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Impact of Dietary Excess Methionine and Lysine with or Without Addition of L-Carnitine on Performance, Blood Lipid Profile and Litter Quality in Broilers

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ABSTRACT

Effects of excess of dietary methionine (6.5 and 8.5 g kg^{-1} diet) and lysine (13.5 and 15.5 g kg^{-1} diet) on performance, blood lipid profile and litter quality of broiler chickens fed corn-soybean meal based diet with or without supplementation of L-carnitine with two levels (175 and 350 mg kg^{-1} diet) were investigated. Two hundred forty one day old chicks (Cobb) of mixed sex were housed in pens littered with horse-bean straw. Three replicates of eight dietary treatments (10 chicks per replicate) were randomly distributed. Dry matter content of litter and excreta were done weekly. The results showed that the group supplemented with surplus of methionine and lysine (6.5-13.5 g kg^{-1} diet) without supplementation of L-carnitine had the highest body weight and body weight gain. L-carnitine either 175 or 350 mg kg^{-1} diet had significantly lower lipid profile parameters in serum than groups without supplementation L-carnitine. Moreover, the broilers fed high levels of lysine and methionine with surplus amount of L-carnitine (350 mg kg^{-1} diet) led to significantly lower cholesterol level vs. low L-carnitine. The experimental group supplemented with highest level of methionine and lysine (8.5-15.5 g kg^{-1}) had lowest malondialdehyde and highest reduced glutathione levels and total antioxidant capacity. No marked differences were found in dry matter content of the excreta and litter among the experimental treatments (mean; 23.8±2.50 and 66.6%±11.2, respectively). Interestingly, dietary L-carnitine supplementation had no effects on foot pad health even with using surplus level of it.

Key words: Methionine and lysine, L-carnitine, serum metabolites, litter quality, broilers

INTRODUCTION

It has been reported that protein is the most important component of feed ingredients. Twenty-two different amino acids are the building block of protein. Among them, 10 are indispensable for monogastric animal production (Baker, 2009; Wu, 2009). Methionine and lysine are usually the most important limiting amino acids in poultry nutrition and are frequently supplemented in the formulated diets (Gill, 2003). There are a number of studies have been conducted to determine the requirements of methionine and lysine as the first two limiting amino acids in practical corn-soybean based diets for broilers. Si *et al*. (2001) suggested that levels of methionine and lysine in excess of NRC (1994) recommendations may result in enhanced

performance, weight gain and feed conversion ratio. Also, Murray *et al*. (1998) found that addition of synthetic amino acids like lysine and methionine at high levels to the diet can stimulate insulin secretion from pancrease by aggregating plasma which in turn releases amino acids and fatty acids from the body saved sources and leads to protein synthesis. Moreover, Bouyeh and Gevorgyan (2011) observed that highest level of lysine and methionine (40% more than NRC) had lowest BWG in comparison with diets supplemented with 10 or 30% more than NRC. However, there was linear decrease of Feed Conversion Ratio (FCR) with increase the supplementation of lysine and methionine. Additionally, L-carnitine can be biosynthesized endogenously from these two amino acids. When diets are not supplemented with these two amino acids, the chicken may not be able to synthesize adequate amounts of L-carnitine (Arslan, 2006).

L-carnitine plays important role in lipid and energy metabolism in poultry (Borum, 1983). L-carnitine (b-OH-g-N-trimethylaminobutyric acid) is a small-molecular-weight water-soluble quaternary amine which occurs naturally in micro-organisms, plants and animals (Bremer, 1983). Its concentrations in animals vary according to species, tissue type and nutritional status of the animal (Rinaudo *et al*., 1991). L-carnitine promotes the mitochondrial β-oxidation of long-chain fatty acids by facilitating their transfer across the inner mitochondrial membrane. 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 (Bremer, 1983; Rebouche, 1992). Arenas *et al*. (1998) reported that L-carnitine represents the second line of cell defence against reactive oxygen species and their derivatives as it breaks free-radical chain reactions (termination of peroxidation) and prevents undesirable oxidation reactions. Furthermore, Yalcin *et al*. (2008) noted that no significant effects of L-carnitine supplementation in Japanese quails' diet on most blood serum parameters. Abdel-Fattah *et al*. (2014) observed significantly increased of serum total protein and globulin with supplementation of L-carnitine. In addition, Winter *et al*. (1995) pointed out that L-carnitine increase the activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase and decline lipid peroxidation.

From another point of view, managing the quality of litter, especially its moisture content (the key factor), has a great interest regarding feeding measures and housing conditions to reduce the incidence of foot pad lesions. Foot-Pad Dermatitis (FPD) is a widespread problem in poultry production with potential effects on animal welfare and economics. Several factors are associated to the incidence and severity of FPD (Kamphues *et al*., 2011), among them: diet composition as proportion of soybean meal; dietary electrolytes (potassium) that force wet litter (Abd El Wahab *et al*., 2013a); management and housing, mainly related to litter type and quality (Abd El-Wahab *et al*., 2011, 2012a); diseases caused by various infections like coccidiosis (Abd El-Wahab *et al*., 2012b, 2013b).

Thus, the purpose of this study was to investigate the potential effects of dietary methionine and lysine in excess of NRC recommendations with or without supplementation of dietary L-carnitine on growth performance, serum metabolites, lipid peroxidation and total antioxidant capacity as well as litter quality in broilers.

MATERIALS AND METHODS

Birds, diets and housing: Two hundred forty one days-old chicks (Cobb) of mixed sex were housed in pens, littered with horse-bean straw. The litter were kept very dry and clean by removing the upper layers of the litter daily and replacing them with fresh dry litter before the experiment started (day 8). After that, the birds were randomly assigned to eight experimental groups. Each

treatment was replicated three times with ten chicks (n = 10). The experimental pens (1.40×0.85 m) were bedded with approximately 1 cm (1 kg m^{-2}) of horse-bean straw (89% DM). The experimental diets were: corn-soybean meal based with normal levels of methionine and lysine according to NRC (1994) without L-carnitine supplementation (control). Second and third groups were fed normal levels of methionine and lysine with addition of L-carnitine (175 and 350 mg kg^{-1} diet, respectively). The fourth group had higher levels of dietary methionine and lysine (6.5 and 13.5 g kg^{-1} diet, respectively) without L-carnitine addition. The fifth and sixth groups contained surplus levels of L-carnitine (175 and 350 mg kg^{-1} diet, respectively), each with dietary methionine and lysine supplementation of 6.5 and 13.5 g kg^{-1} diet, respectively. The seventh and eighth groups had extra supply of dietary methionine and lysine (8.5 and 15.5 g kg^{-1} diet) without and with addition of L-carnitine (350 mg kg^{-1} diet), respectively. The used L-carnitine (as tararate, 350 mg) was produced by Arab Company for Pharmaceuticals and Plants, Egypt. Water and feed were provided *ad libitum*. A lighting program of 16L:8D was used for the entire 35 days growing period. The chicks were not vaccinated throughout the experimental period. Body Weight (BW) and feed intake were recorded weekly. Feed Conversion Ratio (FCR) was estimated on the basis of feed consumed (data from groups) and weight gain of the birds (individual data) throughout the experimental period.

Measurements: Litter samples for measuring Dry Matter (DM) and pH were collected weekly from 3 sites (2 peripheral samples and 1 central one) in each pen. At each area, a sample (~70 g) over the whole bedding height was punched out using a tin with a diameter of 6.5 cm from the full depth of the litter. Samples were oven-dried at 103°C for the time needed to reach constant weight. Litter pH was measured by making a suspension (1 part material: 9 parts water), then measured using a pH meter (HI 2211, ORP meter, HANNA instruments, Romania).

The fresh excreta of the birds were collected from each pen once a week by putting a plastic sheet in each pen for approximately 1 h until ~80-100 g of fresh pure excreta per pen had been obtained. The collected excreta were then removed from each pen, thoroughly mixed and dried at 103°C to determine the DM content.

Sample collection: At day 35, three birds from each replicate were randomly selected and slaughtered. Birds were mechanically de-feathered and manually eviscerated. After that, carcasses and the internal organs (heart, liver and proventriculus) were weighed. The blood samples of every individual were collected in clean sterile test tubes then centrifuged at 3000 rpm for 20 min for serum separation. The collected serum was kept frozen at -30°C until analysis.

Chemical analysis determination

Serum metabolites and antioxidant analyses: Serum total lipid and triacylglycerol were determined according to Zollner and Kirsch (1962) and Fossati and Prencipe (1982), respectively. Serum total cholesterol was estimated by using enzymatic hydrolysis and oxidation of cholesterol for the formation of quinoneimine according to the method of Allain *et al*. (1974). Serum phospholipids were measured spectrophotometrically at wave length 650 nm using the procedure of Connerty *et al*. (1961). Serum High-Density Lipoprotein (HDL) was estimated through the precipitation of all lipoproteins by phosphotungstic acid and magnesium chloride following the technique of Lopes-Virella *et al*. (1977). On the same trend, heparin was used to precipitate Low-Density Lipoprotein (LDL) using the protocol of Wieland and Seidel (1983).

Serum total protein and albumin were determined according to Weissman *et al.* (1950) and Lolekha and Charoenpol (1974), respectively. The concentration of serum globulin was calculated by subtracting serum albumin from serum total protein.

Serum antioxidant capacity and malondialdehyde were determined according to the methods of Koracevic *et al*. (2001) and Satoh (1978), respectively. The concentrations of nitric oxide in serum and the reduced glutathione were determined according to Montgomery and Dymock (1961) and Beutler *et al*. (1963), respectively.

Food pad dermatitis scoring criteria: External assessment of foot pads was made only at the end of the experimental period (days 35) for each bird individually. During the external examination, if the feet were dirty, they were gently washed with a wet cloth and dried before scoring; only the central plantar area was scored and signs of foot pad lesions were recorded on a 7-point scale (0 = normal skin and 7 = over half of the foot pad is covered with necrotic scales) according to Mayne *et al*. (2007).

Statistical analysis: The results were subjected to one-way ANOVA to test the influence of excess dietary of methionine and lysine with and without addition of L-carnitine on performance, lipid profile, antioxidant activity and litter quality of broilers as well as on FPD scores. Data was analyzed using statistical SPSS V20 (SPSS Inc., Chicago, IL, USA). Differences between means were compared using Duncan's multiple range test at significance of differences ($p<0.05$) among dietary treatments.

RESULTS

The chemical composition of the control group is found in Table 1. Briefly, the levels of crude protein, lysine and methionine were 221, 11.8 and 5.2, g kg^{-1} diet respectively. Additionally, no marked differences were found between the other experimental treatments regarding the chemical composition except for the levels of lysine, methionine and L-carnitine.

Performance: Data of BW are shown in Table 2. Birds in G3 and G4 had significantly ($p<0.05$) higher BW (1660 g±29.0 and 1695 g±22.4) compared to G5, G7 and G8 (1482 g± 54.3, 1429 g±82.7 and 1499 g±81.5, respectively), but not significant difference ($p>0.05$) compared to other groups (G1 and G2). No significant differences for FCR were noted among the experimental groups from 28-35 days of growth. However, during that period, BW gain was significantly ($p<0.05$) higher for birds in the G4 (433 g±11.0), compared to all other experimental groups (except for G8; 426 g±28.5). Birds in G7 had significantly ($p<0.05$) lower BW gain (277 g±16.4) compared to other experimental groups (except G5 and G6).

The effect of excess methionine and lysine with and without supplementation of L-carnitine on carcass weight and the percentage of organs weight (internal organs as % of the carcass) are shown in Table 3. There was a significant ($p<0.05$) increase of carcass weight in G4 (1412 g±0.07) compared to other experimental groups. It was noted that the birds in the control group had markedly the highest weights of proventriculus, heart and liver (0.70, 0.78 and 3.55%, respectively) in comparison to other experimental groups. However, no significant differences were found regarding weight (%) of proventriculus, heart among the other experimental groups.

Lipid peroxidation and antioxidant capacity: The results of total antioxidant capacity reduce glutathione and malondialdehyde (MDA) are present in Table 4. Birds in control group had

Table 1: Composition of the experimental diets fed to broilers (8-35 days)

Parameters	Values
Ingredients (%)	
Yellow corn	63.34
Soybean meal	23.5
Corn gluten	6
Soybean oil	3.5
CaCO3	1.36
Dicalcium phosphate	1.46
NaCl	0.3
DL-methionine	0.05
L-lysine	0.24
Premix1	0.25
Chemical composition (g kg^{-1} dry matter)	
DM	881
Crude ash	51.7
Crude protein	221
Crude fat	69
Crude fibre	22.9
NfE2	635
Starch	468
Sugar	46.7
ME3 (MJ kg^{-1})	14.2
Threonine	8.96
Tyrosine	8.06
Cystine	4.51
Methionine	5.29
Lysine	11.8
Macrominerals (g kg^{-1})	
Ca	10.1
P	6.72
Mg	1.62
Na	1.49
Cl	3.52
K	7.9
S	2.67
Macrominerals (mg kg^{-1})	
Cu	12
Zn	75.3
Fe	186
Mn	82.7
Se	0.48

[1]Vitamin and trace elements mixture supplies the following nutrients per kilogram of diet: Vitamin A: 12000 IU, Vitamin D_3: 5000 IU, Vitamin E; 50 mg, Calcium: 8.3 g, Phosphorus: 2.9 g, Sodium: 1.3 g, Copper: 10 mg, Iron: 70 mg, Zinc: 0.0 mg, Manganese: 100 mg, Iodine: 1.87 mg, Selenium: 0.3 mg, [2]NfE (nitrogen free extract), [3]ME (metabolizable energy) calculated by using the official formula for complete diets ME_n (MJ kg^{-1}) = 0.01551, crude protein+0.03431 crude fat+0.01669 starch+0.01301 sugar (FMVO., 2007)

significantly ($p<0.05$) the lowest total antioxidant capacity (0.05 mμ L^{-1}) compared to other experimental groups. Meanwhile, G6 and G7 had the highest total antioxidant capacity (0.29 mμ L^{-1}) than other groups. Birds in control group had significantly ($p<0.05$) the lowest value of reduced glutathione (6.59 mg dL^{-1}±0.53) in comparison to other experimental groups. However, birds in G6 and G7 had the highest value of reduced glutathione (9.66±0.22 and 9.43±0.69 mg dL^{-1}, respectively) compared to other groups. The lowest significant ($p<0.05$) value of MDA (9.89±0.43 nmol mL^{-1}) was observed for birds in G7 compared to other experimental groups (except G2).

Serum metabolites: The influence of excess methionine and lysine with or without different levels of L-carnitine on serum lipid profile is shown in Table 5. The results showed that the groups supplemented with L-carnitine either 175 or 350 mg kg^{-1} diet had significantly ($p<0.05$) lower lipid

Table 2: Influence of dietary excess of methionine and lysine with and without L-carnitine supplementation on growth performance of broilers thought out the experimental period

Growth performance	Experimental diets (groups)							
	G1	G2	G3	G4	G5	G6	G7	G8
Live BW (g)								
14d	375±17.67	411±5.00	391±5.50	386±3.50	383±2.50	380±11.50	373±11.00	390±11.00
21d	803±6.92	826±39.50	824±15.50	815±40.00	767±5.50	794±26.50	737±12.50	743±10.00
28d	1190±23.36^{b}	1240±38.20^{ab}	1309±29.30^{a}	1269±32.87^{a}	1157±34.41^{ab}	1227±20.48^{ab}	1132±73.95^{b}	1133±44.9^{b}
35d	1552±42.59^{ab}	1627±38.58^{ab}	1660±29.00^{a}	1695±22.49^{a}	1482±54.38^{b}	1614±52.26^{ab}	1429±82.71^{b}	1499±81.54^{b}
BW gain (g)								
14-21d	428±10.70^{a}	426±25.00^{a}	433±10.50^{a}	429±36.00^{a}	388±3.50^{ab}	414±14.50^{ab}	364±1.50^{b}	353±1.00^{ab}
21-28d	421± 24.50^{b}	468±11.66^{ab}	528±26.08^{a}	499±15.05^{a}	426±37.82^{ab}	464±14.43^{ab}	476±15.05^{ab}	441±23.38^{ab}
28-35d	378±16.37^{b}	387±12.20^{b}	351±9.21^{b}	433±11.00^{a}	294±7.52^{cb}	323±15.17^{cb}	277±16.40^{c}	426±28.51^{ab}
FCR								
14-21d	1.49±0.03^{ab}	1.60±0.09^{ab}	1.50±0.04^{ab}	1.45±0.12^{b}	1.57±0.02^{ab}	1.51± 0.06^{ab}	1.56±0.01^{ab}	1.67±0.01^{a}
21-28d	1.94±0.06^{b}	1.76±0.04^{cb}	1.62±0.08^{c}	1.63±0.05^{c}	1.71±0.15^{cb}	1.52±0.03^{c}	2.41±0.08^{a}	1.63±0.09^{c}
28-35d	2.02±0.08^{a}	1.95±0.03^{a}	2.11±0.06^{a}	1.84±0.03^{ab}	1.90 ±0.03^{ab}	2.09±0.09^{a}	1.88±0.12^{ab}	1.81±0.01^{ab}

^{a,b}Means in the same row with different superscripts are significantly different ($p<0.05$), G1: Control, G2: Control+175 mg L-carnitine, G3: Control+350 mg L-carnitine, G4: 6.5 g Met+13.5 g lysine, G5: 6.5 g Met+13.5 g lysine+175 mg L-carnitine, G6: 6.5 g Met+13.5 g lysine+350 mg L-carnitine, G7:8.5 g Met+15.5 g lysine, G8: 8.5 g Met+15.5 g lysin+350 mg L-carnitine, BW: Body weight, FCR: Feed conversion ratio

Table 3: Effect of excess of dietary methionine and lysine with or without supplementation of L-carnitine on carcass weight and internal organs percentage of broilers

Parameters	Experimental diets (groups)							
	G1	G2	G3	G4	G5	G6	G7	G8
Carcass weight (g)	1180±0.03^{b}	1202±0.04^{b}	1255±0.03^{b}	1412±0.07^{a}	1220±0.06^{b}	1224±0.08^{b}	1228±0.06^{b}	1220±0.06^{b}
Proventriculus (%)	0.70±0.03^{a}	0.59±0.03^{ab}	0.63±0.03^{ab}	0.56±0.04^{ab}	0.55±0.03^{ab}	0.59±0.02^{ab}	0.54±0.03^{ab}	0.49±0.03^{b}
Heart (%)	0.78±0.02^{a}	0.61±0.02^{b}	0.65±0.03^{b}	0.56±0.05^{b}	0.48±0.03^{cb}	0.59±0.04^{b}	0.54±0.04^{cb}	0.51±0.03^{cb}
Liver (%)	3.55±0.09^{a}	2.83±0.11^{b}	2.70±0.09^{b}	2.45±0.13^{cb}	1.93±0.06^{ce}	2.45±0.05^{cb}	2.92±0.08^{b}	2.55±0.06^{cb}

^{a,b}Means in the same row with different superscripts are significantly different ($p<0.05$), G1: Control, G2: Control+175 mg L-carnitine, G3: Control+350 mg L-carnitine, G4: 6.5 g Met+13.5 g lysine, G5:6.5 g Met+13.5 g lysine+175 mg L-carnitine, G6: 6.5 g Met+13.5 g lysine+350 mg L-carnitine, G7: 8.5 g Met+15.5 g lysine, G8: 8.5 g Met+15.5 g lysine+350 mg L-carnitine

Table 4: Influence of excess of dietary methionine and lysine with or without supplementation of L-carnitine on lipid peroxidation and total antioxidant capacity of broilers

Parameters	Experimental diets (groups)							
	G1	G2	G3	G4	G5	G6	G7	G8
Nitrite (μmol L^{-1})	31.66±1.61^{a}	26.41±0.94^{b}	30.67±1.42^{a}	24.07±0.74^{bc}	23.65±1.20^{c}	20.80±0.45^{d}	29.29±0.45^{a}	23.10±0.92^{cd}
Total antioxidant capacity (μmol L^{-1})	0.05±0.006^{d}	0.17±0.03^{c}	0.23±0.02^{bc}	0.28±0.02^{ab}	0.26±0.02^{ab}	0.29±0.04^{a}	0.29±0.02^{a}	0.15±0.004^{c}
Reduced glutathione (mg dL^{-1})	6.59±0.53^{c}	8.08±0.22^{b}	8.41±0.79^{ab}	8.14±0.49^{b}	9.03±0.41^{ab}	9.66±0.22^{a}	9.43±0.69^{a}	8.13±0.39^{b}
Malondialdehyde (nmol mL^{-1})	12.56±0.49^{a}	10.99±0.45^{bc}	11.68±0.39^{b}	12.22±0.36^{a}	12.10±0.40^{ab}	11.74±0.79^{b}	9.89±0.43^{c}	12.05±0.39^{ab}

^{a,b}Means in the same row with different superscripts are significantly different ($p<0.05$), G1: Control, G2: Control+175 mg L-carnitine, G3: Control+350 mg L-carnitine, G4: 6.5 g Met+13.5 g lysine, G5: 6.5 g Met+13.5 g lysine+175 mg L-carnitine, G6: 6.5 g Met+13.5 g lysine+350 mg L-carnitine, G7: 8.5 g Met+15.5 g lysine, G8:8.5 g Met+15.5 g lysine+350 mg L-carnitine

profile parameters in serum (triglycerides, cholesterol and total lipid) than in groups without supplementation L-carnitine. Moreover, the broilers fed high level of lysine and methionine with surplus amount of L-carnitine (350 mg kg^{-1} diet) led to significantly ($p<0.05$) lower cholesterol level compared to low L-carnitine (175 mg kg^{-1} diet). Additionally, supplementation diet with L-carnitine with high level (350 mg kg^{-1} diet) resulted in significantly lower ($p<0.05$) serum total lipids concentration in comparison with low L-carnitine level (175 mg kg^{-1} diet) at each dietary methionine and lysine level.

No significant effects regarding HDL in serum were observed between the experimental groups (Table 5). However, LDL and Very Low-Density Lipoprotein (VLDL) in groups without addition of

Table 5: Serum lipid profile at end of the experiment in broilers fed excess of dietary methionine and lysine with or without L-carnitine supplementation

	Experimental diets (groups)							
Parameter (mg dL^{-1})	G1	G2	G3	G4	G5	G6	G7	G8
Triglyceride	102±5.62^{a}	99.3±5.26^{b}	87.8±1.85^{cd}	113±6.02^{a}	76.6±1.51^{d}	97.4±4.62^{bc}	101±4.56^{b}	100±3.55^{b}
Cholesterol	216±6.00^{a}	193±5.54^{b}	161±9.55^{c}	214±8.88^{a}	185±3.11^{b}	168±7.74^{c}	155±4.28^{c}	146±3.57^{d}
Total lipid	2061±70.60^{a}	1737±56.10^{b}	1286±43.90^{d}	2117±72.60^{a}	1584±64.40^{c}	1264 ±76.60^{d}	1533±44.90^{c}	1299±52.80^{d}
Phospholipids	111±1.13^{e}	147±8.10^{bd}	134±6.49^{cd}	155±11.90^{b}	153±4.16^{b}	182±3.57^{a}	179±4.12^{a}	185±4.92^{a}
HDL	25.80±0.95	25.00±0.437	25.20±1.80	26.60±1.65	26.20±5.86	25.50±2.75	26.30±2.99	25.70±2.23
LDL	194±2.86^{a}	174±7.88^{b}	160±9.29^{bc}	191±8.93^{a}	156±2.54^{bc}	154±5.26^{bc}	173±37.7^{b}	145±3.62^{bc}
VLDL	25.60±1.32^{a}	20.50±0.96^{b}	17.50±0.37^{c}	24.70±1.07^{a}	19.30±0.30^{b}	17.90±1.31^{c}	21.40±0.99^{b}	15.10±1.32^{c}

^{a,b}Means in the same column in each trial with different superscripts are significantly different ($p<0.05$), G1: Control, G2: Control+175 mg L-carnitine, G3: Control+350 mg L-carnitine, G4:6.5 g Met+13.5 g lysine, G5:6.5 g Met+13.5 g lysine+175 mg L-carnitine, G6:6.5 g Met+13.5 g lysine+350 mg L-carnitine, G7:8.5 g Met+15.5 g lysine, G8:8.5 g Met+15.5 g lysine+350 mg L-carnitine, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein

Table 6: Blood serum protein parameters of broiler chickens fed excess of dietary methionine and lysine with or without supplementation of L-carnitine

	Experimental diets (groups)							
Parameter (g dL^{-1})	G1	G2	G3	G4	G5	G6	G7	G8
Total protein	2.99±0.22^{ab}	3.08±0.29^{ab}	3.23±0.16^{ab}	3.17±0.24^{ab}	3.15±0.05^{ab}	2.74±0.22^{b}	3.35±0.08^{a}	3.06±0.19^{ab}
Albumin	1.08±0.08^{cb}	1.12±0.26^{b}	1.43±0.07^{a}	1.25±0.13^{b}	1.18±0.08^{b}	1.46±0.05^{a}	1.08±0.10^{cb}	1.26±0.12^{b}
Globulin	1.93±0.30^{b}	2.43±0.16^{ab}	1.66±0.10^{c}	2.92±0.33^{a}	1.98±0.06^{b}	1.32±0.10^{c}	2.31±0.10^{b}	2.01±0.03^{b}

^{a,b}Means in the same row with different superscripts are significantly different ($p<0.05$), G1: Control, G2: Control+175 mg L-carnitine, G3: Control+350 mg L-carnitine, G4:6.5 g Met+13.5 g lysine, G5:6.5 g Met+13.5 g lysine+175 mg L-carnitine, G6:6.5 g Met+13.5 g lysine+350 mg L-carnitine, G7:8.5 g Met+15.5 g lysine, G8:8.5 g Met+15.5 g lysine+350 mg L-carnitine

Table 7: Impact of excess of dietary methionine and lysine with or without L-carnitine on dry matter content and pH value of litter and excreta throughout the fattening period and the foot-pad dermatitis scores of broilers

	Experimental diets (groups)							
Parameters	G1	G2	G3	G4	G5	G6	G7	G8
Excreta, DM (%)	23.70±2.01	23.20±2.80	25.10±3.0	22.50±3.75	25.30±3.51	23.80±1.81	23.90±1.25	23.10±1.90
pH of excreta	6.47±0.28	6.35±0.29	6.53±0.43	6.30±0.51	6.11±0.32	6.41±0.44	6.27±0.32	6.19±0.29
Litter, DM (%)	66.40±9.10	66.60±9.27	65.80±13.80	66.20±13.90	67.90±12.90	67.60±8.77	66.20±11.10	66.50±11.10
pH of litter	7.46±0.78	7.13±0.78	7.19±0.56	7.35±0.69	7.43±0.72	7.49±0.86	7.32±0.57	7.23±0.55
FPD score at d 35	4.60±0.84^{a}	4.45±0.52^{a}	4.40±0.84^{a}	3.45±0.52^{b}	3.60±0.97^{b}	3.50±0.97^{b}	3.18±0.60^{b}	3.00±0.47^{b}

^{a,b}Means in the same row with different superscripts are significantly different ($p<0.05$), G1: Control, G2: Control+175 mg L-carnitine, G3: Control+350 mg L-carnitine, G4: 6.5 g Met+13.5 g lysine, G5: 6.5 g Met+13.5g lysine+175 mg L-carnitine, G6: 6.5 g Met+13.5 g lysine+350 mg L-carnitine, G7: 8.5 g Met+15.5 g lysine, G8: 8.5 g Met+15.5 g lysine+350 mg L-carnitine, FPD: Foot-pad dermatitis

L-carnitine had significantly ($p<0.05$) higher levels (191-194 and 24.7-25.6 mg dL^{-1}, respectively) compared to other groups (145-174 and 15.1-21.4 mg dL^{-1}, respectively). Furthermore, using surplus levels of L-carnitine (350 mg kg^{-1} diet) was associated with significantly lower serum VLDL content in comparison with low level of L-carnitine level (175 mg kg^{-1} diet).

Total serum protein, albumin and globulin of the experimental groups are presented in Table 6. Birds in G7 had the highest value of total protein in serum (3.35 g dL^{-1}) compared to other experimental groups. Meanwhile, no significant differences for serum total protein were found among the other experimental groups. Birds in G3 and G6 had significantly ($p<0.05$) the highest contents of serum albumin (1.43 and 1.46 g dL^{-1}, respectively) compared to other experimental groups.

Litter condition: The means of the different moisture contents of excreta and litter are shown in Table 7. No marked differences were found in the means of dry matter of the excreta or in pH values among the experimental treatments (23.8%±2.50 and 6.32%±0.360, respectively).

Throughout the experimental period, there were no marked differences in the dry matter content of the litter or in the pH values (66.6%± 11.2 and 7.32%±0.688) for any of the groups.

Foot pad lesions: The means of external scores of foot pads during the experimental period of rearing birds are presented in Table 7. At the beginning of the experiment (day 8) there was no evidence of external FPD lesions. Surplus levels of methionine and lysine (8.5 and 15.5 g kg^{-1} diet), regardless level of L-carnitine supplementation (G7 and G8) resulted in the lowest FPD scores (3.18 and 3.00, respectively) compared to the other experimental groups. Moreover, birds in G1, G2 and G3 had significantly ($p<0.05$) the highest FPD scores (4.60, 4.45 and 4.40, respectively) in comparison to other experimental groups.

DISCUSSION

Growth performance and carcass characteristics: L-carnitine is a natural, vitamin-like substance that acts in the cells as a receptor molecule for activated fatty acids. The major metabolic role of it appears to be the transport of long-chain fatty acids into the mitochondria for B-oxidation (Coulter, 1995). The effects of L-carnitine on the growth parameters were inconsistent. Some authors found beneficial effects of supplementation of L-carnitine on broiler diets, while others found no effects. Carrol and Core (2001) reported that L-carnitine has favourable effects on animal performance by enhancing resistance to metabolic diseases, preventing some diseases, strengthening immune system and playing an important role in metabolic and physiological processes. Moreover, Lettner *et al*. (1992) observed improvement in weight gain, feed conversion ratio, carcass characteristics or decrease in serum triglyceride in birds fed supplemented L-carnitine. However, no effect of L-carnitine on broiler performance was found in some other studies (Xu *et al*., 2003; Daskiran and Teeter, 2001). Recent researches have suggested that levels of lysine and methionine in excess of NRC (1994) recommendations may result in enhanced performance, especially in regard to breast meat yield, weight gain and feed conversion ratio (Si *et al*., 2001, 2004).

In this study, the experimental group with surplus level of L-carnitine with normal levels of lysine and methionine (G3) had high BW. This results in agreement with that found by Michalczuk *et al*. (2012), who observed a high BW of experimental group supplemented with excess level of L-carnitine in water. Nevertheless, in our study using only surplus levels of methionine and lysine (6.5 and 13.5 g kg^{-1} diet, respectively) without supplementation of L-carnitine (G4) had highest BW and BWG during the experiment period 28-35 days.

The data of carcass weight and internal organs percentages illustrated that the liver and heart weight percentages were higher in the broilers fed control diet than other experimental groups. However, birds fed 6.5 g Met+13.5 g lysin kg^{-1} diet (G4) had the highest carcass weight. With the same concept, Rezaei *et al*. (2007) observed liver weight decreased and heart percentage remained unchanged in the experimental group receiving L-carnitine. On the contrary, Bouyeh and Gevorgyan (2011) noticed that the weight of liver and heart (as percentage of carcass weight) was linearly increased in response to supplementation of lysine and methionine. Also, Buyse *et al*. (2001) found that supplementation L-carnitine to the broiler's diet caused increase heart weight. This might be due to a positive response to faster rate of metabolism for synthesis L-carnitine, glucose, cholesterol and protein (Harmeyer, 2002).

Serum metabolites: Analysis of variance showed that broilers fed diets supplemented with high levels of lysine and methionine with supplementation of L-carnitine had lower levels of serum

triglycerides, cholesterol and lipid compared with control and other experimental groups. Supplementation of lysine and methionine to diets as precursors of L-carnitine could be used to supply of L-carnitine for use in metabolism. With the same concept, Abdel-Fattah *et al*. (2014) observed that the diets of Japanese quail supplemented with excess level of L-carnitine (400 ppm) followed by mid one (200 ppm) reduced the serum concentrations of total lipid, cholesterol, triglycerides and LDL. Also, Arslan (2006) postulated that L-carnitine is known as a hypolipidemic drug which able to reduce the circulating concentration of cholesterol, triglycerides, free fatty acids, phospholipid and VLDL.

The serum total protein of the diet supplemented with highest level of lysine and methionine without L-carnitine (G7) was higher than other dietary treatments. Sahir *et al.* (2006) found that serum total protein concentration increased with increasing dietary lysine in broiler diets.

Antioxidant activity and lipid peroxidation: Citil *et al*. (2005) postulated that lipid peroxidation is an indicator of cell damage caused by toxic effects, ageing and stress. The induction of lipid peroxidation gave rise to an increase in MDA content. Moreover, there is a negative relationship between lipid oxidation and reduced glutathione (GSH). The MDA, which is the primary stable by product of lipid peroxidation was significantly decreased in G2 and G7. However, there were significantly increase of both reduced glutathione and total antioxidant capacity in G6 and G7. These results are in agreement with those found of Japanese quail by Abdel-Fattah *et al*. (2014). Those authors found that there was highly significant reduction of MDA of Japanese quail whose diets supplemented with various levels of L-carnitine. Also, Citil *et al*. (2005) observed reduction of plasma MDA and increase whole blood reduced glutathione of Japanese quail supplemented with 200 mg kg^{-1} L-carnitine in drinking water. Arockia Rani and Panneerselvam (2001) suggested that the increase of reduced glutathione may be due to the decrease of lipid peroxidation caused by L-carnitine supplementation.

Litter quality and FPD: The pH values of the litter were not affected by the diets. It was stated that wet litter was associated with a higher pH compared with dry litter (Lerner, 1996). On the contrary, Martland (1985) found a higher pH in the dry litter compared with wet litter. Moisture is the key factor influencing litter quality and managing litter is a crucial step in promoting flock health and well-being (Kamphues *et al*., 2011). Previous research (Abd El-Wahab *et al*., 2012a) has shown that the first significant increase in FPD lesion was observed after exposure of young turkeys for only 4 h day^{-1}-35% moisture content of litter.

Dietary changes that reduce FPD are of special interest for poultry health and welfare. The present results suggest that increasing dietary methionine and lysine supplementation above normal recommendations for broilers will reduce the severity of FPD at almost identical litter DM contents (measured weekly). It means that level of dietary methionine and lysine play an important role for health of skin rather than moisture content in the litter. Chavez and Kratzer (1972, 1974) reported that the source and not only the dosage- of methionine might play an important role for the foot pad health of turkeys. Furthermore, Abd El-Wahab *et al*. (2014) observed that increasing dietary methionine supplementation (above normal recommendations for young turkeys) will reduce the severity of FPD.

CONCLUSION

It was concluded that using only surplus levels of Met and lysine (6.5 and 13.5 g kg^{-1} diet, respectively) is enough to have the highest BW and BW gain. Supplementation of the diet with

higher level of L-carnitine has a marked effect on reducing blood lipid profile. Moreover, dietary levels of methionine and lysine play an important role for health of skin rather than moisture content in the litter. Dietary L-carnitine supplementation had no effects on foot pad health even with using surplus level of it.

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