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Effect of *In ovo* Injection of L-Carnitine at Different Incubational Ages on Egg Hatchability in Broiler Breeders and Post-Hatch Performance

M.H. Rabie, F.S.A. Ismail and A.A.S. Ahmed
Department of Poultry Production, Faculty of Agriculture, Mansoura University, Egypt

Corresponding Author: M.H. Rabie, Department of Poultry Production, Faculty of Agriculture, Mansoura University, Egypt

ABSTRACT

An experiment was conducted to investigate the effect of *in ovo* L-carnitine injection at three incubational ages on hatchability of broiler breeders and post-hatch performance. Four doses of L-carnitine (0.0, 4, 8 and 12 mg/100 μL) were injected into fertile eggs at days 14, 16 and 18 of incubation. Hatched chicks were individually weighed and fertile hatchability was calculated. Birds were kept in batteries and fed common starter and grower diets. Means of weight gain, feed intake and feed conversion were estimated. Mortality was also monitored. A slaughter test was made to evaluate the relative weights of carcass yield, front and hind parts and the edible organs. No deaths occurred during a 7-week feeding trial. Hatchability was unaffected by the injected dose of L-carnitine but it significantly improved when eggs were injected on day 14 of incubation compared with those injected on days 16 or 18. Although, total feed intake of chicks was not affected by *in ovo* L-carnitine injection, cumulative weight gain was positively affected. Birds hatched from eggs that were injected with L-carnitine at 8.0 or 12.0 mg/100 μL achieved significantly better feed conversion compared with the control group. Injection with 12.0 mg/100 μL positively affected the percentages of front parts but decreased those of liver and gizzard and had no effect on other traits examined. It is concluded that *in ovo* L-carnitine injection is beneficial for hatchability on day 14 while its injection at 8.0 mg/100 μL on day 16 of incubation is advantageous to subsequent performance.

Key words: L-carnitine, *In ovo* injection, hatchability, post-hatch performance, cumulative weight gain

INTRODUCTION

L-carnitine (β-OH-γ-N-trimethylamino-butyrate) is a water soluble quaternary amine that naturally occurs in micro-organisms, plants and animals. It functions as an essential acyl carrier in the mitochondrial beta-oxidation of long-chain fatty acids for energy production (Bremer, 1983). It also facilitates the removal from mitochondria of short-chain and medium-chain fatty acids that accumulate as a result of normal and abnormal metabolism (Rebouche, 1992). L-carnitine is biosynthesized from the essential amino acids lysine and methionine in the presence of ferrous ions (Fe^{++}) and three vitamins: ascorbate, niacin and pyridoxine that are required as cofactors for the enzymes involved in the metabolic pathway of carnitine (Rebouche, 1992).

In fact, chicken embryos start to utilize yolk lipids in lieu of glucose as a prevalent source of energy after the first week of egg incubation (Rinaudo *et al*., 1991). In addition, it has been reported that about 90% of the total energy requirement of the developing chicken embryos is derived from fatty acid oxidation (Noble and Cocchi, 1990). In this regard, L-carnitine has a specific role in

facilitating the uptake of fatty acyl groups from the egg yolk into the tissues of embryonic chicks via the yolk sac membrane, which in turn increases their efficiency of energy utilization (Casillas and Newburgh, 1969). According to Rinaudo *et al*. (1991), the ratio of esterified short-chain L-carnitine to free L-carnitine reaches a maximum in tissues (liver, brain and heart) of the chicken embryos on the 18th day of incubation, thereby suggesting that the fatty acid oxidation is inevitable for energy production in embryos. Furthermore, freshly laid eggs, especially those produced by hens fed plant-originated diets, have been reported to contain low concentrations of L-carnitine (Chiodi *et al*., 1994). It was also documented that chicken embryos have a limited capacity to synthesize L-carnitine during incubation (Casillas and Newburgh, 1969) due to the lower activity of the enzyme γ-butyrobetaine hydroxylase, which is essential for L-carnitine biosynthesis (Rebouche, 1992).

As regards the effect of *in ovo* injection of L-carnitine on hatchability of eggs, some studies failed to find a beneficial effect for *in ovo* injection of L-carnitine on hatchability of eggs (Keralapurath *et al*., 2010; Nouboukpo *et al*., 2010; Dooley *et al*., 2011). However, Salmanzadeh *et al*. (2013) found that injection of L-carnitine into eggs of turkey breeder hens on day 5 of incubation caused significant reductions in hatchability of fertilized eggs. On the other hand, dietary supplementation with L-carnitine has been reported to positively affect the hatchability of eggs (Oso *et al*., 2014; Al-Daraji and Tahir, 2014) while some investigators found no effect (Salmanzadeh, 2011).

Therefore, the aim of the present study was to investigate the effect of *in ovo* injection of L-carnitine at different incubational ages on hatchability of broiler breeders and subsequent performance of their progeny.

MATERIALS AND METHODS

The first part of this study was carried out at a local commercial hatchery, Dakahlia Governorate, Egypt but the second part (feeding trial for the hatched chicks) was undertaken at the Poultry Research Unit, Center of Agricultural Researches and Experiments, Faculty of Agriculture, Mansoura University, Egypt.

Injection and incubation of eggs: In this study, four doses of L-carnitine (0.0, 40, 80 and 120 mg dissolved in 1 mL of sterilized saline) were injected in fertilized eggs at three incubational ages (14, 16 and 18 days of incubation). A total of 600 fertilized eggs (produced by 56 weeks old Cobb-500 broiler breeders), obtained from a local commercial hatchery, were labeled and randomly divided to twelve equal treatments, each with five replications. L-carnitine used in this study has a declared purity of 98%, as stated by the Global Napi Pharmaceuticals Company, Egypt.

For each incubational age, one third of these eggs (200 eggs) was subdivided into four equal groups. Egg shells of each group were carefully bored at the large end of egg and *in ovo* injected with L-carnitine within the air cell (at about 5 mm depth) by a ten-member team of experienced operators using insulin syringes, according to the following procedure: Individual eggs of the control group were injected with 100 μL of sterilized saline, the 2nd group was injected with 4 mg L-carnitine dissolved in 100 μL sterilized saline, the 3rd group was injected with 8 mg L-carnitine dissolved in 100 μL sterilized saline and the 4th group was injected with 12 mg L-carnitine dissolved in 100 μL sterilized saline. In order to avoid cross contamination among eggs of different treatments, the used insulin syringes have been changed repeatedly with new ones after performing five injections. The injection sites of eggs were disinfected with ethyl alcohol and sealed

using adhesive cellophane and transferred again to the same incubator (a single-stage incubator equipped with a system of automatic turning for eggs) to complete the embryonic development and hatching process, according to specified hatchery practices (incubator temperature and relative humidity were maintained at 37.6°C and 52% from day one to day 18 of incubation but during the last three days of the incubation period, the temperature was decreased to 36.9°C coincided with relative humidity values of 60, 75 and 80% for the 19th, 20th and 21st, respectively). All hatched chicks were used to calculate the hatchability of fertile eggs.

Birds, management and diets: At hatch, chicks of each replicate group per treatment were randomly selected, around the average body weight of treatment, kept in a separate compartment in the brooding batteries and fed a common commercial starter diet (0-21 days of age) and then, transferred to the rearing batteries and fed a common grower diet (21-49 days of age). All birds had free access to feed and water and were subjected to similar managerial and hygienic conditions. Both starter and grower diets, applied herein, were formulated to meet or exceed the nutrient requirements of broiler chicks, as recommended by the National Research Council (NRC., 1994). Ingredient composition and calculated analyses of these basal starter and grower diets are shown in Table 1.

Growth performance of chicks: Data on Live Body Weight (LBW) and Feed Intake (FI) of chicks hatched from eggs that have been injected with L-carnitine were recorded on a replicate group basis and thus, their Body Weight Gain (BWG) and Feed Conversion Ratio (FCR, g feed consumed: g weight gain) were determined. The cumulative means of LBW, FI, BWG and FCR were also calculated for the whole experimental period (0-49 days of age). Mortality of chicks was also monitored throughout the duration of the feeding trial.

Carcass traits of chicks: At the end of study (49 days of age), 3 chicks from each treatment, with approximately similar body weights, were selected to evaluate the carrying-over effects of *in ovo*

Table 1: Composition and calculated analyses of the basal starter and grower diets

Ingredients (%)	Starter diet	Grower diet
Ground yellow corn	59.00	65.50
Soybean meal (44% CP)	25.80	20.95
Corn gluten meal (60% CP)	6.90	6.40
Sunflower oil	1.00	2.50
Ground limestone	1.40	1.40
Dicalcium phosphate	2.30	2.30
Common salt (NaCl)	0.30	0.30
Vit. Min. Premix	0.30	0.30
DL-methionine	0.10	0.10
L-lysine.HCl	0.20	0.25
Total	100.00	100.00
Calculated analysis (as fed basis, NRC., 1994)		
Metabolizable energy, (kcal kg^{-1})	3016.00	3154.00
Crude protein (%)	22.56	18.05
Calcium (%)	1.10	1.09
Nonphytate phosphorus (%)	0.56	0.55
Lysine (%)	1.10	0.99
Methionine (%)	0.51	0.44
Methionine+cystine (%)	0.89	0.77

Inclusion of 0.3% premix into the diet supplies the following per kg diet, Vit. A: 1000 IU, Vit. D_3: 2000 IU, Vit. E: 10 mg, Vit. K: 1.0 mg, Vit. B_1: 5.0 mg, Vit. B_2: 5.0 mg, Vit. B_6: 1.5 mg, Vit. B_{12}: 0.01 mg, Folic acid: 0.35 mg, Biotin: 0.05 mg, Pantothenic acid: 10 mg, Niacin: 30 mg, Choline chloride: 250 mg, Fe: 30 mg, Zn: 50 mg, Cu: 4 mg, Se: 0.1 mg, CP: Crude protein

injection with L-carnitine on their carcass traits. Before slaughter, the birds were fasted for 16 h. Individual Live Body Weights (LBW) of birds were recorded just prior to slaughter and reweighed after slaughter and complete bleeding. Immediately after scalding, their feathers were plucked and evisceration was performed. Procedures of cleaning out was performed on the hot carcasses. Records on the individual weights of Carcass Yield (CY), Front Parts (FP, including breast yield plus wings and neck), Hind Parts (HP; including thigh plus drumstick yield) and edible organs (i.e., heart, liver without gall bladder and skinned empty gizzard) were maintained. Dissection of the carcasses was performed according to the standard procedures. Carcass yield and its components and the edible organs were expressed as a percentage of LBW at slaughter.

Statistical analysis: A completely randomized block design in a factorial arrangement of treatments (4×3), four *in ovo* injection doses of L-carnitine (0.0, 4, 8 and 12 mg/100 µL dissolved in sterilized saline) at three incubational ages (14, 16 and 18 days of incubation), was used in this study. Data were statistically processed by a two-way analysis of variance using Statgraphics Program (Statistical Graphics Corporation, 1991). The significant differences among means of treatments, for each criterion, were separated at $p \leq 0.05$ by LSD-multiple range test.

RESULTS AND DISCUSSION

Hatchability traits: The effects of *in ovo* injection of L-carnitine at three incubational ages on hatchling weight and hatchability of fertile eggs are presented in Table 2. It is interesting to note that hatchling weight was not affected ($p>0.05$) by either *in ovo* injection dose of L-carnitine or injection day during the incubation period (Table 2). At the same time, hatchability of fertile eggs was not affected ($p>0.05$) by injected dose of L-carnitine, regardless of injection day. However, hatchability of fertile eggs was significantly increased ($p<0.01$) when eggs were injected with L-carnitine on day 14 of incubation compared with hatching rates of eggs injected on days 16 or 18 of incubation. The effect of L-carnitine injection dose by injection day interaction was significant on hatchability of fertile eggs but was insignificant on hatchling weight.

The present results are in harmony with the findings of Nouboukpo *et al*. (2010), who reported that hatchability of fertile eggs was not affected by *in ovo* L-carnitine administration (500 and 1000 µmol dissolved in 0.9% saline) at day 18 of incubation. Similar results were also obtained by

Table 2: Effect of *in ovo* injection of L-carnitine at three incubational ages on hatchling weight and hatchability of fertile eggs

Treatments	Hatchability of eggs (%)	Hatchling weight (g)
L-carnitine dose (A)		
0.0 mg/100 µL saline (A1)	74.33	40.75
4.0 mg/100 µL saline (A2)	74.84	40.00
8.0 mg/100 µL saline (A3)	77.81	40.50
12.0 mg/100 µL saline (A4)	76.59	40.00
SEM	1.53	1.12
Significance level	NS	NS
Incubational age (B)		
14th day (B1)	81.73[a]	40.02
16th day (B2)	74.02[b]	40.38
18th day (B3)	71.93[b]	40.54
SEM	1.02	0.89
Significance level	**	NS
AB interaction		
SEM	2.65	1.24
Significance level	*	NS

[a-b]For each of the main effects, means bearing different superscripts differ significantly ($p \leq 0.05$), NS: Not significant, *Significant at $p<0.05$, **Significant at $p<0.01$, SEM: Standard error of the means

Shafey *et al*. (2010), who investigated the effects of *in ovo* administration of L-carnitine (at levels of 25 up to 500 μg/egg) on hatchability traits of broiler chickens. They found that *in ovo* carnitine administration did not influence hatchability traits but increased the absolute and relative weights of hatchling. In addition, Keralapurath *et al*. (2010) observed no significant effect for the *in ovo* L-carnitine injection (up to 8 mg dissolved in 100 μL of a commercial diluent) at the 18th day of incubation on hatchability of fertilized eggs in a young broiler breeders. Moreover, Dooley *et al*. (2011) indicated that *in ovo* injection with L-carnitine (8, 16, or 32 mg/100 μL) had no effect on incubation time or hatchability of fertilized eggs of broiler breeders.

However, Salmanzadeh *et al*. (2013) found that injection of L-carnitine (from 10 to 30 mg dissolved in 0.5 mL of sterilized saline) into eggs of turkey breeder hens on day 5 of incubation caused significant reductions in hatchability of fertilized eggs. On the other side, Al-Daraji and Tahir (2014) reported that dietary supplementation with L-carnitine (50, 100 and 150 mg kg^{-1} diet) caused significant increases in egg fertility and hatchability and significantly decreased the embryonic mortality during incubation. Also, Oso *et al*. (2014) found that dietary L-carnitine supplementation for turkey breeder hens produced the highest egg fertility and significantly reduced the late embryonic mortality. Such positive effects of dietary L-carnitine on hatchability of eggs might have been related to the increased egg fertility, the decreased late embryonic mortality and/or the prolonged incubation period. These inconsistent results, appeared in the literature, concerning the responsiveness to *in ovo* injection of L-carnitine might have been resulted from many factors such as differences in strain and age of breeder hens, injection technique, site of *in ovo* injection, timing of injection (incubational age), dose and purity of L-carnitine used, diluent type in which L-carnitine is dissolved, incubator conditions, diet composition and metabolic fate of L-carnitine.

Live body weight and weight gain of chicks: Data on live body weight and weight gain of Cobb-500 broilers fed common starter and grower diets, in response to *in ovo* injection with L-carnitine at three incubational ages, are presented in Table 3 and 4, respectively.

It was surprising to note that no mortality occurred during the course of the feeding trial (0-7 weeks of age). *In ovo* injection with L-carnitine, particularly when the dose of injection was 8.0

Table 3: Live body weights of broilers fed common starter and grower diets in response to *in ovo* injection with L-carnitine at three incubational ages

Treatments	Day old	1 week old	2 weeks old	3 weeks old	4 weeks old	5 weeks old	6 weeks old	7 weeks old
L-carnitine dose (A)								
0.0 mg/100 μL saline (A1)	40.75	155	384^{b}	708^{c}	1085^{c}	1377^{c}	1756^{c}	2088^{c}
4.0 mg/100 μL saline (A2)	40.00	157	389^{ab}	698^{c}	1093^{c}	1412^{c}	1772^{c}	2120^{c}
8.0 mg/100 μL saline (A3)	40.50	158	394^{ab}	736^{b}	1140^{b}	1492^{b}	1916^{b}	2249^{b}
12.0 mg/100 μL saline (A4)	40.00	159	403^{a}	755^{a}	1174^{a}	1574^{a}	2060^{a}	2410^{a}
SEM	1.12	4.02	5.08	7.05	14.0	14.0	16.7	24.7
Significance level	NS	NS	*	*	*	*	*	*
Incubational age (B)								
14th day (B1)	40.02	158	392	722.5	1127^{ab}	1470^{a}	1865^{b}	2195^{b}
16th day (B2)	40.38	157	392	730.9	1138^{a}	1497^{a}	1927^{a}	2295^{a}
18th day (B3)	40.54	157	393	718.4	1104^{b}	1425^{b}	1836^{b}	2161^{b}
SEM	0.89	3.25	4.58	6.58	11.1	10.9	13.3	21.6
Significance level	NS	NS	NS	NS	*	*	*	*
AB interaction								
SEM	1.24	6.33	11.3	14.2	22.0	29.0	39.2	34.4
Significance level	NS	NS	*	*	*	*	*	*

$^{a-c}$For each of the main effects, means bearing different superscripts differ significantly ($p \leq 0.05$), NS: Not significant, *Significant at $p<0.05$, SEM: Standard error of the means

or 12 mg 100 µL saline, resulted in significant improvements ($p<0.05$) in LBW of broilers during the period from the 2nd to the 7th weeks of life as compared to their controls injected with saline only, regardless of day of injection (Table 3). Similarly, BWG of birds significantly improved for the 2nd, 3rd, 4th, 5th and 6th weeks of age and for the whole feeding trial due to *in ovo* injection with L-carnitine whatever was the day of injection (Table 4). Apart from the injected dose of L-carnitine, birds produced from eggs that had been injected on day 16 of incubation exhibited higher final LBW and cumulative BWG (0-7 weeks old) compared with those injected on days 14 or 18 of incubation but injection day had no effect on LBW or BWG of broilers at the 1st, 2nd or the 3rd week of life (Table 3 and 4).

Birds hatched from eggs that had been injected with L-carnitine on day 16 of incubation achieved significantly higher ($p<0.05$) BWG for the 5th, 6th and 7th weeks of life and for the entire growth phase (0-7 weeks of age) compared with those injected on days 14 or 18 of incubation (Table 4). The interactions between the injected dose of L-carnitine and injection day were significant on LBW of broilers at the 2nd, 3rd, 4th, 5th, 6th and 7th weeks of life but had no effect on LBW at the first week of life (Table 3). Injected dose of L-carnitine by injection day interactions were significant on BWG of broilers for the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th weeks of age and for the whole growth phase (Table 4).

Feed intake and feed conversion of chicks: Data on feed intake and feed conversion ratio of Cobb-500 broilers fed common starter and grower diets, in response to *in ovo* injection with L-carnitine at three incubational ages, are given in Table 5 and 6, respectively. As presented in Table 5, FI of broilers for the entire growth phase was not affected ($p>0.05$) by the injected dose of L-carnitine, injection day or their interaction. Apart from the effect of injection day, birds hatched from eggs that had been injected with L-carnitine at 8.0 or 12.0 mg/100 µL saline exhibited significantly better ($p<0.05$) FCR as compared to the control group or the group produced from eggs injected with 4.0 mg/100 µL saline (Table 6). This improvement in FCR of broilers in response to *in ovo* injection with L-carnitine may partly explained by the improved final LBW and cumulative BWG, since feed intake of birds was not affected. Day of L-carnitine injection into eggs did not

Table 4: Body weight gain of broilers fed common starter and grower diets in response to *in ovo* injection with L-carnitine at three incubational ages

	Weeks							
Treatments	Ist	2nd	3rd	4th	5th	6th	7th	0-7
L-carnitine dose (A)								
0.0 mg/100 µL saline (A1)	114	230^{b}	323^{b}	378^{c}	291^{d}	380^{c}	332	2048^{d}
4.0 mg/100 µL saline (A2)	117	232^{b}	309^{c}	395^{bc}	320^{c}	360^{c}	348	2080^{c}
8.0 mg/100 µL saline (A3)	117	236^{b}	345^{a}	404^{ab}	352^{b}	424^{b}	333	2212^{b}
12.0 mg/100 µL saline (A4)	119	244^{a}	352^{a}	420^{a}	400^{a}	485^{a}	350	2370^{a}
SEM	3.84	8.86	9.15	10.1	8.33	8.54	9.43	19.8
Significance level	NS	*	*	*	*	*	NS	*
Incubational age (B)								
14th day (B1)	118	234	330^{ab}	404^{a}	343^{b}	396^{bc}	329^{b}	2155^{b}
16th day (B2)	117	235	339^{a}	408^{a}	358^{a}	430^{a}	368^{a}	2254^{a}
18th day (B3)	116	236	326^{b}	385^{b}	321^{c}	411^{ab}	325^{b}	2120^{b}
SEM	3.33	7.32	8.09	10.9	15.0	6.15	11.3	18.8
Significance level	NS	NS	*	*	*	*	*	*
AB interaction								
SEM	6.66	7.53	9.86	5.54	12.0	18.8	16.6	26.3
Significance level	0	*	*	*	*	*	*	*

$^{a-d}$For each of the main effects, means bearing different superscripts differ significantly ($p\leq0.05$). NS: Not significant, *Significant at $p<0.05$, SEM: Standard error of the means.

Table 5: Feed intake of broilers fed common starter and grower diets in response to *in ovo* injection with L-carnitine at three incubational ages

	Weeks							
Treatments	Ist	2nd	3rd	4th	5th	6th	7th	0-7
L-carnitine dose (A)								
0.0 mg/100 µL saline (A1)	147	361	480	624	539	702	666	3517
4.0 mg/100 µL saline (A2)	151	365	480	638	575	665	692	3536
8.0 mg/100 µL saline (A3)	149	361	495	640	613	774	650	3683
12.0 mg/100 µL saline (A4)	150	351	510	640	664	867	675	3857
SEM	2.02	9.56	10.1	12.4	14.7	14.9	24.8	35.0
Significance level	NS	NS	NS	NS	NS	NS	NS	NS
Incubational age (B)								
14th day (B1)	147	358	490	647	608	726	650	3626
16th day (B2)	152	360	508	646	628	785	723	3802
18th day (B3)	150	360	510	605	557	745	639	3566
SEM	1.75	7.35	9.81	7.47	13.6	19.2	12.2	38.6
Significance level	NS	NS	NS	NS	NS	NS	NS	NS
AB interaction								
SEM	3.49	2.69	6.81	7.77	10.6	13.4	22.2	40.1
Significance level	NS	NS	NS	NS	NS	NS	NS	NS

NS: Not significant, SEM: Standard error of the means

Table 6: Feed conversion ratio of broilers fed common starter and grower diets in response to *in ovo* L-carnitine injection at three incubational ages

	Weeks							
Treatments	Ist	2nd	3rd	4th	5th	6th	7th	0-7
L-carnitine dose (A)								
0.0 mg/100 µL saline (A1)	1.29	1.57	1.49	1.65	1.85	1.85	2.00	1.71[a]
4.0 mg/100 µL saline (A2)	1.28	1.58	1.58	1.62	1.80	1.84	1.99	1.70[a]
8.0 mg/100 µL saline (A3)	1.27	1.53	1.45	1.56	1.73	1.82	1.95	1.67[b]
12.0 mg/100 µL saline (A4)	1.26	1.44	1.43	1.53	1.65	1.78	1.92	1.62[c]
SEM	0.18	0.08	0.07	0.07	0.04	0.04	0.04	0.04
Significance level	NS	NS	NS	NS	NS	NS	NS	*
Incubational age (B)								
14th day (B1)	1.25	1.53	1.48	1.60	1.78	1.83	1.98	1.68
16th day (B2)	1.29	1.53	1.50	1.59	1.76	1.82	1.96	1.68
18th day (B3)	1.29	1.53	1.56	1.57	1.74	1.81	1.96	1.68
SEM	0.02	0.07	0.06	0.13	0.18	0.07	0.09	0.11
Significance level	NS	NS	NS	NS	NS	NS	NS	NS
AB interaction:								
SEM	0.03	0.02	0.08	0.14	0.06	0.04	0.06	0.02
Significance level	NS	NS	NS	NS	NS	NS	NS	NS

[a-c]For each of the main effects, means bearing different superscripts differ significantly ($p \leq 0.05$). NS: Not significant, *Significant at $p<0.05$, SEM: Standard error of the means

affect FCR of broilers during the whole fattening period, regardless of the injected dose of L-carnitine. The interactions between the injected dose of L-carnitine and injection day were not significant on FCR of broilers for the entire growth phase.

The observed positive effect of *in ovo* injection with L-carnitine on final LBW and BWG of broilers, reported herein, harmonizes with the findings of Salmanzadeh *et al*. (2013), who found that body weight gain of turkey poults hatched from L-carnitine injected eggs was significantly higher than those of the control group. In line also with the present results, Oladele *et al*. (2011) investigated the growth response of broiler chickens to supplemental L-carnitine (added in diet or drinking water) and found that added L-carnitine positively affected final body weight and weight gain of birds but negatively affected feed intake. The lack of a beneficial effect of *in ovo* injection with L-carnitine on feed intake and feed conversion of chicks, observed in this study, is in

agreement with the results obtained by Keralapurath *et al*. (2010), who observed no significant effect of *in ovo* injection with L-carnitine on feed intake, feed conversion ratio or mortality of Ross×Ross 308 broilers.

The present results harmonize also with the findings of Kheiri *et al*. (2011) and Wang *et al*. (2013), who observed no positive effects of dietary L-carnitine supplementation on growth performance of broiler chickens. However, Zhang *et al*. (2010) investigated the effects of dietary supplementation with acetyl-L-carnitine (at levels of 300, 600 and 900 mg kg^{-1}) on growth performance of broilers and found that added carnitine linearly reduced average feed intake and average daily weight gain of broiler chicks compared with their control counterparts. But we must bear in mind the fact that comparing studies dealing with the effect of dietary supplementation with L-carnitine on broiler growth performance with others that deal with the effect of *in ovo* L-carnitine injection on posthatch performance are invalid mainly because the large differences between the chicken embryos and the growing or adult chickens in lipid metabolism and in L-carnitine concentrations in different tissues or organs.

Carcass traits of chicks: Data on carcass traits of Cobb-500 broilers fed common starter and grower diets, in response to *in ovo* injection with L-carnitine at three incubational ages, are given in Table 7.

These results indicate that *in ovo* injection with the highest dose of L-carnitine resulted in a significant increase ($p<0.05$) in the relative weight of carcass front parts (including breast yield, wings and neck) and significantly decreased ($p<0.05$) the percentages of liver and gizzard but other carcass traits examined were not affected, irrespective of the injection day. Day of L-carnitine injection into eggs had no effect on carcass traits of broilers, except relative gizzard weight which was significantly higher in chicks hatched from eggs that were injected with L-carnitine on day 16 of incubation compared with those injected on the days 14 or 18 of incubation, regardless of the injected dose of L-carnitine. Injection dose with L-carnitine by injection day interactions were significant only on the relative weights of the front parts of carcass, liver and gizzard.

Table 7: Carcass traits of broilers fed common starter and grower diets in response to *in ovo* injection with L-carnitine at three incubational ages

Treatments	LBW (g)	CY (%)	FP (%)	HP (%)	Liver (%)	Heart (%)	Gizzard (%)
L-carnitine dose (A)							
0.0 mg/100 µL saline (A1)	2131	70.22	39.79^{b}	30.03	2.16^{a}	0.45	1.79^{a}
4.0 mg/100 µL saline (A2)	2158	69.28	39.20^{ab}	29.34	1.93^{ab}	0.42	1.69^{ab}
8.0 mg/100 µL saline (A3)	2177	69.68	41.11^{ab}	29.15	1.99^{ab}	0.45	1.65^{ab}
12.0 mg/100 µL saline (A4)	2148	70.90	41.59^{a}	30.24	1.86^{b}	0.46	1.53^{b}
SEM	23.8	0.65	0.63	0.61	0.09	0.02	0.08
Significance level	NS	NS	*	NS	*	NS	*
Incubational age (B)							
14th day (B1)	2130	69.75	40.05	29.53	1.98	0.46	1.62^{b}
16th day (B2)	2170	69.78	40.17	29.49	1.98	0.44	1.84^{a}
18th day (B3)	2160	70.53	41.03	30.04	2.01	0.44	1.54^{b}
SEM	24.5	0.67	0.41	0.54	0.06	0.01	0.07
Significance level	NS	NS	NS	NS	NS	NS	*
AB interaction:							
SEM	41.2	0.55	0.45	0.53	0.08	0.02	0.08
Significance level	NS	NS	*	NS	*	NS	*

LBW: Live body weight at slaughter, CY: Carcass yield, FP: Front parts of carcass, HP: Hind parts of carcass, $^{a-b}$For each of the main effects, means bearing different superscripts differ significantly ($p \leq 0.05$). NS: Not significant, *Significant at $p<0.05$ SEM: Standard error of the means

The beneficial effect of *in ovo* injection with L-carnitine on the front parts of the carcass, observed in this study, harmonizes with the findings of Salmanzadeh *et al*. (2013), who found that *in ovo* injection with L-carnitine caused a significant increase in relative weight of breast of turkey poults. In partial agreement with the present results, Keralapurath *et al*. (2010) observed no significant effect for the *in ovo* injection of L-carnitine on all slaughter yield parameters investigated of 47 days old turkey poults. In addition, Parsaeimehr *et al*. (2014) evaluated the effects of adding L-carnitine to animal fat-containing diets on carcass characteristics of broiler chicks and reported that birds fed the L-carnitine-supplemented diets, particularly those containing 4 or 5% animal fat, displayed significantly higher percentages of thigh meat and breast muscle and lower percentage of abdominal fat compared with the control group. However, Zhang *et al*. (2010) found that increasing supplemental dietary carnitine caused a significant reduction in percentage of abdominal fat but addition of carnitine had no effect on the percentages of carcass yield, breast muscle or thigh muscle.

CONCLUSION

Based on the obtained results, it is concluded that *in ovo* L-carnitine injection is beneficial for hatchability when it is applied on day 14 of incubation. In addition, L-carnitine injection within the fertile eggs at a level of 8.0 mg/100 μL on day 16 of incubation is advantageous to subsequent performance.

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