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EFFECTS *IN OVO* INJECTION OF L-CARNITINE ON HATCHABILITY, PRODUCTIVE AND PHYSIOLOGICAL TRAITS OF JAPANESE QUAIL (*COTURNIX JAPONICA*)

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Abstract

In the chicks, L-carnitine helps the body turn fat into energy and body may convert L-carnitine to different amino acids. This study was conducted in the Research Center of Tikrit University to determine the effects of early feeding *in ovo* injection of L-carnitine on the hatching traits, growth performance, and carcass characteristics of quail chicks. This experiment was carried out using 450 fertile quail eggs at 14 days of incubation in completely randomized design with three levels of L-carnitine (0, 5, and 10 %). These fertile eggs were allotted to 3 groups in 3 replicates and 50 eggs in each replicate. The L-carnitine was *in ovo* injected into the large end of egg and monitored for its hatchability rate, performance, physiological traits, and relative weight of liver, heart, and intestine. After hatching, 180 quail chicks were placed in 9 experimental flour pens, and three replicates with 60 quail chicks per replicate were divided to three treatments 20 each. The results showed that there is significant differences in hatchability rate, initial-final body weights, glutathione (GSH) level, and feed conversion ratio among experimental treatments ($p<0.05$). However, the effect of L-carnitine on dressing percentage, the total protein, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Malondialdehyde (MDA), and relative weight of liver, heart, and intestinal hatched was not significant difference among experimental treatments ($p>0.05$). Based on current results, it could be concluded that the early feeding of L-carnitine by *in ovo* injection on the 14th day of incubation may improve the hatchability, productive performance, and antioxidant status of the quail chicks post hatching period.

Keywords **:** In ovo injection, L-carnitine, Quail; productive performance; physiology traits; antioxidant parameters.

Introduction

During embryogenesis, all the nutrients that required in the developing embryo were presented in the fertile eggs so that any nutrient deficiency could result to serious complications during the embryo formation stages (Molenaar *et al.*, 2017; Peña-Villalobos *et al.*, 2017). Quail diets are mainly made from corn and soy, which have low in L-carnitine concentration causing lack or no L-carnitine content in quail eggs (Adabi *et al.*, 2011; Rehman *et al.*, 2017). Additionally, there is a limited chance in an avian embryo to synthesize L-carnitine during the incubation period because of the limited availability of γ -butyrobetaine, an intermediate for the biosynthesis of L-carnitine in embryos (Arslan, 2006). At early stages of life, low level of γ-butyrobetaine is attributed to the low γ-butyrobetaine hydroxylase activity, so It may be necessary to provide L-carnitine supplements to compensate lack of it (Rebouche, 1992). By other hand, there is a high level of polyunsaturated fatty acids in avian embryonic tissues, and this of polyunsaturated fatty acid is one of the important components of cell membrane .As normal fact, lipid peroxidation which is rised from free radicals that developed from mitochondria due to the high metabolic rate of rapidly improving embryos, is sensitive to polyunsaturated fatty acids (Kermanshahi, 2017). L-carnitine as any water-soluble amine is caused transfer of long chain fatty acids from the cytoplasm of a cell to the mitochondria for energy generation and subsequent β-oxidation. Exogenous L-carnitine supplementation may be beneficial and important in the chick hatching process, and it can do as an antioxidant to scavenge free radicals (Agarwal *et al.,* 2005; Esfahani *et al.,* 2018).

The maximum incubation day of free L-carnitine and its esterified form in the avian embryos organs is 18th day, and this suggests the inevitability of fatty acid oxidation to the embryonic energy generation. Furthermore, fresh eggs from hens fed with plant-based feed contains low levels of L-carnitine (Chiodi *et al.*, 1994).

It is hypothesized that *in ovo* injection of L-carnitine into quail chick eggs on the day 14th of incubation may provide the required energy for embryo activities and thus increase the growth performance and carcass weight of the quail chick .Indeed, add energy supply to L-carnitine may reduce the level of decrease oxidative stress during the hatching process thus decrease embryonic mortality and improve hatch rate. Hence, the aim of this study is to determine effects of early feeding with L-carnitine by *in ovo* injection on the hatchability, growth performance, carcass characteristics, and antioxidant levels of quail chicks.

Materials and Methods

In ovo injection procedure

In this experiment, 450 fertile eggs obtained at 14th day of incubation from the same quail breed collected from the College of Agriculture, Tikrit University, Iraq. Fertile eggs, on based completely randomized design were allotted to three treatment groups with three replicates per treatment groups and 50 eggs per replicate. At the 14th day of incubation, the eggs were inoculated with L-carnitine via *in ovo* supplementation at the following concentrations: 0% (control group was inoculated with sterile distilled water and designated as T1); 5% L-carnitine (T2 group) and 10% L-carnitine (T3 group). The L-carnitine was purchased from Qualikems Fine Chemicals (Delhi, India). Prior to inoculation, the eggs were incised with the aid of an automatic needle and infused with 100 µL of L-carnitine solution (different concentrations) using a needle (26-gauge). The injection site was sterilized by ethanol (70%) and sealed with nail paint before proceeding the hatching process.

Growth performance

This section of the study was carried out at the poultry farm of the College of Agricultural, Tikrit University, Iraq. From each treatment group, healthy hatched quail chicks were equally allocated into 9-floor pens, with 3 pens per treatment group and 20 chicks per pen. According to NRC (1994), the chicks' diet was formulated and their body weight and feed intake were weekly monitored and recorded. The feed intake, feed conversion ratio (FCR), and body weight of the chicks were also recorded on the last day of the experiments (35th day post-hatch).

Physiological parameters

Six birds per experimental groups (2 birds per replicate) were randomly selected, and free-flowing blood samples were drawn from the jugular vein (Campbell, 1995). The collected blood samples from each treatment group were pooled and analyzed for serum biochemistry traits. First, the samples were centrifuged at 3000 *g* for 15 min and the separated serum was sent to the laboratory for total protein, GOT, and GPT enzyme activity analyses. The blood analyses in this study were performed following the recommendations of the companies that supplied the reagents and equipment. The antioxidant status was determined by measuring the MAD and GSH level in serum, and they were analyzed according to the methods reported by (Taha, 2008).

On the 35th day post-hatching, the birds were kept away from feed to ensure gut clearance. Then, the birds were individually weighed to determine live body weights prior to slaughtering, de-feathering, and processing. Finally, the liver, heart, and gastrointestinal tract were harvested from the chicks and weighed to determine relative percentage weights.

Statistical analysis

The data of all experiments were presented to ANOVA procedures for completely randomized designs through general linear model (GLM) procedure of the SAS program (SAS Institute, 2011). The differences among the treatments were compared by Duncan's multiple range test while P-values where estimated statistically different at $p \leq 0.05$.

Results

Hatching

The studies on the hatchability of the quail eggs and the hatching weights are shown in Figures 1and 2. The hatchability of the treatment groups injected with L- carnitine was significantly higher compared to the control group ($p \leq 0.05$). Meanwhile, there was no observable difference between the hatchability rate of T2 and T3 groups. The chicks from T2 and T3 groups presented a higher hatching weight compared to the chicks from the control group.

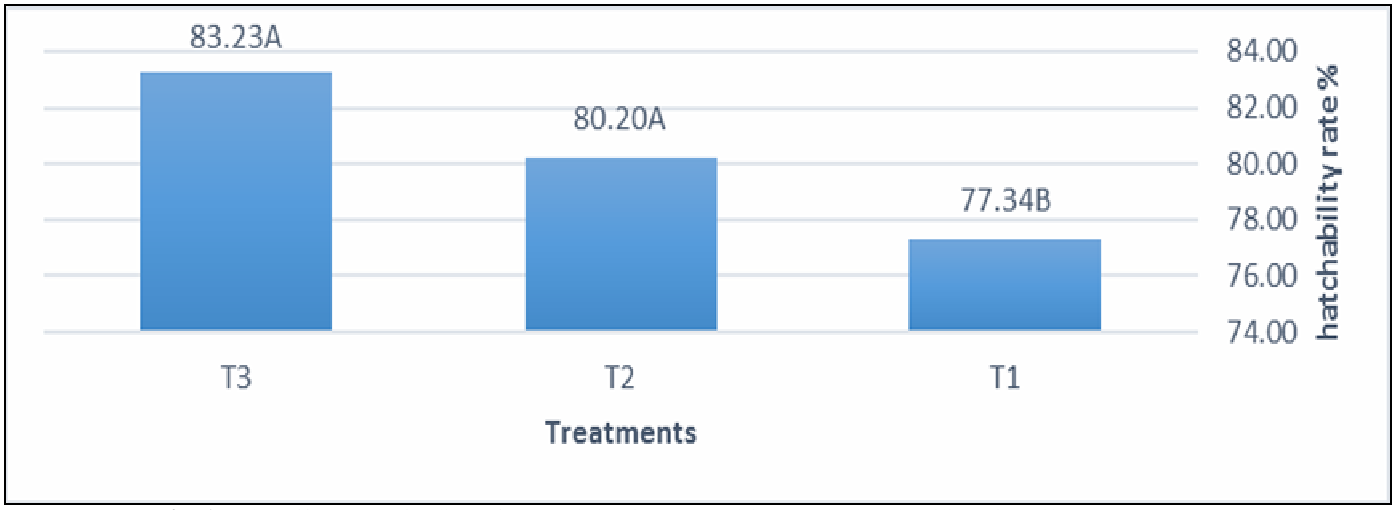

Fig. 1 : The effect early feeding of *in ovo* injection L-carnitine on the hatchability of quail eggs (mean ± SE)
Different superscripts (a, b) on the same column indicate statistically significant differences between the treatment groups at $p \leq 0.05$.
T1 -Control group (injected with distilled water); T2 and T3 -Test groups injected with 5% and 10% L-carnitine, respectively.

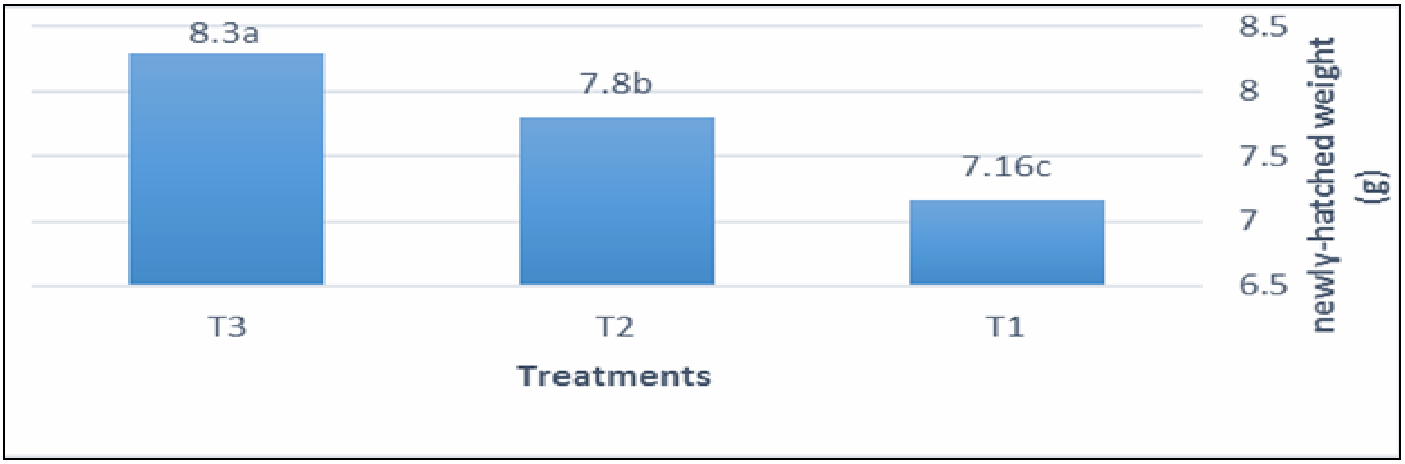

Fig. 2 : The effect early feeding of *in ovo* injection L-carnitine on the weight (g) of newly–hatched quail chicks (mean ± SE)
Different superscripts (a, b) on the same column indicate statistically significant differences between the treatment groups at $p \leq 0.05$.
T1 -Control group (injected with distilled water); T2 and T3 -Test groups injected with 5% and 10% L-carnitine, respectively.

Growth performance

Table 1 shows the weekly body weight, final body weight, feed intake, weight gain, and feed conversion ratio after 5 weeks of hatching. The results showed that chicks from L-carnitine injected eggs had higher body weights, weight gains and feed consumption ($p \leq 0.05$) compared to control group, with better-feed conversion ratio.

Table 1 : The effect early feeding of *in ovo* injection L-carnitine on some productive traits of quail (mean ± SE)

Age	1 week	2 week	3 week	4 week	5 week	
Treatments	Live body weight(g)					
T1	29.6 ± 0.88 b	59.4 ± 1.3b	91.4 ± 1.3b		124.7 ± 1.3 b	158.6 ± 1.1 c
T2	32.4 ± 0.5 a	61.0 ± 1.4 b	93.9 ± 1.3 ab		127.2 ± 1.4 b	165.8 ± 2.6 b
T3	34.3 ± 0.8 a	66.4 ± 1.4 a	98.5 ± 1.5 a		131.4 ± 1.4 a	175.7 ± 2.0 a
Body weight gain (g/bird)						
Treatments	1 week	2 week	3 week	4 week	5 week	Total period
T1	22.4 ± 0.6 b	29.8 ± 0.5 b	32.0 ± 0.5	33.3 ± 0.3	33.8 ± 0.7 b	151.4 ± 0.9 b
T2	24.6 ± 0.3 a	28.5 ± 0.9 ab	32.9 ± 0.1	33.3 ± 0.3	38.5 ± 2.2 ab	158.0 ± 2.5 b
T3	25.9 ± 0.7 a	32.1 ± 0.7 a	32.1 ± 0.2	32.9 ± 0.1	44.2 ± 2.2 a	167.3 ± 2 a
Feed consumption (g/bird/week)						
Treatments	1 week	2 week	3 week	4 week	5 week	Total period
T1	51.3 ± 1.0 b	75.2 ±0.9 b	93.5 ± 1.7 b	113.7 ± 0.4 b	136.7 ± 1.5 b	470.4 ± 0.7 b
T2	55.7 ± 1.0 a	67.7 ± 2.9 ab	95.2 ± 1.4 ab	110.9 ± 0.7 ab	153.6 ± 10.6ab	483.1 ±10.1 ab
T3	58.0 ± 1.2 a	78.1 ± 2.6 a	89.2 ± 1.0 a	107.8 ± 1.4 a	171.2 ± 8.6 a	504.3 ± 8.5 a
Feed conversation ratio						
Treatments	1 week	2 week	3 week	4 week	5 week	Total period
T1	2.29 ± 0.03	2.52 ± 0.03 a	2.92 ± 0.03 a	3.41 ± 0.02 a	4.04 ± 0.04	3.10 ± 0.02 a
T2	2.2 ± 0.01	2.3 ± 0.02 b	2.8 ± 0.02 ab	3.3 ± 0.05 ab	3.9± 0.07	3.05± .01 ab
T3	2.2 ± 0.02	2.4 ± 0.03 ab	2.7 ± 0.02 b	3.2 ± 0.02 b	3.8 ± 0.04	3.01 ± 0.01 b

Different superscripts (a, b) on the same column indicate statistically significant differences between the treatment groups at $p \leq 0.05$.
T1 -Control group (injected with distilled water); T2 and T3 -Test groups injected with 5% and 10% L-carnitine, respectively.

Physiological parameters

The total serum protein, GPT, and MDA levels of the chicks after 5 weeks showed no variation across the treatment groups as shown in Table 2 However, there were significant differences between the GOT and GSH level among the study groups ($p \leq 0.05$). Third treatment was recorded decrease in GOT enzyme activity with height level of GSH compared to control group.

Table 2: The effect early feeding of *in ovo* injection L-carnitine on some physiological traits of the quails (mean ±SE)

Traits / Treatments	Total protein g/100ml	GOT u/l	GPT u/l	MDA	GSH
T1	3.8 ± 0.20a	115.7 ± 4.3 a	41.4 ± 1.5a	199.7 ±12.3 a	3.3 ± 0.26 b
T2	4.3 ± 0.26a	116.5 ± 3.1 a	38.3 ± 3.4a	189.1 ± 5.8 a	3.9 ± 0.2 ab
T3	4.5 ± 0.17a	103.4 ± 2.3 b	33.5 ± 2.0a	172.9 ± 2.5 a	4.2 ± 0.22 a

Different superscripts (a, b) on the same column indicate statistically significant differences between the treatment groups at $p \leq 0.05$.
T1 -Control group (injected with distilled water); T2 and T3 -Test groups injected with 5% and 10% L-carnitine, respectively.

As shown in Table 3, the differences among the treatment groups in terms of dressing percentage and organ relative weights were not significant.

Table 3: The effect of L-carnitine injection on the dressing percentage and organ relative weights (means±SE)

Traits / Treatments	Dressing %	Liver %	Heart %	gastrointestinal tract%
T1	68.5 ±1.0	2.71 ±0.1	0.8 ±0.08	6.3± 0.5
T2	68.5 ± 1.8	2.5 ±0.4	0.8 ±0.03	5.5 ±0.7
T3	69.0 ±1.1	2.9 ± 0.2	0.8± 0.06	5.5± 0. 2

Different superscripts (a, b) on the same column indicate statistically significant differences between the treatment groups at $p \leq 0.05$.
T1 -Control group (injected with distilled water); T2 and T3 -Test groups injected with 5% and 10% L-carnitine, respectively.

Discussion

Hatching

The positive effects of dietary L-carnitine supplementation on egg hatchability can manifest reduced late embryonic dead rate at late incubation periods. At the last 3 days of the hatching period, the quail embryos are prone to lipid peroxidation as the embryonic tissues contain a comparatively higher level of polyunsaturated fatty acids (PUFA) (Keralapurath *et al.*, 2010; Oso *et al.*, 2014). Similarly, the level of natural antioxidants has not attained a level to sustain innate protection. Moreover, "internal piping" occurs at this critical stage, and oxygen availability increases as beginning of pulmonary respiration. The low level of

antioxidants, combined with high temperatures, humidity, and PUFAs increase the embryo's exposure to lipid peroxidation (Yigit *et al.*, 2014). In the eggs, the yolks' lipid content provides essential energy to the growing embryos, and a significant amount of the total energy required by the developing embryo, which is sourced from the oxidation of yolk lipids PUFA. Chick embryos require a high level of L-carnitine, and the level of L-carnitine in the yolk is low. Hence, the injection of L-carnitine into the eggs could enhance lipid circulation from the fat storage sites such as the yolk sac, and it may increase the breakdown of fatty acids. An increase in the level of fatty acid oxidation can also reduce the embryonic dependency on gluconeogenesis, so that may preserve the muscle tissue protein in the chicks after hatching. This protein of tissue muscle could result to increase muscle yield during the growing phase. Since that the skeletal muscles are a major fatty acid oxidation site, there could be higher effects of exogenous L-carnitine on lipid utilization in various muscles (Silva *et al.,* 2018). In this study, the *in ovo* injection of L-carnitine was found to significantly increase the body weight of the chicks. Additionally, the L-carnitine acted as an antioxidant to protect the main organs in the embryo especially in brain where the level of vitamin E in the brain was approximately 100-fold lower than that in the liver (Klasing, 1998). This apparent reduction in the brain's antioxidant level is further compound by the relatively low levels of vitamin A and carotenoids (Surai *et al*, 2016). Okura *et al.* (2014) reported a reduction in the blood-brain barrier membrane activity with L-carnitine, and this may account for the reduced hatchability rate in the control group.

Growth performance

As shown in the present study, there are positive effects of *in ovo* injection with L-carnitine on the body weight, final body weight, and body weight gain of the quail chick. This is in agreement with the report of Colucci *et al.* (2005) they found a significant correlation between L-carnitine level and an increasing level of osteocalcin, which is responsible for bone calcification. This may lead to the building of a strong skeletal system. Gonz´alez-Cer´on *et al.* (2015) found a positive correlation between live body weight and the development of the skeletal system.

Physiological parameters

Acting of L-carnitine seems as an antioxidant, and that helps to scavenging of Reactive Oxygen Species (ROS) through the removal excessive levels of acetyl-CoA within the cells. Acetyl CoA is a molecule that induces the production of mitochondrial ROS (Agarwal& Said, 2004; Vicari *et al.*, 2001). L-carnitine scavenges the free radicals, which are responsible for lipid peroxidation, and it possibly increases the level of GSH, as witnessed in this study (Rani & Panneerselvam, 2002). Additionally, L-carnitine facilitates transfer a long chain of fatty acids across the mitochondrial membranes for β-oxidation. When level of metabolism is an increased, the body's demand for energy is increased too. Meanwhile, the presence of L-carnitine could limit the β -oxidation of fatty acids, and there would be a need for exogenous L-carnitine supplementation, which could also facilitate the hatching process (Buyse *et al.*, 2001).

Conclusion

The outcome of this study showed that in *ovo* injection of L-carnitine into quail eggs on the 14th day of incubation could influence the hatchability rate, hatching weight, post-hatching performances, and parameters of the liver functions. Therefore, L-carnitine can be used in poultry farms to decrease embryonic death rate, increase hatching weight, and achieve optimal post-hatch performance as it serves as an antioxidant.

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Author Contributions.

Tariq Kh ALjomaily designed and arranged the structure of the article and performed the experimental work, Ahmed Taha the data preview the previous work in this field and wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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