect between strain and in-ovo injection on the immunological response of chicks at hatched period. The study conducted by[11] revealed that the humoral immune response against Newcastle disease and infectious bronchitis in broiler chicks improved when a nutritional solution was injected into the amniotic membrane at 18 days of incubation. Additionally[1], discovered that the system immune activity, growth performance, and growth of bones in broiler chicks were enhanced through in-ovo injection of vitamins D3, and K3 at 18 days of incubation.

**Table 4:** The mean ± standard error (SE) impact of in-ovo injection with L-carnitine on hatched chicks' immunity of two broiler strains.

| Treatments | Newcastle disease antibody titer (ND) | Infectious bronchitis antibody titer (IB) |
|---|---|---|
| L-carnitine dose (LC) | | |
| Negative control | 10645.00±369.84$^{e}$ | 15677.20±365.81$^{c}$ |
| Positive control | 12246.40±442.28$^{d}$ | 15963.10±391.95$^{c}$ |
| 2% L-Carnitine | 14005.40±408.80$^{c}$ | 16962.60±315.43$^{b}$ |
| 4% L-Carnitine | 15437.95±309.80$^{b}$ | 17452.80±252.43$^{ab}$ |
| 6% L-Carnitine | 16531.65±469.23$^{a}$ | 18189.40±177.94$^{a}$ |
| *P-Value* | <.0001 | <.0001 |
| Strains (S) | | |
| Indian River | 14649.50±503.85$^{a}$ | 16322.20±280.56$^{b}$ |
| Ross 308 | 12897.06±424.68$^{b}$ | 17375.84±206.66$^{a}$ |
| *P-Value* | <.0001 | <.0001 |
| Interaction (LC*S) | | |
| I×TO | 11317.80±455.19$^{e}$ | 15240.00±704.70$^{f}$ |
| I×T1 | 12895.70±727.89$^{d}$ | 15351.80±675.33$^{ef}$ |
| I×T2 | 15096.60±339.08$^{bc}$ | 16475.20±482.04$^{cde}$ |
| I×T3 | 16255.30±126.81$^{b}$ | 16766.80±187.51$^{bcd}$ |
| I×T4 | 17682.10±458.41$^{a}$ | 17777.20±168.64$^{ab}$ |
| R×TO | 9972.20±426.60$^{f}$ | 16114.40±100.03$^{def}$ |
| R×T1 | 11597.10±373.69$^{e}$ | 16574.40±219.96$^{cd}$ |
| R×T2 | 12914.20±204.15$^{d}$ | 17450.00±310.81$^{abc}$ |
| R×T3 | 14620.60±285.96$^{c}$ | 18138.80±127.60$^{a}$ |
| R×T4 | 15381.20±344.77$^{bc}$ | 18601.60±170.53$^{a}$ |
| *P-Value* | 0.6214 | 0.9420 |

$^{a-f}$ There are significant differences between means in columns with different superscripts ($P \leq 0.05$).

## Conclusions

Considering findings, the study concluded that administration of L-carnitine in–ovo injection on day 13 of incubation positively influences the hatching rate, reduces mortality, and enhances hatched chicks immunity response against Newcastle and Infectious Bronchitis in both strains of broiler breeders. This improvement is attributed to the superior characteristics observed in the hatched chicks. Furthermore, the study recommends injection 2% L-carnitine to fertilized eggs on day 13 of hatching as an effective method to study broiler characteristics.

## Conflict of interests

We affirm that there are no competing interests related to this manuscript

## Authors contribution

Lajan S. Ahmad: Supervised, Conceived, designed the analysis, and participated in editing the paper writing.

Mekail H. Mustafa: Collected samples, conducted characterizations, performed the analysis, and contributed to writing paper drafts.

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