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Effect of adding L-carnitine and methionine on growth performance, carcass traits, blood metabolites, and tibia characteristics in broiler chickens

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Abstract This study examined the interactive effects of dietary L-carnitine and methionine (Met) supplementation on growth performance, carcass characteristics, serum biochemical parameters, and tibial bone properties in broiler chickens. A total of 144 one-day-old Ross 308 male broiler chicks was distributed among four experimental treatments, with four replicates of nine birds each. Dietary treatments included two levels of L-carnitine (0 and 250 mg/kg of diet) and Met (Ross recommendation, 2014) and Met at 20% above Ross recommendation (H-Met), as a 2×2 factorial arrangement. Growth performance parameters, including feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR), were determined at the end of each rearing phase (d 1–10, 11–25, and 26–42); at d 42, two birds per replicate, nearest to the pen mean body weight, were designated for blood collection, carcass assessment, and tibial bone characterization. The results revealed that L-carnitine supplementation enhanced BWG, relative carcass and breast weights, and tibial dry weight, length, and breaking strength, as well as serum total protein and albumin concentrations, while concurrently reducing FI, FCR, abdominal fat deposition, and serum cholesterol and triglyceride levels ($P \leq 0.05$). Birds allocated to the H-Met treatment exhibited the greatest BWG, relative carcass and breast weights, tibial breaking strength, and serum total protein and albumin, alongside the lowest FCR and abdominal fat proportion ($P \leq 0.05$). Overall, these findings highlighted the synergistic effects of L-carnitine and H-Met on enhancing metabolic health and production efficiency in broilers.

Keywords: body weight, broilers, L-carnitine, methionine, tibia

Introduction

Excessive abdominal fat deposition and poor skeletal quality remain major concerns in modern commercial broiler production systems (Akter et al., 2020; Dixon, 2020). Continuous genetic improvement for rapid growth and increased breast meat yield has substantially enhanced production efficiency; however, these advances have also increased the prevalence of metabolic disorders and leg abnormalities in fast-growing broiler strains (Dixon, 2020). As a result, considerable attention has been directed toward nutritional interventions capable of improving nutrient utilization, regulating lipid metabolism, and supporting skeletal development in broiler chickens (Surai, 2015; Fagundes et al., 2020).

L-carnitine is a naturally occurring quaternary amine synthesized endogenously from lysine and methionine and is widely recognized for its central role in mitochondrial fatty acid metabolism. This compound facilitates the transport of long-chain fatty acids into mitochondrial matrix, where β-oxidation and ATP production occur, potentially reducing lipid accumulation in adipose tissues (Adabi et al., 2011). In addition to its metabolic role, L-carnitine possesses antioxidant properties that may help protect tissues against oxidative damage under intensive production conditions (Surai, 2015; Canbolat et al., 2017). Several studies have reported positive effects of dietary L-carnitine supplementation on BWG, carcass yield, and serum lipid profile in broiler chickens (Panahi et al., 2019; Azizi-Chekosari et al., 2021). Furthermore, recent evidence suggests that L-carnitine may contribute to improved bone metabolism by supporting mitochondrial activity in osteogenic cells and enhancing skeletal integrity and bone strength (Orsal et al., 2013; Kwiecień et al., 2023).

Methionine (Met) is considered as the first limiting amino acid in corn–soybean meal-based broiler diets and plays a critical role in protein accretion, methyl-group transfer reactions, and antioxidant defense mechanisms. In addition to its contribution to muscle protein synthesis, Met is involved in glutathione and carnitine biosynthesis, thereby linking amino acid metabolism with oxidative status and lipid utilization (Park et al., 2018; Fagundes et al., 2020). Adequate dietary Met supply has been associated with improved productive performance, carcass yield, and serum protein concentrations in broiler chickens (Brahmaiah et al., 2018; Çenesiz et al., 2022). Moreover, Met may influence skeletal development through its involvement in collagen formation and mineral metabolism (Fabà et al., 2019; Akter et al., 2020).

Although previous studies have investigated L-carnitine and Met supplementation in poultry, information on their interactive effects on serum biochemical responses and tibia characteristics in broiler chickens remains limited. Given the contribution of Met-derived methyl groups to endogenous L-carnitine biosynthesis, a Met level 20% above the Ross recommendation was selected to investigate its potential effects in combination with dietary L-carnitine. Therefore, this study evaluated the individual and interactive effects of L-carnitine and H-Met on growth performance, carcass traits, serum biochemical parameters, and tibia characteristics in broiler chickens.

Materials and methods

Experimental birds, design, and dietary treatments

The present study was conducted at Shahid Beheshti Station of University of Jiroft, Iran. All experimental procedures involving broiler chickens were approved by the Animal Care Committee of University of Jiroft (Approval No. 2812-30009). A total of 144 one-day-old Ross 308 male broiler chicks with a uniform initial body weight (42.91±0.72 g) were used in the experiment. Birds were randomly distributed into four experimental treatments based on a completely randomized design with a 2×2 factorial arrangement. Each treatment consisted of four replicate pens containing nine birds per replicate. The experiment lasted 42 days and included starter (1-10 d), grower (11-25 d), and finisher (26-42 d) phases. Dietary treatments were consisted of two levels of L-carnitine (0 and 250 mg/kg diet) and two Met (equal to Ross recommendation, 2014) and Met at 20% above the Ross recommendation (H-Met). The ingredient composition and nutrient contents of the experimental diets are presented in Tables 1 and 2. Experimental diets were formulated according to Ross 308 nutrient specifications (Aviagen, 2014). Birds were reared in floor pens (1.5 × 1 m) covered with approximately 10 cm of fresh sawdust litter. Ambient temperature was maintained at 33 °C during the first three days post-hatch and gradually was reduced by nearly 3 °C each week until reaching 22±2 °C. Relative humidity was maintained between 50 and 60% throughout the trial. A lighting program of 23 h light and 1 h dark (23L:1D) was provided during the experimental period, and birds had ad libitum access to feed and water. Vaccination and flock management procedures were conducted according to Ross 308 management guidelines. The DL-methionine used in this experiment was supplied by Degussa (Germany), whereas L-carnitine was obtained from Stargan Nik Pharmaceutical Company (Tehran, Iran).

Table 1. Ingredients and composition of the control diet

Ingredients (%)	Starter (1-10 d)	Grower (11-25 d)	Finisher (26-42 d)
Corn	50.60	54.40	58.41
Soybean meal	41.77	37.81	33.68
Soybean oil	3.52	3.73	4.43
Di-Calcium phosphate	1.62	1.66	1.27
Limestone	1.28	1.29	1.14
Sodium chloride	0.31	0.31	0.30
DL-Methionine	0.16	0.13	0.10
L-Lysine	0.23	0.17	0.17
Vitamin and mineral premix [1]	0.50	0.50	0.50
Calculated analysis (DM basis)			
Metabolizable energy (kcal/kg)	3000	3050	3150
Crude protein (%)	23.00	21.50	20.00
Calcium (%)	0.96	0.96	0.81
Available phosphorus (%)	0.48	0.48	0.41
Sodium (%)	0.16	0.16	0.16
Chlorine (%)	0.23	0.23	0.23
Lysine (%)	1.44	1.29	1.19
Methionine (%)	0.56	0.51	0.45

[1] Vitamin premix supplied the following per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 1500 IU; tocopherol, 60 IU; Phyllo Quinone, 2 mg; thiamine, 2.4 mg; riboflavin, 4.8 mg; niacin, 30 mg; pantothenic acid, 16 mg; pyridoxine, 3 mg; folic acid, 1 mg; vitamin B12, 0.03 mg; biotin, 0.15 mg; and choline chloride, 50 mg. Mineral premix supplied the following per kilogram of diet: Mn, 80 mg; Fe, 120 mg; Zn, 60 mg; Cu, 100 mg; I, 0.95 mg; and Se, 0.25 mg.

Growth performance

During starter, grower, finisher, and overall rearing periods, FI and BWG were recorded on a pen basis. The FCR was calculated using FI and BWG values. Mortality was recorded daily throughout the experiment, and the performance data were adjusted accordingly.

Carcass traits

Upon completion of the 42-day trial, two birds per replicate nearest to the pen mean body weight were selected and slaughtered following blood collection. After feather removal, the carcass was eviscerated and internal organs including the spleen, bursa of Fabricius, and abdominal fat were weighed using a calibrated digital balance (A&D Company, GF3000, Japan). Carcass (with viscera), breast, and thigh weights were also recorded.

Table 2. Ingredients and compositions of the control diet+20% Met over ROSS recommendation

Ingredients (%)	Starter (1-10 d)	Grower (11-25 d)	Finisher (26-42 d)
Corn	50.71	55.24	58.69
Soybean meal	41.59	37.50	33.49
Soybean oil	3.48	3.54	4.33
Di-Calcium phosphate	1.62	1.40	1.20
Limestone	1.28	1.20	1.12
Sodium chloride	0.31	0.31	0.31
DL-Methionine	0.27	0.23	0.19
L-Lysine	0.24	0.17	0.17
Vitamin and mineral premix [1]	0.50	0.50	0.50
Calculated analysis (DM basis)			
Metabolizable energy (kcal/kg)	3000	3000	3150
Crude protein (%)	23.00	21.50	20.00
Calcium (%)	0.96	0.87	0.81
Available phosphorus (%)	0.48	0.43	0.40
Sodium (%)	0.16	0.16	0.16
Chlorine (%)	0.23	0.23	0.23
Lysine (%)	1.44	1.29	1.19
Methionine (%)	0.67	0.61	0.54

[1] Vitamin premix supplied the following per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 1500 IU; tocoferol, 60 IU; Phyllo Quinone, 2 mg; thiamine, 2.4 mg; riboflavin, 4.8 mg; niacin, 30 mg; pantothenic acid, 16 mg; pyridoxine, 3 mg; folic acid, 1 mg; vitamin B12, 0.03 mg; biotin, 0.15 mg; and cholin chloride, 50 mg. Mineral premix supplied the following per kilogram of diet: Mn, 80 mg; Fe, 120 mg; Zn, 60 mg; Cu, 100 mg; I, 0.95 mg; and Se, 0.25 mg.

Blood biochemistry

At the end of the experimental period, blood samples were obtained from the brachial vein of selected broilers at 42 days of age. Approximately 3 mL of blood was collected from each bird and centrifuged at 3,000 rpm for 10 min to separate serum. Serum samples were transferred into sterile microtubes and stored at −20 °C until biochemical analyses were performed. Serum glucose (GLU), total cholesterol (CHO), triglycerides (TRI), total protein (TP), and albumin (ALB) concentrations were determined spectrophotometrically using commercial diagnostic kits (Pars Azmoon, Tehran, Iran) according to the manufacturer's analytical procedures.

Tibia bone characteristics

For skeletal assessment, two broiler chickens per replicate whose body weight approximated the pen mean were selected and slaughtered at the end of the experimental period (42 days of age). The right tibia was harvested following careful removal of all non-osseous tissues (muscle, fat, tendon, etc.). Tibial length and diaphysis diameter were determined with a digital caliper (GENERAL, USA; ±0.01 mm). Following morphometric assessment, tibia samples were subjected to lipid extraction in a chloroform–methanol solution (2:1 ratio) for 72 hours (Calik et al., 2019), then oven-dried at 105°C for 24 hours (Fan Azma Gostar, BF120S, Iran). Fat-free dry weight was determined on a precision digital balance (A&D, GF3000, Japan; ±0.01 g). Breaking strength (N) was assessed using a universal materials testing apparatus (Santam STM-5, Korea) at a crosshead speed of 20 mm/s with a 50-mm inter-support span (Santos et al., 2022). Ash content was established gravimetrically following incineration in a muffle furnace (Fan Azma Gostar, FM6P, Iran) at 550°C for 24 hours (Sohail et al., 2024).

Statistical analysis

All experimental data were analyzed using the GLM procedure of SAS software (version 9.4) based on a completely randomized design with a 2×2 factorial arrangement. The statistical model included the main effects of dietary L-carnitine and Met levels as well as their interaction. Differences among treatment means were compared using the Tukey's multiple comparison test, and statistical significance was set at P≤0.05.

Results

Growth performance

Whole-period growth performance data are presented in Table 3. Supplementing the diets with 250 mg of L-carnitine significantly reduced FI and FCR and improved BWG (P≤0.05). Birds fed with the H-Met diet also showed greater BWG and lower FCR (P≤0.05), with no significant change in FI (P>0.05). The interaction between L-carnitine and Met significantly affected BWG and FCR but no FI. The greatest BWG and most favorable FCR were recorded in birds receiving the combined 250 mg L-carnitine and H-Met diet.

Table 3. The effects of L-carnitine and high Met level on growth performance of broilers at 42 days of age

Treatments	Feed intake (g/bird)	Weight gain (g/bird)	FCR
L-carnitine (mg/kg)			
0	4148.19	2299.62	1.81
250	4070.74	2370.03	1.73
SEM	15.01	8.37	0.0065
P-value	0.003	<.0001	<.0001
Methionine			
ROSS	4123.95	2284.58	1.80
ROSS + 20%	4094.98	2358.06	1.72
SEM	15.01	8.37	0.005
P-value	0.19	<.0001	<.0001
L-carnitine × Methionine			
0 × ROSS	4179.74	2233.12[c]	1.87[a]
250 × ROSS	4068.15	2336.04[b]	1.75[b]
0 × ROSS + 20%	4116.64	2366.10[a]	1.74[b]
250 × ROSS + 20%	4073.33	2404.03[a]	1.70[c]
SEM	21.22	11.83	0.007
P-value	0.13	0.02	<.0001

[a,b]: Within column, means with common superscript (s) are not different (P>0.05).

Carcass traits

Data on carcass traits at 42 days of age are presented in Table 4. Broilers fed with the diets supplemented with 250 mg/kg L-carnitine and H-Met showed greater relative carcass and breast weights and lower abdominal fat percentage compared with the control treatment. Dietary treatments did not affect the relative weights of the thigh, spleen, or bursa of Fabricius. A significant interaction between L-carnitine and Met supplementation was observed for the relative carcass weight and abdominal fat percentage (P≤0.05). The combined L-carnitine and H-Met treatment resulted in the greatest carcass yield and the lowest abdominal fat deposition. No interaction was detected for breast, thigh, spleen, or bursa relative weights.

Table 4. The effects of L-carnitine and high Met level on relative organ weight (relative weight: g/100 g of live weight) of broilers at 42 days of age

Treatments	Carcass[1]	Breast	Thigh	Spleen	Bursa of Fabricius	Abdominal fat
L-carnitine (mg/kg)						
0	84.48	22.39	23.34	0.12	0.15	1.18
250	86.65	25.22	23.30	0.11	0.15	0.98
SEM	0.17	0.25	0.40	0.006	0.01	0.05
P-value	<.0001	<.0001	0.95	0.10	0.82	0.01
Methionine						
ROSS	84.71	22.98	23.39	0.12	0.15	1.32
ROSS + 20%	86.42	24.63	23.25	0.11	0.15	0.84
SEM	0.17	0.25	0.40	0.006	0.01	0.05
P-value	<.0001	0.0005	0.81	0.08	0.85	<.0001
L-carnitine × Methionine						
0 × ROSS	83.32[b]	21.43	23.38	0.13	0.14	1.54[a]
250 × ROSS	86.11[a]	24.53	23.39	0.12	0.15	1.09[b]
0 × ROSS + 20%	85.65[a]	23.36	23.29	0.11	0.15	0.82[c]
250 × ROSS + 20%	87.19[a]	25.90	23.21	0.10	0.15	0.86[c]
SEM	0.24	0.35	0.56	0.008	0.02	0.07
P-value	0.02	0.44	0.94	0.73	0.79	0.003

[a,b]: Within column, means with common superscript (s) are not different (P>0.05).
[1] Whole carcass with viscera.

Blood metabolites

Serum biochemical data are shown in Table 5. Dietary supplementation with L-carnitine and H-Met did not influence serum GLU concentration. Broilers fed with diets containing 250 mg/kg L-carnitine exhibited lower serum CHO and TRI concentrations than the control birds. Serum TP and ALB concentrations increased due to dietary supplementation with L-carnitine and H-Met. A significant interaction effect was observed only for ALB concentration (P≤0.05). The greatest ALB level was detected in broilers receiving both L-carnitine and H-Met. No interaction effects were observed for GLU, CHO, TRI, or TP concentrations.

Table 5. The effects of L-carnitine and high Met level on blood metabolites of broilers at 42 days of age

Treatments	GLU (mg/dl)	CHO (mg/dl)	TRI (mg/dl)	TP (g/dl)	ALB (g/dl)
L-carnitine (mg/kg)					
0	284.62	119.50	49.00	4.47	1.21
250	286.62	114.87	45.25	4.72	1.39
SEM	6.65	1.08	0.86	0.05	0.04
P-value	0.83	0.01	0.009	0.004	0.006
Methionine					
ROSS	286.12	116.37	46.00	4.44	1.20
ROSS + 20%	285.12	118.00	48.25	4.76	1.40
SEM	6.65	1.08	0.86	0.05	0.04
P-value	0.92	0.31	0.09	0.0007	0.003
L-carnitine × Methionine					
0 × ROSS	283.50	120.00	48.50	4.25	1.05[c]
250 × ROSS	288.75	112.75	43.50	4.62	1.35[b]
0 × ROSS + 20%	285.75	119.00	49.50	4.70	1.37[b]
250 × ROSS + 20%	284.50	117.00	47.00	4.82	1.42[a]
SEM	9.41	1.53	1.22	0.07	0.05
P-value	0.73	0.11	0.32	0.10	0.04

[a,b]: Within column, means with common superscript (s) are not different (P>0.05).

Tibial bone characteristics

Dietary L-carnitine supplementation increased tibial dry weight, tibial length, and bone breaking strength (P≤0.05; Table 6). However, tibial dry matter and ash percentage, and diaphysis diameter were not affected by L-carnitine supplementation. Broilers fed H-Met diets showed greater bone breaking strength than that of the control group. In contrast, tibial dry weight, dry matter content, ash percentage and length, and diaphysis diameter were not influenced by dietary Met level. A significant interaction effect between L-carnitine and Met supplementation was observed only for diaphysis diameter (P≤0.05). No interaction effects were detected for tibial dry weight, dry matter percentage, ash content and length, and bone breaking strength.

Table 6. Effects of L-carnitine and high Met level on tibial bone characteristics of broilers at 42 days of age

Treatments	Dry weight (g)	Dry matter (%)	Ash (%)	Length (mm)	Diaphysis diameter (mm)	Breaking strength (N)
L-carnitine (mg/kg)						
0	5.07	97.49	46.52	82.63	7.20	157.01
250	6.48	95.64	48.05	90.23	7.18	247.27
SEM	0.31	1.28	1.55	1.20	0.22	21.65
P-value	0.008	0.33	0.50	0.0008	0.96	0.01
Methionine						
ROSS	5.62	96.22	46.34	85.79	7.20	168.04
ROSS + 20%	5.94	96.91	48.23	87.07	7.19	236.25
SEM	0.31	1.28	1.55	1.20	0.22	21.65
P-value	0.48	0.71	0.41	0.4668	0.99	0.04
L-carnitine × Methionine						
0 × ROSS	5.06	97.52	45.25	80.83	7.56^{a}	121.37
250 × ROSS	6.17	94.91	47.43	90.74	6.83^{b}	214.70
0 × ROSS + 20%	5.09	97.45	47.79	84.43	6.84^{b}	192.65
250 × ROSS + 20%	6.79	96.36	48.67	89.70	7.54^{a}	279.85
SEM	0.44	1.81	2.20	1.70	0.31	30.61
P-value	0.52	0.68	0.77	0.19	0.04	0.92

^{a,b}: Within column, means with common superscript (s) are not different (P>0.05).

Discussion

Broilers receiving 250 mg/kg dietary L-carnitine demonstrated notable improvements in growth indices, as evidenced by decreased FI and FCR and increased BWG. Comparable responses were reported by Azizi-Chekosari et al. (2021) who observed increased BWG and reduced FI and FCR when 200 and 400 mg/kg L-carnitine were added to the diets of broilers. These improvements are primarily explained by the role of L-carnitine in transporting long-chain fatty acids into the mitochondria for β-oxidation, thereby increasing ATP production and promoting lean tissue accretion rather than fat deposition (Mosa et al., 2018; Kuter and Önol, 2021). Furthermore, L-carnitine has been reported to increase circulating IGF-I levels, a key anabolic hormone in poultry, which may further contribute to the improved BWG observed in supplemented broilers (Xu et al., 2003). El-Kelawy (2017) and Panahi et al. (2019) also reported improved BWG and reduced FI and FCR in broiler chickens following L-carnitine supplementation. Moreover, reductions in FCR were observed when broilers received 100 mg/kg L-carnitine combined with methionine at 25% above the NRC recommendations (Kuter and Önol, 2021), suggesting that improved feed efficiency may result from decreased lipogenesis or enhanced lipolysis and β-oxidation, ultimately leading to greater protein deposition (Kuter and Önol, 2021).

Broilers fed H-Met diets showed greater BWG and lower FCR without changes in FI. Similar findings were reported by Ghoreyshi et al. (2019) who observed improved BWG and FCR in broilers fed diets containing different levels of Met (equal to Ross, 15 and 30% Ross recommendation) in combination with L-carnitine (equal to Ross, 15 and 75% Ross recommendation). Previous studies have likewise demonstrated positive effects of dietary Met supplementation on BWG and FCR in broiler chickens (Wang et al., 2019a; b). The beneficial effects on these production indices are largely attributed to the capacity of synthetic methionine to stimulate protein biosynthesis (Ghoreyshi et al., 2019). Beyond its essential role in protein synthesis, Met is converted to S-adenosylmethionine (SAM), the principal methyl-group donor involved in cellular methylation reactions. Moreover, through the transsulfuration pathway, Met contributes to the synthesis of cysteine and glutathione, thereby supporting cellular antioxidant defense. These metabolic functions may improve cellular redox balance and nutrient utilization, potentially contributing to the improved growth performance observed in H-Met-fed broilers (Park et al., 2018; Fagundes et al., 2020).

Dietary supplementation with L-carnitine increased the relative carcass and breast yields while reducing abdominal fat deposition in broiler chickens. Similar responses were reported by Panahi et al. (2019) who observed improved carcass and breast yield following supplementation with 300 mg/kg L-carnitine. Azizi-Chekosari et al. (2021) also demonstrated that inclusion of 400 mg/kg L-carnitine increased breast yield and reduced abdominal fat without affecting the relative weights of the thigh, heart, liver, or pancreas. Likewise, Jaffari Golrokh et al. (2016) reported greater carcass and breast yields in broilers receiving 150 and 300 mg/kg dietary L-carnitine. The reduction in abdominal fat may be attributed to the role of L-carnitine in facilitating the mitochondrial transport of long-chain fatty acids for β-oxidation, thereby promoting fatty acid utilization for energy production rather than storage in adipose tissue (Kuter and Önol, 2021).

The relative weights of carcass and breast were significantly greater in H-Met-fed broilers, while abdominal fat deposition was significantly reduced. Similar findings were reported by Wang et al. (2019a) who demonstrated that dietary supplementation with different levels of L-Met and DL-Met (75, 80, 85, 90, 95, and 100% of Ross requirements) improved carcass, breast, and thigh yields while reducing abdominal fat in broilers at 21 d of age. Increased carcass and breast yields following Met supplementation have also been documented previously (Linh et al., 2021). The improvement in carcass characteristics may be related to the role of Met in maintaining amino acid balance and stimulating muscle protein synthesis (Si et al., 2004). Because breast muscle growth is highly responsive to dietary amino acid supply, elevated Met levels may enhance breast muscle accretion in rapidly growing broilers (Si et al., 2004). In agreement with the present findings, Elsharkawy et al. (2021) reported reduced abdominal fat deposition in broilers supplemented with dietary Met. Mechanistically, this reduction may be partly associated with the contribution of Met to endogenous L-carnitine synthesis and the stimulation of hormone-sensitive lipase activity. Increased carnitine availability may facilitate mitochondrial fatty acid utilization, while enhanced lipolytic activity may promote the mobilization of stored lipids, collectively favoring fatty acid oxidation over lipid deposition (Zhan et al., 2006). However, as these metabolic pathways were not directly evaluated in the present study, they should be considered potential mechanisms underlying the observed reduction in abdominal fat.

Regarding serum biochemical responses, dietary supplementation with L-carnitine reduced serum CHO and TRI concentrations without significantly affecting GLU levels, which is consistent with previous findings in broiler chickens (El-Kelawy, 2017; Wang et al., 2019b). El-Kelawy (2017) likewise reported that dietary inclusion of 0, 50, 100, and 150 mg/kg L-carnitine decreased serum CHO and TRI concentrations while increasing serum TP in broilers. Similar reductions in circulating lipids following L-carnitine supplementation have also been documented by Azizi-Chekosari et al. (2021) who observed lower CHO and TRI together with elevated TP and ALB concentrations in broiler chickens. The reduction in serum lipid concentrations may be attributed to the role of L-carnitine in facilitating mitochondrial transport and β-oxidation of long-chain fatty acids, thereby enhancing lipid utilization and contributing to lower circulating CHO and TRI concentrations (Zhang et al., 2010). In addition, L-carnitine may promote TRI hydrolysis through modulation of lipolytic enzyme activity, which could further contribute to lower serum TRI concentrations. The higher TP and ALB concentrations observed in L-carnitine-supplemented broilers may also indicate improved protein utilization and reduced protein degradation (Azizi-Chekosari et al., 2021). Therefore, the favorable changes in serum biochemical parameters observed in the present study likely reflect improved lipid metabolism and metabolic efficiency in broiler chickens receiving dietary L-carnitine supplementation.

In this study, H-Met supplementation increased the serum total protein and albumin but had no significant effect on serum GLU, CHO, and TRI, which is in agreement with earlier studies (Brahmaiah et al., 2018). Similarly, Mirzaei et al. (2022) reported that dietary supplementation with 75 mg/kg L-carnitine and lysine–methionine (100% of the NRC recommendations) increased serum TP and ALB in breeder ducks, suggesting a similar response of these protein metabolism-related parameters in another poultry species. This might be due to increasing protein biosynthesis by supplementation of synthetic Met (Brahmaiah et al., 2018). Likewise, dietary supplementation with 0.32%, 0.37%, and 0.42% DL-methionine increased serum TP and ALB without affecting CHO in broiler chickens (Attia et al., 2005). Therefore, the increased serum TP and ALB concentrations observed in the present study may be associated with enhanced protein biosynthesis following H-Met supplementation (Brahmaiah et al., 2018).

The link between genetic selection for rapid growth and greater vulnerability to leg disorders in broiler chickens has been well established (Dixon, 2020). Bone strength represents a key determinant in maintaining the welfare of broilers intended for slaughter and carcass processing. Adequate bone development is influenced by proper growth and the dietary availability of calcium and phosphorus (Wang et al., 2014). Depending on the degree of bone mineralization, physical properties such as strength can be modulated through nutritional strategies (Akter et al., 2020; Kwiecień et al., 2023). Consistent with the findings of the present study, Kwiecień et al. (2023) reported that supplementation of L-carnitine (30 g/L) in drinking water enhanced femoral bone weight, length, and strength parameters, without affecting crude ash percentage in female turkeys. Although evidence in broiler chickens is limited, Hooshmand et al. (2008) demonstrated that dietary supplementation with 150 mg/kg L-carnitine increased tibial dry weight and bone density in rats, suggesting that L-carnitine may play a beneficial role in bone metabolism. Mechanistically, L-carnitine may contribute to improved bone strength by promoting osteoblastic activity and osteogenesis while attenuating mitochondrial-derived oxidative stress, thereby enhancing bone formation and providing a plausible biological explanation for the favorable bone responses observed in the present study (Colucci et al., 2005; Terruzzi et al., 2019).

The contribution of Met to bone development seems to be restricted and is mainly associated with its sulfur-donating capacity, which may affect cartilage growth via augmented proteoglycan synthesis (Huang et al., 2014). It has been demonstrated that Met deficiency negatively affects bone growth and development, leading to the formation of smaller and thinner bones (Huang et al., 2014). A study examining varying levels of L-Met supplementation (DL-Met recommended requirement, 0.20, 0.25, and 0.30% L-Met) on tibial characteristics in broilers found that L-Met did not produce any significant changes in bone weight, length, width, bone head width, or bone calcium and phosphorus content (Akter et al., 2020). However, dietary inclusion of different levels of DL-Met and L-lysine (0.2% and 0.4%) in broiler diets increased tibial calcium, phosphorus, and potassium content, and the tibiotarsal index, without significantly affecting bone weight-to-length ratio, bone strength index, ash content, or magnesium levels (Ogunwole et al., 2016). Methionine supplementation also enhanced mechanical properties and tibial strength in rearing gilts (Fabà et al., 2019). The improvement in tibial strength observed in H-Met-fed broilers may therefore be partly attributed to the role of adequate Met supply in supporting cartilage matrix formation and bone mineralization, thereby contributing to the structural development and mechanical integrity of the tibia (Huang et al., 2014; Ogunwole et al., 2016).

Conclusion

Dietary supplementation with 250 mg/kg L-carnitine and H-Met significantly improved broiler performance by reducing FI and FCR while increasing BWG. The combined supplementation was associated with greater carcass and breast weights and lower abdominal fat without affecting the lymphoid organs. L-carnitine lowered serum CHO and TRI and, together with H-Met, elevated TP and ALB, with their interaction further increasing the ALB levels. Additionally, L-carnitine improved tibial dry weight, length, and strength, while H-Met enhanced bone strength. A significant interaction between L-carnitine and H-Met was observed for diaphysis diameter. Accordingly, dietary supplementation with L-carnitine and H-Met may improve growth performance, carcass traits, selected serum biochemical parameters, and tibial characteristics in broiler chickens.

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Conflict of interest

The authors declare no conflict of interest.

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