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Growth Performance, Immune Response, Serum Metabolites and Digestive Enzyme Activities of Japanese Quail Fed Supplemental L-Carnitine

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Abstract: The aim of this study was to evaluate the effect of using graded levels of L-carnitine on growth performance, serum metabolites, humoral and cell mediated immune responses and intestinal digestive enzymes activity of Japanese quail chicks. Two hundred forty 7d old Japanese quail chicks were randomly divided into four groups of 60 chicks. The first group was fed on the basal diet (control), while groups 2, 3 and 4 were given the basal diet supplemented with 100, 200 and 400 ppm L-carnitine, respectively. The results showed significant improvement in body weight, feed conversion and dressing percent of quails, particularly with the high level (400ppm) followed by those of the mid one (200pm). Similar trend was noted for the relative lymphoid organ weights (bursa and spleen values). Moreover, supplemental L-carnitine with different levels enhanced the humoral and cell mediated immune responses in Japanese quail as evidenced with better antibody titers against Newcastle disease virus and greater wing web swelling in response to PHA-L injection, respectively. The serum level of total proteins, globulin, high density lipoprotein (HDL), triiodothyronine (T_3) and thyroxin (T_4) was significantly increased for quails fed on diets contained either 200 or 400ppm L-carnitine. But, the concentrations of total lipid, cholesterol, triglycerides, low density lipoprotein (LDL) and malondialdehyde (MDA), which is the primary stable by-product of lipid peroxidation, were reduced. On the other hand, the albumin level didn't significantly affected by dietary L-carnitine supplementation. A significant increase in the activities of amylase, lipase, trypsin and chymotrypsin enzymes was found throughout the small intestine portions with supplemental L-carnitine. It is concluded that, L-carnitine supplementation with 200ppm up to 400ppm was sufficient to enhance the growth performance traits, immune response, thyroid and digestive enzyme activities and antioxidant system in growing Japanese quails.

Key words: Carnitine · Quail · Growth · Serum · Immune · Digestive Enzyme

INTRODUCTION

In the later decades, poultry producers and researchers; in several areas all over the world; tended to feed poultry stocks on diets formulated based mainly on plant sources, in an attempt to reduce the inclusion percentage of feedstuffs from animal sources. Although, this purpose seemed to be valuable, especially with the concern of consumers' health, it could be negatively affect the net outcome of producers. Where, poultry diets have a high percentage of cereal grains that are poorly contain amino acids essential for better performance. Thus, the livestock researchers and producers tended to examine new feed additives that can compensate for this shortage and consequently, benefit the poultry health and production. This presents considerable opportunities for the use of a recent physiological feed additive; L-carnitine.

A potential additive, enhances domestic animal production because of its metabolic functions. Hence, plants and plant-based feedstuffs generally contain very little carnitine, compared with animals, resulting a barrier for optimum metabolic requirement [1].

L-carnitine (β-hydroxy γ-trimethylaminobutyrate) is an amino acid derivative compound exists in cells of all living organism in various amount [2]. It is synthesized *in vivo* from the amino acids lysine and methionine that act as precursors [3]. The endogenous synthesis of

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L-carnitine requires the ferrous ions and a number of vitamins; ascorbate, folate, niacin and pyridoxine [4]. The principal function of L-carnitine is the transport of long-chain fatty acids from the cytosol into mitochondria for β-oxidation; consequently it sustains the supply of energy [5]. Moreover, L-carnitine plays a role in some physiological processes in humans and animals.

The dietary incorporation of L-carnitine is found to receive much interest with the increase of metabolic rate, energy demands, as well as using plant dietary sources, particularly during the growth and laying periods, environmental stress and fat enriched diets. In these cases, insufficient endogenous L-carnitine synthesis coupled to a low dietary L-carnitine supply could become limiting for metabolic requirements. Harmeyer, 2002 [6] reported that, a deficiency of this compound results primarily in impaired energy metabolism and membrane function. Dietary supplementation of L-carnitine resulted in better protection against cold stress [7] and acute heat stress [8].

In addition, carnitine has beneficial effects on preventing some diseases, strengthening immune system, improving poultry performance and playing role in metabolic and physiological processes [9, 10]. Thus, L-carnitine act as reactive oxygen species (ROS) scavengers [11] and known to has immunomodulary properties in mammalian as well as in avian species. Mast *et al*. [12] reported that supplemental L-carnitine significantly ameliorated the primary and secondary antigen-specific IgG response to bovine serum albumin in broiler chickens. Also, Deng *et al*. [13] in laying-type chickens and Buyse *et al*. [14] in broiler chickens confirmed the stimulating properties of dietary L-carnitine on humoral immunity. However, the former authors failed to find evidence for any effects of L-carnitine on cell mediated immunity.

Several studies on broiler and quail chicks have shown that productive performance traits were improved by dietary supplementation with L-carnitine [9, 15-17]. Conversely, others failed to observe any favorable effects [13, 18, 19].

Therefore, the present study was conducted due to the scarcity of information concerning the effect of dietary L-carnitine supplementation in quails, particularly with the consideration of humoral and cell mediated immune response. As well, the activity of digestive enzyme activities and some serum metabolites and their relation to the growth performance.

MATERIALS AND METHODS

The present study was carried out at Quail Production Unit, Agricultural Experiment and Researches Unit, Faculty of Agriculture, Ain Shams University.

Birds, Diet and Experimental Design: Two hundred forty 7d old Japanese quail chicks were randomly divided into four groups of 60 chicks, with similar initial average body weight, three replicates of 20 birds each. The chicks were reared in starter batteries. The first group was fed on the basal diet (control), while groups 2, 3 and 4 were given the basal diet supplemented with 100, 200 and 400 ppm L-carnitine, respectively. All chicks were kept under the same managerial, hygienic and environmental conditions till the age of 42 day. Feed and water were provided for ad libitum consumption throughout the experimental period. The grower diet (Table 1) was formulated to meet all requirements recommended by NRC [20]. Chicks were maintained on a light cycle of 16L: 8D.

Measurements and Observations: Chicks were individually weighed at weekly intervals until the end of the experiment at the 6th week of age (WOA). Live body weight (LBW), body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR, g feed/g gain) were recorded during these periods.

All chicks were vaccinated at 21 day of age (DOA) and again at 35 DOA with Lasota strain vaccine. At 28 and 42 DOA chicks were bled via heart puncture, blood was centrifuged at 4000 rpm for 15 minutes. Serum was stored at -20°C until the detection of primary and secondary humoral immune responses. The detection was conducted by Haemagglutination inhibition (HI) test according to Hitchner et al. [21]. The total, mercaptoethanol-sensitive (MES-presumably IgM) and mercaptoethanol-resistant (MER-presumably IgG) anti–NDV were determined using a micro-hemagglutination technique [22]. The antibodies were expressed as the log2 of the reciprocal of the highest dilution giving visible agglutination. At 35 DOA, nine chicks from each treatment were injected in the right wing web with 0.1ml of phytohemagglutinin–L, (Sigma Chemical Co., St. Louis, U 063178), (each 1 ml contains 100 µg PHA-L dissolved in sterile saline). The swelling of the wings were measured using micrometer before injection and 24, 48 and 72 hrs post injection. While the left wing was injected with 0.1 ml of the sterile saline and kept as control.

Table 1: Composition and calculated analysis of basal diet

Ingredients (%)	Grower (0-6 wks)
Yellow corn	54.55
Soybean meal (44%CP)	36.15
Corn gluten meal	5.60
Plant oil	0.65
Dicalcium phosphate	0.94
Limestone	1.23
Vit & Min Premix*	0.3
NaCl	0.34
Lysine	0.136
DL-Methionine	0.104
Total	100
Calculated analysis:	
ME (Kcal/Kg diet)	2905
Crude protein %	24.01
Calcium %	0.80
Available phosphorous %	0.30
Lysine %	1.30
Methionine %	0.51
Cystine %	0.40

*Each kilogram of diet contains = A, 12000 I.U., D3, 2500 I.U., E, 10mg., B1, 2mg., B2, 5mg., B6, 4mg., B12, 10µg., Niacin, 25mg., Pantothenic acid, 10mg., Biotin, 50µg., Folic acid, 1000µg. and Coline chloride, 255mg. Selenium, 350µg., Copper, 10mg., Iodine, 1.0mg., K, 2.0mg., Iron, 33mg., Manganese, 60mg.and , 100mg. Zinc.

At the end of the experimental period (42 day) bird samples (males and females) were randomly taken from each treatment, weighed, slaughtered by severing the carotid arteries and jugular veins, then scalded and defeathered. Carcasses were manually eviscerated and weighed. Liver, gizzard, spleen, bursa and kidney were removed and their relative percentages of live body weight were estimated.

Blood samples were collected from the slaughtered birds, during their exsanguinations and centrifuged. Serum samples were harvested, decanted and stored at -20 until the biochemical analyses were done. They were assigned for the determination of total protein, albumin, total lipids, triglycerides (TG), cholesterol, LDL-cholesterol and HDL-cholesterol, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using available commercial kits. Globulin was calculated by subtraction of serum albumin from total protein and the albumin/globulin (A/G) ratio was calculated. Concentration of triiodothyronine (T3) and thyroxin (T4) were determined using commercial enzyme immunoassay test kit purchased from Taytec Incorporation (7278 Aldercrest Dr., Mississauga, ON, L5N 7N8, Canada).

The determination of serum malondialdehyde (MDA) as a marker of lipid peroxidation was based on a colorimetric method described by Uchiyama and Mihara [23]. Spectrophotometer was adjusted to read the absorbance at a wave length of 535 and 520 nm. The contents of duodenum, jejunum and ileum were collected, form the slaughtered chicks, weighed and kept in equal volumes of sterilized physiological saline. They were then individually centrifuged and the supernatant fluids were decanted and used for determination of some digestive enzymes activity (amylase, lipase, trypsin and chymotrypsin) as described by Nitsan *et al*. [24].

Data were subjected to one way analysis of variance using the General Linear Models (GLM) procedure of SAS User's guide [25]. Duncan's Multiple Range Test [26] was used to separate means when separation was relevant. Percentages of slaughter traits were divided by 100 and subjected to arc sin transformation of the square root before analysis; however actual percentage means are presented. Statistical significance was accepted at a probability level of 0.05 ($P \leq 0.05$).

RESULTS AND DISCUSSION

The effects of dietary L-carnitine supplementation at different levels on LBW, BWG and FCR of Japanese quail chicks are listed in Table 2. Results showed significant improvement in all studied productive performance data as the level of dietary L-carnitine level increased. However, the group of 200 ppm L-carnitine didn't significantly differ from those of 100ppm. These results are in agreement with those of Yalçin *et al*. [9] who showed that supplementation of Japanese quail diet with 200 ppm L-carnitine from one till 4 WOA, significantly increased body weights and cumulative body weight gains. Also, Rabie *et al.* and Rabie and Szilaggi [15-17] indicated that supplementation of L-carnitine at either 50, 100, or 150 ppm significantly increased body weight and weight gain and improved feed conversion of broiler chickens. However, Sarica *et al.* [19] found no significant differences in the growth performance of quail chicks fed diet contained either 0, 30, 40, or 50 ppm L-carnitine from 0 to 35 DOA. Also, Xu *et al.* [18] revealed that dietary supplementation of L-carnitine to commercial male broilers at 0, 25, 50, 75, or 100 ppm had no significant effect on daily body gain or feed conversion. Similarly, Deng *et al.* [13] found that short-term supplementation of L-carnitine at levels of 0 (control), 100 or 1000 ppm of egg Leghorn-type chickens after hatching for 4 weeks induced no difference in growth rates, feed intake or feed utilization efficiency among the dietary treatments throughout the study.

Table 2: Productive performance of Japanese quail chicks fed supplemental L-carnitine

	Variable					
	LBW (g)			BWG (g)		FCR
L-carnitine level (ppm)	Initial (1 wk)	4 wk	6wk	1- 4 wk	1- 6 wk	1- 6 wk
0	21.70	112.70 c	177.60 c	91.00 c	155.90 c	4.48 a
100	21.18	117.03 b	191.16 b	95.85 b	169.98 b	3.70 b
200	21.11	119.34 ab	192.72 b	98.23 ab	171.61 b	3.78 b
400	21.16	121.96 a	203.60 a	100.80 a	182.44 a	3.56 c
MSE	0.23	4.52	11.73	4.99	11.47	0.01
Probability	0.216	0.0001	0.0001	0.0001	0.0001	0.0001

a-c Means within a row with different superscripts are significantly different at ($p \leq 0.05$).

The valuable effect of dietary addition of L-carnitine, achieved herein, on the productive performance of Japanese quail could be account for the increase in the efficiency of fatty acids oxidation. Consequently, improvement the utilization of dietary nitrogen thereafter. Moreover, carnitine is well known as a crucial for the transfer of long-chain fatty acids across the inner mitochondrial membrane and controls the rates of β-oxidation of long-chain fatty acids, playing a pivotal role in energy metabolism [27]. This Indicates better utilization of dietary ingredients as a consequence, improvement of the gain and feed efficiency, especially in young individuals where L-carnitine synthesis is insufficient to meet endogenous requirements. Also, in this context, Sarica *et al.* [19] pointed out that L-carnitine has the ability to improve the use of dietary nitrogen, whether directly through sparing its precursors (methionine and lysine) for protein biosynthesis and other cellular functions or indirectly by optimizing the balance between essential and nonessential amino acids within the cell. The observed inconsistency among various studies could be related to using different levels of supplemental L-carnitine, nutritional conditions, sex, age, species and physiological status of the birds.

Dressing and organ percentages of 42 day old Japanese quail fed diets supplemented with L-carnitine at different levels during the experimental period from 7 up to 42 DOA are shown in Table 3. Data illustrated that supplemental L-carnitine significantly increased the dressing percent of quails, particularly with the high level (400ppm) followed by those of the mid one (200pm). Whereas, the relative values of liver, heart, gizzard and kidneys were numerically increased comparable to the control group. The enlargement, however insignificant, of these organs may be account for their hyperactivity. Where dietary carnitine appears to be extracted from the portal circulation into the systemic circulation by the liver, after being traversed the mucosal intestinal membrane by both passive and active transport mechanisms [28, 29]. Furthermore, Mc Dowell[30] added that carnitine is not carried in blood in any tightly bound forms, in contrast to other water-soluble vitamins. So, free carnitine is excreted by kidneys. Another possible interpretation may be due to the anabolic effect of L-carnitine on muscle building, where gizzard and heart consists mainly of muscles.

The current findings are in full agreement with those of Yalçin *et al.* [9]. They found that, weights and yields of carcasses tended to be insignificantly improved in Japanese quail fed on diet contained 200 ppm L carnitine. However, Sarica *et al.* [19] reported that supplemental L-carnitine did not significantly affect carcass yields, as well as the relative values of heart, liver and gizzard of Japanese quail at the age of 35 day old. Also, Daskiran and Teeter [31] observed no significant effect in dressing percentage of broilers in response to dietary L-carnitine.

Dietary addition of L-carnitine had significantly increased the relative lymphoid organ weight (bursa and spleen values) indicating that carnitine may have an immunomodulator action compared with control chicks. Indeed, the effect was more prominent with the mid level (200ppm). This result disagreed with those found at 12 WOA in Leghorn-type chickens by Deng *et al.* [13]. Also, in this connection, Karadeniz *et al.* [32] demonstrated in broiler chickens that L-carnitine supplementation had significantly modified the cellularity of some lymphoid organs (thymus, spleen and caecal tonsil), whereas the bursa of Fabricius was slightly affected.

As shown in Table 4, the total antibody titers and mercaptoethanol-resistant (MER, presumably IgG) anti-NDV vaccines were significantly increased whether estimated 7 days post primary or secondary immunization in response to supplementary L-carnitine at any level. Similar trend, however not significant, was observed for the mercaptoethanol-sensitive (MES, presumably IgM), indicating that L-carnitine treatment was shown to exert

Table 3: Relative percentage weights of dressing and some organs of Japanese quail chicks fed supplemental L-carnitine

	L-carnitine level (ppm)					
Variable	0	100	200	400	MSE	Prob.
Dressing	69.13 c	69.91 bc	71.04 ab	72.48a	2.070	0.005
Liver	2.38	2.49	2.55	2.52	0.042	0.446
Heart	0.83	0.86	0.85	0.86	0.011	0.545
gizzard	2.54	2.59	2.71	2.67	0.069	0.513
Kidney	0.63	0.67	0.65	0.70	0.011	0.293
Bursa	0.122 b	0.132b	0.165 a	0.156 b	0.0003	0.001
Spleen	0.089c	0.105b	0.108ab	0.121a	0.0002	0.001

a-c Means within a row with different superscripts are significantly different at ($p \leq 0.05$).

Table 4: Total antibody titers against Newcastle disease virus (NDV) and cell mediated immunity of Japanese quail chicks fed supplemental L-carnitine.

	L-carnitine level (ppm)					
	0	100	200	400	MSE	Prob.
Variable	Primary					
Total antibody	3.244 b	4.244 a	4.600 a	4.600a	0.401	0.0001
IgM	1.778	2.022	2.250	2.200	0.082	0.415
IgG	1.467 b	2.222 a	2.350 a	2.400 a	0.245	0.002
	Secondary					
Total antibody	4.322 b	5.967 a	6.200 a	6.300 a	0.531	0.0001
IgM	1.300	1.522	1.500	1.500	0.098	0.685
IgG	3.022 b	4.444 a	4.700 a	4.800 a	0.308	0.0001
	Cell Mediated Immunity (CMI)					
24h post-injection	0.884 b	1.042 a	1.067 a	1.088 a	0.005	0.0001
48h post-injection	0.819 c	0.907 b	0.958 a	0.947 a	0.001	0.0001
72h post-injection	0.750 c	0.817 b	0.889 a	0.861 a	0.001	0.0001

a-c Means within a row with different superscripts are significantly different at ($p \leq 0.05$).

an immunomodulatory effect on antigen-specific total immunoglobulins and IgG responses. This finding concurred with those of Mast *et al.*, Arslan and Yalcin *et al.* [12, 27, 33]. It is worth that, the achieved increase in the IgG titers may be of major practical consequence in the improvement of protecting immune response on vaccination.

Qureshi *et al.* [34] stated that phytoheamagglutinin is considered to be a good *in vivo* measure of T-lymphocyte function through stimulating T-cell proliferation with minimal effect on B cells. As well known, cell-mediated immunity (CMI) plays a major role in the response against intracellular bacteria and virus.

It is clear form Table 4 that, injection of PHA-L significantly increased the wing-web swelling of the L-carnitine quail groups comparable to control one. Furthermore, the swelling was more pronounced with both mid and high L-carnitine levels.

The mechanism(s) responsible for the affirmative effect of L-carnitine on antibody production is still needs further clarifications, particularly in poultry species. L-carnitine can exhibit immunomodulatory effects, through lowering the levels of cytokines, tumour necrosis factor-a in man [35] and interleukin-1b, interleukin-6 and tumour necrosis factor-a in rat models [36]. These cytokines not only have an essential role in energy homeostasis, but also in the modulation of antibody productions. Moreover, L-carnitine could prevent apoptotic cell death of B and T lymphocytes during the immune response of broiler chickens [12]. In this connection, Lohninger *et al.* [37] suggested that L-carnitine inhibits apoptosis by interacting with cytokines. Furthermore, some immunostimulator antioxidants such as ascorbic acid and vitamin E, as well as antioxidant enzymes are protected by L-carnitine [38].

In addition, L-carnitine may contribute to the white cell activation via enhancing their energetic metabolism throughout lipid oxidation with promoting secretion and release of immunomodulatory hormones such as insulin and insulin-like growth factor-I (IGF-I) and triiodothyronine (T_3). Furthermore, L-carnitine may support lymphocyte survival by inhibiting apoptosis and increasing the proliferative response to mitogenes [32].

Table 5: Serum biochemical constituents and liver functions of Japanese quail chicks fed supplemental L-carnitine

	L-carnitine level (ppm)					
Variable	0	100	200	400	MSE	Prob.
Total protein (g/dL)	5.03 b	5.25 b	5.62 a	5.63a	0.072	0.006
Albumin (A) (g/dL)	1.95	2.01	2.01	1.99	0.018	0.863
Globulin (G) (g/dL)	3.09 b	3.24 b	3.61 a	3.64a	0.077	0.013
A/G ratio	0.635	0.629	0.557	0.547	0.005	0.162
Total lipid (mg/dL)	992.45 a	922.72 ab	896.52 b	865.96 b	66.23	0.010
Cholesterol (mg/dL))	330.95 a	279.43 b	267.60 b	253.80 c	19.60	0.0001
TG (mg/dL)	230.44 a	218.21 ab	203.84 b	202.67 b	17.75	0.027
HDL (mg/dL)	32.90 b	38.60 ab	41.69 a	43.40 a	3.46	0.017
LDL (mg/dL)	174.40 a	154.03 b	156.05 b	151.00 b	13.36	0.002
AST (U/I)	110.97	104.21	100.62	94.67	4.33	0.142
ALT (U/I)	36.32	34.71	34.82	32.52	1.72	0.324

a-c Means within a row with different superscripts are significantly different at ($p \leq 0.05$).

Serum Constituents: Data presented in Table 5, indicated that dietary addition of L-carnitine significantly increased serum total proteins and globulin, especially with the mid and high dosages. Whereas, chicks whose diet was contained the low L-carnitine level had slightly higher serum total protein and globulin when compared with the unsupplemented control ones. Nevertheless, the albumin level and the albumin globulin ratio were insignificantly different among whole groups including the control one. These results may be attributed to the useful effect of L-carnitine in sparing both methionine and lysine (its precursors) for protein biosynthesis and other cellular functions moreover, optimizing the balance between essential and nonessential amino acids [19]. Besides, the suggested immunostimulator role of L-carnitine, induces the production of immunoglobulins, in particular IgG, as evidenced in the current experiment, consequently elevated the total serum globulin. Thereby, the total serum protein was increased especially with the absence of significant differences in the level of serum albumin among all experimental groups. These results are in partial agreement with those reported in local laying hens by Nofal *et al.* [39].

With respect to serum concentration of total lipid and its fractions. Results in Table 5 illustrated that, supplemental L-carnitine could effectively reduced the serum concentrations of total lipid, cholesterol, triglycerides and LDL. Contrary, the level of serum HDL was raised due to L-carnitine addition. Moreover, the effect was markedly observed with the high dosage (400ppm) followed by the mid one (200ppm). On the other hand, the activities of serum AST and ALT, as an indication of liver function, didn't significantly differ among all treatments due to dietary L-carnitine inclusion indicating that it had no deleterious effect on liver cells. Our findings coincided with those found in quail by Uysal *et al.* [40] who detected in 42 day old male quails that dietary 500 ppm L-carnitine caused no effect on ALT activity while a significant decrease in serum triglyceride concentration was observed. Similarly, the results of Xu *et al.* [18]. Also, Arslan [27] reported that L-carnitine is known as a hypolipidemic drug, able to reduce the circulating concentration of cholesterol, triglycerides, free fatty acids, phospholipids and very low density lipoproteins (VLDL). As well as increasing the concentration of both high and intermediate density lipoproteins [10]. On the contrary, several previous studies conducted on Japanese quail whether during the growing or laying period failed to demonstrate significant effects of supplemental L-carnitine, either through the water or diet, on most blood serum constituents [9, 33, 41].

Through its action in reducing the lipoprotein lipase (LPL) activity, L-carnitine could decrease the lipid fractions. Thus, catalyses the conversion of triglycerides to glycerol and fatty acids [18]. Moreover with the reduction of LPL activity, L-carnitine increases the hydrolysis of LDL, which plays a major role in regulating the deposition of fat in bird body and thus, minimizes fat deposition in the subcutaneous tissues. Additionally, Arslan *et al.* [41] revealed that the reduction in serum total lipid and triglyceride is resulted from an acceleration of long chain fatty acid oxidation within the mitochondrial matrix. Thus, spare serum glucose from tissue energy production [27]. This is related to the increase in activity of carnitine palmitoyl transferase enzyme, which is associated with transfer of long chain fatty acids into the mitochondria. The elevation in this enzyme level is L-carnitine dependent [42]. Besides, this increase of long

chain fatty acid catabolism and the consecutive accumulation of acetyl-coA residues could induce retardation in the endogenous cholesterol synthesis. The contradictions between our present findings and those of the other previous studies could be related to factors concerned with either birds (species, age and sex) or the experimental procedure (duration of treatment, seasonal conditions, nutrimental status and L-carnitine dosages).

Thyroid Gland Activity and Lipid Peroxidation: T_3, T_4, as well as their calculated ratios were significantly increased in a linear manner due to L-carnitine supplementation. In addition, it is noticeable the lack of statistical significance between the high (400ppm) and the mid (200ppm) level. Our results are in full agreement with those reported in a study conducted on broiler chicks submitted to either normal temperature or thermal stress by Buyse *et al.* [43]. They found that L-carnitine supplementation (100 ppm) resulted in marked increases of heart weights and plasma triiodothyronine (T_3), particularly in the stressed birds. Similar trend was also shown by Abdel-Fattah and Shourrap[10] in broiler chicks hatched from eggs in ovo administered with L-carnitine. In partial accordance with our results, Janssens *et al.* [44] found that the plasma level of T_3 and T_4 were insignificantly increased in pigeon after being fed supplemental L-carnitine.

In this respect, Roncero and Goodridge [45] established the synergic effect of L-carnitine on the T_3 provoked accumulation of mRNA of fatty acid synthase and malic enzyme. Malic enzyme promotes the conversion of malate to pyruvate. Thus, accelerating the citric acid cycle as a consequence of higher capacities for aerobic acyl CoA combustion is created.

It has been well established that, free radicals or reactive oxygen species (ROS) are harmful to cell membranes. In which, it promotes lipid peroxidation causing membrane breakdown and loss of function. Lipid peroxidation, production and accumulation in the plasma membrane, results when the intracellular production of ROS rises above the antioxidant defense mechanisms utilized by cells, resulting in cell membranes damage [46].

As presented in Table 6, a highly significant reduction was obtained in the serum level of (MDA), which is the primary stable by-product of lipid peroxidation, in quail chicks whose diets were containing various L-carnitine levels in particular, the mid and high levels comparable to chicks fed the control diet.

Interestingly, L-carnitine has the ability of free radical scavenger acting to reduce lipids available for peroxidation by transferring fatty acids into the mitochondria for the production of adenosine triphosphate (ATP) through β-oxidation process [46,47]. Moreover, L-carnitine seemed to increase the plasma concentrations of ascorbic acid and vitamin E, as well increases the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). While, lipid peroxides was declined with L-carnitine supplementation [38]. Furthermore, it is well known that, circulating free iron is able to catalyze ROS leading to lipid membrane degradation. Carnitine has iron-chelating properties, which may cause carnitine and acetylcarnitine to partially prevent the generation of ROS by binding with free iron [48].

Digestive Enzyme Activities: Effect of feeding Japanese quail chicks on diets contained various L-carnitine dosages on the digestive enzyme activities in different segments of small intestine (duodenum, jejunum and ileum) are presented in Table 7.

It is clearly observable that, the activity of amylase enzyme in the duodenum was significantly lower in the control group than the groups of L-carnitine. Similar trend was achieved in the jejunum portion, although the absence of significant differences between the control chicks and those of 100ppm L-carnitine. The activity values within the ileum were only significant for chicks received the highest L-carnitine level (400ppm).

Concerning lipase enzyme the current findings indicated that, its activity was slightly affected with the addition of L-carnitine. Where, the statistical significant differences was noted only between the group control group and the highest L-carnitine level (400ppm) in both the jejunum and ileum. This may be associated with the action of L-carnitine in β-oxidation of fatty acids as previously mentioned herein.

Results of trypsin enzyme activity showed that, it was elevated significantly with supplemental L-carnitine with 400ppm compared with the control treatment within the first small intestinal portion (duodenum). Similar trend, however, non significant was obtained in the second and third portions (jejunum and ileum).

The activity of chymotrypsin was numerically increased in the first two small intestine portions as indicted in Table 7. While it was markedly elevated with dietary addition of 400ppm L-carnitine regarding the last portion of small intestine (ileum).

Table 6: Thyroid hormone activities and lipid peroxidase of Japanese quail chicks fed supplemental L-carnitine

	L-carnitine level (ppm)					
Variable	0	100	200	400	MSE	Prob.
T3 (ng/ml)	1.277 c	1.627 b	1.937 a	2.023a	0.007	0.0001
T4 (ng/ml)	7.941 c	8.999 b	9.737 a	9.918 a	0.081	0.0001
T3/T4	0.161 c	0.181 b	0.199 a	0.204 a	0.001	0.0001
MDA nmol/ml (535nm)	0.198 a	0.178 b	0.113 c	0.107c	0.001	0.0001
MDA nmol/ml (525nm)	0.171 a	0.124 b	0.090 c	0.086 c	0.001	0.0001

a-c Means within a row with different superscripts are significantly different at ($p \leq 0.05$).

Table 7: Digestive enzyme activities (Unit/dl) throughout the small intestine of Japanese quail chicks fed supplemental L-carnitine

	L-carnitine level (ppm)					
Enzyme type (U/dl)	0	100	200	400	MSE	Prob.
Duodenum						
Amylase	1.961 b	2.626 a	2.768 a	2.780a	0.077	0.001
Lipase	8.390	9.804	10.074	9.350	1.031	0.084
Trypsin	25.010 b	26.674 ab	27.620 ab	28.674 a	2.193	0.002
Chymotrypsin	14.194	15.182	16.908	16.118	1.892	0.107
Jejunum						
Amylase	1.570 c	2.596 a	1.730 bc	2.131 b	0.093	0.0003
Lipase	9.198 bc	9.928 b	10.388 b	12.214 a	0.679	0.0001
Trypsin	26.156 ab	28.778 a	28.152 a	27.974 a	2.900	0.042
Chymotrypsin	14.540	15.956	15.358	15.252	1.891	0.466
Ileum						
Amylase	2.522 b	2.348 b	2.538 b	2.998 a	0.036	0.0005
Lipase	9.584 b	9.364 b	10.254 ab	11.064 a	0.647	0.016
Trypsin	25.826	25.660	27.460	27.112	1.912	0.260
Chymotrypsin	13.776 b	15.910 a	16.866 a	17.468 a	1.315	0.008

a-c Means within a row with different superscripts are significantly different at ($p \leq 0.05$).

In general, the results showed that there was a tendency toward increasing the activity of all tested digestive enzymes (amylase, lipase, trypsin and chymotrypsin) throughout the small intestine portions with supplemental L-carnitine. Thereby, improving productive performance of chicks by altering metabolism. These results may interpret, to a great extent, the current growth performance data.

To our knowledge, the enhancement mechanisms of L-carnitine on the digestive enzyme activity are not yet clearly identified and needs further investigations. However, this beneficial effects may be account, from our view, for the presence of useful impact of L-carnitine on the reabsorption of several minerals (i.e., Ca, P, K, Na), involved in many digestive, physiological and biosynthetic processes within the animal body. These minerals may function as catalysts in the endogenous enzyme systems. This assumption may be confirmed by the previous finding of Teeter *et al.* [49] who postulated that, L-carnitine supplementation could prevent the sudden death syndrome (SDS) that is characterized by high mortality (12-18%) in broiler breeder strain. This high mortality percentage is closely related to an imbalance or deficit of minerals such as, potassium, calcium or phosphorus on metabolism during egg production.

It is concluded that, dietary L-carnitine supplementation with 200ppm up to 400ppm in quails was sufficient to enhance the growth performance traits, thyroid activities and antioxidant system. Moreover, however, the present study pointed out that, L-carnitine has the potentiality to modulate the immune response and digestive enzyme activities. Further researches are needed to throw more clarification of mechanisms associated with these valuable effects.

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