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ORIGINAL ARTICLE

The Effects of *in ovo* Injection of L-carnitine on Hatching traits, growth Performance, and carcass Characteristics of turkey Poults

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ABSTRACT
The current research was designed to determine the effects of in ovo injection of L-carnitine on hatching traits, growth performance, and carcass characteristics of turkey poults. On the 5th of incubation, 840 fertile eggs, on based completely randomized design were allotted to seven treatments with four replicates per treatment and 30 eggs per replicate. Fertilized eggs were injected into the yolk sac with L-carnitine (10, 15, 20, 25, or 30 mg dissolved in 0.5 ml of sterilized saline) at 5 days of incubation. Two control egg groups (not injected and injected with 0.5 ml of sterilized saline) were also included. Hatchability, performance, and carcass characteristics (carcass weight and relative weights of breast, and liver) on day 21 and 42 post-hatching were assigned. Hatchability was slightly but significantly depressed in all injected eggs compared to the not injected ones, but significantly increased in eggs injected with L-carnitine compared to the sham egg controls. Moreover, weight of newly-hatched poults, and weight gains were significantly increased in turkey poults from L-carnitine injected eggs compared to the control groups. In addition, carcass weights and relative weight of breast were also significantly increased in turkey poults treated in ovo injection with L-carnitine. In conclusion, the in ovo injection of L-carnitine may improve weights at hatching, which can increase growth performance of turkey poults on days 21 and 42 post-hatching.
***Key-words**: L-carnitine, in ovo injection, hatchability, performance, carcass, turkey poults*

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INTRODUCTION

L-carnitine transports long chain fatty acids across mitochondrial membrances for β-oxidation of fatty acids [1]. β-oxidation is essential as a source of energy and as a base for developmental processes in the bird embryo [2, 3, 4]. In other hand, the chick embryo may have limited capability to synthesize L-carnitine during incubation [5]. The γ-butyrobetaine, an intermediate substance required for L-carnitine biosynthesis, is limited in embryos and young birds due to the low activity of γ-butyrobetaine hydroxylase [6, 7]. In such situations, exogenous supplementation of L-carnitine could prove advantageous [1] and could in turn be used by the chick during hatching.

Avian embryonic tissues contain high levels of polyunsaturated fatty acids, a necessary component of the phospholipid content in cell membranes [8, 9]. Polyunsaturated fatty acids are sensitive to lipid peroxidation caused by free radicals, which are produced by mitochondria because of the high metabolic rate of rapidly developing embryos [10]. In addition, L-carnitine may work as an antioxidant to scavenge free radicals [11, 12, 13]. We hypothesized that *in ovo* injection of L-carnitine into turkey breeder eggs may provide energy for embryo activity and consequently increase growth performance and carcass weight of turkeys. Together with supplying energy, the addition of L-carnitine in the fertile egg may decrease embryonic mortality by reducing oxidative stress during the hatch process, therefore improving hatch rate. For this purpose, the aim of present study was to test the effects of *in ovo* injection of L-carnitine on hatchability, growth performance and carcass characteristics of turkey poults.

MATERIALS AND METHODS

Incubation and in ovo injection

In this experiment, 840 fertile eggs obtained at 4 days of incubation from a commercial hatchery and incubated according to standard hatchery practices (37.7 °C and %67 RH). Fertile eggs, on based completely randomized design were allotted to seven treatment groups with four replicates per treatment groups and 30 eggs per replicate. At 5 days of incubation, each egg was candled to identify the location of the injection. A hole was incised using a gauge needle, and 0.5 ml of *in ovo* injection solution was injected into the yolk sac. The injection site was disinfected with ethyl alcohol, sealed with cellophane tape and transferred to hatching. The *in ovo* injection solutions were the following: 1) Non-injection (control group), 2) 0.5 ml of sterilized saline (sham group), 3) L-carnitine 10 mg dissolved in 0.5 ml of sterilized saline, 4) L-carnitine 15 mg dissolved in 0.5 ml of sterilized saline, 5) L-carnitine 20 mg dissolved in 0.5 ml of sterilized saline, 6) L-carnitine 25 mg dissolved in 0.5 ml of sterilized saline, 7) L-carnitine 30 mg dissolved in 0.5 ml of sterilized saline. Non injection group was subjected to the same handling procedures as the IOF treatment groups. Injected solutions containing L-carnitine were prepared by directly dissolving L-carnitine hydrochloride (~98% purity; Sigma Chemical Co) in the deionized water. All of the treatment solutions were prepared in autoclaved water.

Birds

In each cage, total poult body weight, poult numbers and the weight of unconsumed and added feed were recorded on days 0, 21 and 42. Mean body weight gains, feed consumption and feed conversion ratios were calculated for each cage (replicate) between 0 and 21 and 22 and 42 days. For each time period, body weight gain was calculated and expressed as grams per bird. Food intake (g of food intake/bird) over the entire grow-out period was calculated by totalling food consumption in each time interval between each bird sampling. Food conversion ratio (g of food intake /g of body weight gain) was calculated by dividing total food intake by total weight gain in each cage.

Carcass characteristics

On day 42, ten poults per experimental groups were randomly selected for organ weights and intestinal measurements. Poults were weighted and slaughtered by cervical dislocation then the abdominal cavity was opened. The weight of carcass, breast and liver, were recorded and the corresponding percentages (% of live body weight) were calculated.

Statistical analysis

All the data were subjected to ANOVA procedures for completely randomized designs using the general linear model (GLM) procedure of the SAS program (SAS Institute, 2003) [14]. When data were percentages they were transformed by arc sin square root. Differences between treatments were compared by the Duncan's multiple range tests following ANOVA, and values were considered statistically different at $p < 0.05$.

RESULTS AND DISCUSSION

Hatchability was slightly but significantly depressed in all injected eggs compared to the not injected ones, but significantly increased in eggs treated with L-carnitine compared to the sham egg controls. The weight of newly-hatched poults was significantly higher when L-carnitine was received than sham and control group (Table 1). Based on the results of present study, the in ovo injection of L-carnitine in the yolk sac can be seen as an effective tool to increase the weight of newly hatched poults. It has previously been shown that in ovo injection of L-carnitine on day 6 of incubation improved embryonic development and subsequently increased total poults body weight at hatch [15]. Also, the results of the other study indicate that, dietary L-carnitine supplementation didn't show any significant influence on hatchability of eggs from broiler breeder hens from 32 to 36 weeks of age. But, weight of newly-hatched chickens was significantly higher when supplemental L-carnitine in broiler breeder hen diets from 32 to 36 weeks of age were received 400 and 500 mg than control group. In other hand, Zhai et al. [16] demonstrate that in ovo injection of L-carnitine in a 0.05 to 10 μmol/egg dose range into fertile Single Comb White Leghorn eggs at the 17th-18th days of incubation did not affect hatchability and body weight gains. Moreover, Keralapurath et al. [17] demonstrate that in ovo injection of L-carnitine (0.5, 2.0, or 8.0 mg dissolved in 100 μL of a commercial diluent) into broiler breeder eggs on day 18 of incubation had no significant effect on the hatchability.

As shown in Table 2, there were no significant treatment effects on feed intake throughout the experimental period. But, poults from in ovo injection of L-carnitine showed improved body weight gain and feed conversion ratio compared to Poults hatched from sham and control group between 0 and 21, 22 and 42 post hatch. These results were in agreement with report of Salmanzadeh *et al*. [15] that have stated weight gains and food efficiency were significantly increased in Puolts from L-carnitine treated eggs compared to the control poults. By contrast, Keralapurath *et al.* [17] showed no significant effects of L-carnitine (0.5, 2.0, or 8.0 mg) injection in the amnion of fertilized eggs at the 18th day of incubation on weight gains, food intake and food conversion ratio determined on days 3, 10, 28 or 46 post hatching.

Poults from groups in ovo injection of L-carnitine showed carcass yield and breast were significantly increased compared to the control (not injected and sham) groups whereas the liver was not affected (Table 3).

In eggs, yolk lipids provide essential energy to growing embryos. In fact, approximately 90% of the total energy requirement of the developing embryo is derived from fatty acid oxidation of yolk lipids [8]. As chick embryos show a high requirement for L-carnitine whereas the L-carnitine content is low in yolk, the injection of L-carnitine into the yolk sac would promote lipid circulation from fat storage areas such as the yolk sac, leading to an increase in fatty acid catabolism. An increased efficiency in fatty acid oxidation may, likewise, reduce the dependency of the embryo upon gluconeogenesis, thereby sparing muscle tissue protein in the post-hatched chick. This could subsequently lead to an increase in muscle yield during grow-out. Because skeletal muscle is a major site for fatty acid oxidation [18], the effects of exogenous L-carnitine on lipid utilization may become most evident in various muscle groups. In the present study, the *in ovo* injection of Lcarnitine has significantly increased the breast muscle size. This result generally agrees with our hypothesis that exogenous nutrition provision can substitute for amino acids from the pectoral for glucose gluconeogenesis; i.e. exogenous nutrient supply increases protein deposition, probably by attenuating muscle wasting. Yalcin *et al*. [19] demonstrate that, the carcass mean weight and the carcass mean yield obtained from male quails at the end of the experiment tended to increase in groups supplemented with 150 and 200 mg/kg diet L-carnitine. Also, Salmanzadeh *et al*., 200 showed that carcass weights and relative weight of breast was also markedly increased in Poults treated *in ovo* with L-carnitine whereas liver was not significantly altered throughout the whole experimental period. These data suggest that the in ovo injection of L-carnitine may improve growth performance in turkey poults.

Table 1: Effect of *in ovo* injection of L-carnitine on poult weight and percent hatchability in newly-hatched poults.

Item	**Hatchability (%)**	**Hatch Weight (g)**
Control	86.20[a]	52.85[b]
Sham group	80.14[c]	52.72[b]
L-carnitine 10 mg	82.27[b]	53.27[a]
L-carnitine 15 mg	83.03[b]	53.54[a]
L-carnitine 20 mg	83.06[b]	53.51[a]
L-carnitine 25 mg	82.85[b]	53.56[a]
L-carnitine 30 mg	83.18[b]	53.56[a]
SEM	0.285	0.117
P-Value	0.0001	0.0001

[a–c] Averages in a column with different superscript letters are significantly different

Table 2. Effect of in ovo injection of L-carnitine on body weight gain, feed intake and Food Conversion ratio of poults in different period

Item	**Starting period (day 1- day21)**			**Growing period (day 22- day 42)**		
	BWG	FI	FCR	BWG	FI	FCR
Control	669.05[b]	915.23	1.368[a]	1528[b]	3054	1.998[a]
Sham group	667.12[b]	917.58	1.375[a]	1517[b]	3049	2.009[a]
L-carnitine 10 mg	687.25[a]	901.15	1.311[b]	1562[a]	3034	1.949[b]
L-carnitine 15 mg	686.55[a]	897.80	1.307[b]	1574[a]	3036	1.928[b]
L-carnitine 20 mg	688.25[a]	896.53	1.302[b]	1569[a]	3031	1.932[b]
L-carnitine 25 mg	687.50[a]	896.44	1.304[b]	1574[a]	3031	1.925[b]
L-carnitine 30 mg	691.50[a]	892.68	1.291[b]	1576[a]	3030	1.922[b]
SEM	5.997	7.641	0.015	8.637	8.830	0.013
P-Value	0.038	0.180	0.002	0.0002	0.362	0.0003

[a–b] Averages in a column with different superscript letters are significantly different

Table 3: Effect of *in ovo* injection of L-carnitine on carcass, breast and liver of poults

Item	**Carcass (%)**	**Breast (%)**	**Liver (%)**
Control	64.65[b]	29.21[c]	1.950
Sham group	64.49[b]	29.38[c]	2.045
L-carnitine 10 mg	65.28[a]	29.45[b]	1.842
L-carnitine 15 mg	65.33[a]	29.53[b]	2.155
L-carnitine 20 mg	65.31[a]	29.88[a]	1.940
L-carnitine 25 mg	65.49[a]	29.85[a]	1.920
L-carnitine 30 mg	65.51[a]	29.94[a]	2.070
SEM	0.113	0.067	0.057
P-Value	0.0001	0.0001	0.056

[a–c] Averages in a column with different superscript letters are significantly different

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