BMC Veterinary Research

RESEARCH

Open Access

Dietary L-carnitine modulates oxidative stress, lipid metabolism, and pulmonary hypertension in chickens raised at high-altitude under cold stress

Behnam Ahmadipour[1*], Masoud Hayat Isfahani[1], Samira Abaszadeh[1], Hossein Hassanpour[2,3] and Fariborz Khajali[1]

Abstract

Background This study evaluated L-carnitine's impact on oxidative stress, lipid metabolism, and pulmonary hypertensive response in broilers under high-altitude and cold stress.

Method A total of 225 male broiler chicks (Ross 308, 38.5 ± 0.5 g) were raised at 2100 m altitude and exposed to cold stress. Birds were divided into three groups: control, LC-50 (50 mg/kg L-carnitine), and LC-100 (100 mg/kg L-carnitine). At 42 days, blood and tissues were collected to measure malondialdehyde (MDA), nitric oxide (NO), hematocrit, and right ventricular to total ventricular weight (RV: TV) ratio (PHS indicator). Gene expression of *superoxide dismutase 1 (SOD1)*, *glutathione peroxidase (GPX)*, *catalase (CAT)*, *carnitine palmitoyltransferase 1 (CPT1)*, *carnitine palmitoyltransferase 2 (CPT2)*, and *inducible nitric oxide synthase (iNOS)* was analyzed in the heart, lung, and hindbrain using real-time quantitative polymerase chain reaction (RT-qPCR).

Results The RV: TV ratio, MDA levels, and hematocrit (%) significantly decreased in the LC-50 and LC-100 groups compared to the control group, while nitric oxide levels increased (For all $P < 0.05$). The expression of *SOD1*, *GPX*, *CAT*, and *iNOS* genes was significantly increased in the heart, lung, and hindbrain of the LC-100 group compared to the control group ($P < 0.05$), while *CPT1* and *CPT2* expression increased significantly only in the heart and hindbrain ($P < 0.05$).

Conclusion Dietary L-carnitine, especially at 100 mg/kg, effectively alleviates oxidative stress, enhances vasodilation, improves lipid metabolism, and mitigates pulmonary hypertensive responses in broilers exposed to high-altitude hypoxia and cold stress.

Keywords Ascites, Gene expression, L-carnitine, Lipid metabolism, Oxidative stress

*Correspondence:
Behnam Ahmadipour
behnam.ahmadipour@gmail.com
[1]Department of Animal Sciences, Faculty of Agriculture, Shahrekord University, Shahrekord, Iran
[2]Department of Basic Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
[3]Department of Health Equity Research Center, Shahed University, Tehran, Iran

Introduction

Broiler chickens raised at high-altitude are exposed to hypobaric hypoxia which negatively affects their growth, metabolism, and overall health [1]. Hypoxia disrupts normal metabolic processes and induces oxidative stress, marked by an imbalance between reactive oxygen species (ROS) production and antioxidant defenses [2]. This imbalance can lead to cellular damage, reduced growth performance, and induction of pulmonary hypertension syndrome (PHS) in these birds [3]. Concurrently, cold stress increases energy demands as birds expend more energy to maintain their body temperature, diverting resources away from growth and productivity [4]. This heightened metabolic rate also accelerates the production of ROS, exacerbating oxidative stress. The combined effect of hypoxia and cold stress creates a synergistic burden, increasing the birds' vulnerability to PHS [5].

PHS arises when the cardiopulmonary system fails to meet the oxygen demands of rapidly growing tissues, particularly under hypoxic conditions leading to pulmonary vasoconstriction, increasing the workload on the right ventricle of the heart. Over time, this results in right ventricular hypertrophy and, eventually, right-sided heart failure [6].

Nitric oxide (NO), is a critical signaling molecule produced by endothelium and other cells. NO plays a vital role in regulating vascular tone by promoting vasodilation and maintaining blood flow. Under hypoxic conditions, NO is often reduced due to its scavenging by ROS. This reduction in NO disrupts vascular homeostasis, leading to increased vasoconstriction and PHS development [7, 8].

The body's defense against oxidative stress relies on endogenous antioxidant enzymes, such as superoxide dismutase 1 (SOD1), glutathione peroxidase (GPX), and catalase (CAT). SOD1 catalyzes the dismutation of superoxide radicals ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2) and oxygen (O_2). GPX then reduces hydrogen peroxide and lipid hydroperoxides to water and alcohol. CAT further breaks down hydrogen peroxide into water and oxygen, preventing the formation of highly reactive hydroxyl radicals through the Fenton reaction. In high-altitude environments, the overproduction of ROS overwhelms the antioxidant defense system, leading to oxidative damage to lipids, proteins, and DNA [9, 10].

Moreover, hypoxia and oxidative stress impair lipid metabolism which plays a central role in energy production [11, 12]. A critical aspect of lipid metabolism involves the transport of fatty acids into the mitochondria for β-oxidation, a process facilitated by carnitine palmitoyltransferases (CPT1 and CPT2). CPT1 converts fatty acids to acylcarnitines, which are shuttled into the mitochondria by the translocase and reconverted back to fatty acyl-CoA by CPT2 for β-oxidation and ATP production [13, 14]. Under hypoxic conditions, the activity of CPT1 and CPT2 can be impaired, leading to reduced fatty acid oxidation, accumulation of toxic lipid intermediates, and energy deficiency. This metabolic dysfunction exacerbates the strain on the heart and liver, further contributing to the development of PHS [15]. In this context, L-carnitine supplementation emerges as a potential therapeutic strategy to mitigate the pathophysiological mechanisms underlying PHS. L-carnitine supplementation in broiler chicken diets has been shown to influence various performance and health parameters. While low doses generally do not significantly affect growth performance, higher doses can improve body weight and feed conversion ratio. Additionally, L-carnitine reduces abdominal fat percentage, lowers blood triglycerides, cholesterol, and LDL levels, and enhances immune responses as indicated by higher antibody titers against Newcastle and influenza viruses [16]. L-carnitine alleviates the metabolic system by enhancing fatty acid oxidation and energy production [17, 18]. Its role in supporting CPT1 and CPT2 activity ensures efficient transport and utilization of fatty acids in the mitochondria, reducing the accumulation of toxic lipid intermediates and improving energy homeostasis. Furthermore, L-carnitine's antioxidant properties help neutralize ROS, reducing oxidative damage [19].

This study aims to assess the effects of dietary L-carnitine on lipid peroxidation, serum nitric oxide levels, and the expression of *SOD1*, *GPX*, *CAT*, *CPT1*, *CPT2*, and *iNOS* genes in broiler chickens raised at high altitude under cold stress, with a focus on pulmonary hypertensive responses. While previous studies have established L-carnitine's benefits under hypoxia or cold stress independently, our work reveals its synergistic role in mitigating combined stressors through tissue-specific modulation of oxidative, metabolic, and neural pathways. The upregulation of *CPT1* and *CPT2* in the hindbrain, along with dose-dependent antioxidant effects, provides novel mechanistic insights into its cardiopulmonary protective properties.

Methods

Bird management and experimental facility

225 day-old male broiler chicks (Ross 308, 38.5 ± 0.5 g) were purchased from SepidMakian Co. (Iran, Gilan) and randomly assigned to 45 floor pens (1.5 m^2, 15 birds/pen, 5 replicates/treatment), each equipped with a bell drinker and feed trough. After a 5-day acclimation period on a commercial diet, runts were removed, and chicks were re-allocated to ensure equal average body weight per pen. To induce cold stress, environmental temperatures were gradually reduced (32 ± 1 °C to 15 ± 1 °C) from day 1 to 42, based on Ahmadipour, et al. [20]. Chicks had ad libitum access to feed and water under a 23 L:1D lighting

schedule throughout the trial. The control diet, based on corn and soybean meal, was formulated according to Aviagen [21] nutritional requirements for three growth phases: starter (1–10 days), grower (11–22 days), and finisher (23–42 days) (Table 1). In addition to the control diet, two experimental diets were prepared with the addition of L-carnitine (Carniking®, Lohmann Co. Ltd., Germany) at concentrations of 50 mg/kg (LC-50) and 100 mg/kg (LC-100). These selected doses were based on findings from our prior studies and other research, which demonstrated the impact of L-carnitine on oxidative stress, lipid metabolism, growth performance, and immune responses in broiler chickens [22–24].

Mortality rate from ascites was recorded daily throughout the experimental period (1–42 days) to assess the impact of dietary treatments on broiler survival.

Tissue/plasma sampling and PHS index Estimation

At 42 days, 10 birds per group (2 birds/pen) were randomly selected for blood and tissue collection. Blood samples (4 mL) were collected in EDTA tubes from the brachial vein, centrifuged at 2500 g for 10 min to obtain plasma, and stored at -20 °C for biochemical analysis. Birds were then euthanized by cervical dislocation, and the brain, lungs, and heart were dissected. After removing vessels/atria from the heart ventricles, the RV: TV ratio was calculated. RV: TV ratios > 0.25 indicated developmental PHS [25], while ratios > 0.29 indicated full-blown PHS and ascites [26]. The whole brain was removed from the skull, then the hindbrain was separated according to Hassanpour et al. [27]. The heart (right ventricle), hindbrain, and lung tissues were immediately snap-frozen in liquid nitrogen and stored at -70 °C for RNA extraction.

Measurement of blood nitric oxide, malondialdehyde, and hematocrit levels

Nitric oxide is an unstable gas in blood that rapidly degrades into stable metabolites. This study measured nitrite, a major NO metabolite, as an indicator of nitric oxide levels. Nitrite was quantified using a Griess reaction after reducing nitrate to nitrite with activated

Table 1 Composition of the basal diet fed to broilers (Ross 308) from 1 to 42 days of age

Item (% unless noted)	Starter (1–10d)	Grower (11-22d)	Finisher (23–42d)
Corn	53.76	56.76	62.48
Soybean meal (44% CP)	36.90	36.52	30.32
Corn Gluten Meal	3.46	-	-
Soy oil	1.34	2.67	3.47
Dicalcium phosphate	2.15	1.91	1.65
CaCO3	0.68	0.61	0.57
Salt	0.16	0.20	0.19
Na-Bicarbonate	0.29	0.24	0.25
DL-Methionine	0.31	0.29	0.27
L-Lysine	0.28	0.17	0.18
L-Threonine	0.11	0.09	0.07
Choline Chloride	0.06	0.04	0.05
Mineral supplement*	0.25	0.25	0.25
Vitamin supplement**	0.25	0.25	0.25
Calculated composition			
Dry Matter	89.026	88.934	88.920
AME (kcal/kg)	2890.00	2960.00	3085.0
CP	22.75	20.59	18.27
Met	0.631	0.574	0.528
Met + Cys	0.910	0.833	0.764
Lys	1.23	1.110	0.980
Thr	0.824	0.744	0.657
Arg	1.31	1.252	1.087
Na	0.157	0.157	0.157
Cl	0.204	0.204	0.204
K	0.931	0.926	0.817
Na + K–Cl (mEq/kg)	249.10	247.82	219.92

* Provided the following per kg of diet: vitamin A (trans retinyl acetate), 3600IU; vitamin D3 (cholecalciferol), 800 IU; vitamin E (dl-α-tocopheryl acetate), 7.2 mg; vitamin K3, 1.6 mg; thiamine, 0.72 mg; riboflavin, 3.3 mg; niacin, 0.4 mg; pyridoxin, 1.2 mg; cobalamine, 0.6 mg; folicacid, 0.5 mg; choline chloride, 200 mg

** Provided the following per kg of diet: Mn (from MnSO4-H2O), 40 mg; Zn (from ZnO), 40 mg; Fe (from FeSO4-7H2O), 20 mg; Cu (from CuSO4-5H2O), 4 mg; I [from Ca (IO3)2-H2O], 0.64 mg; Se (from sodium selenite),0.08 mg

cadmium as previously described [28]. Plasma samples were deproteinized by mixing 300 µL of plasma with 250 µL of 75 mmol/L zinc sulfate, vortexed, and centrifuged at 10,000 g for 1 min at room temperature. Then, 350 µL of 55 mmol/L NaOH was added, followed by a second centrifugation at 10,000 g for 3 min. The clear supernatant (750 µL) was diluted with 250 µL of glycine buffer (45 g/L, pH 9.7). Activated cadmium granules, pre-treated with 5 mmol/L $CuSO_4$ in glycine-NaOH buffer, were added to the mixture and stirred for 10 min to reduce nitrate to nitrite. For the Griess reaction, 75 µL of Reagent 1 (1% sulfanilamide in 5% phosphoric acid) and 80 µL of Reagent 2 (0.1% N-naphthylethylenediamine dihydrochloride) were added sequentially. Absorbance was measured at 540 nm using a spectrophotometer. Nitrite concentrations were calculated from a standard curve and expressed as µmol/L.

Malondialdehyde, a key biomarker for lipid peroxidation, was measured in plasma samples using the thiobarbituric acid reactive substances (TBARS) assay as decribed previously [29]. Briefly, This assay was performed by mixing 100 µL plasma with 200 µL 8.1% SDS, 1.5 mL sodium acetate buffer (3.5 M, pH 4), and 1.5 mL 0.8% thiobarbituric acid. Samples were heated at 95 °C for 1 h, cooled on ice for 30 min, centrifuged (1,500 × g, 10 min, 4 °C), and supernatant absorbance measured at 532 nm. A standard curve was prepared alongside samples. The results from the TBARS assay were converted to µmol/L.

The hematocrit, expressed as a percentage, was determined by centrifuging blood samples in heparinized capillary tubes at 12,000 g for 5 min.

RNA extraction and cDNA synthesis

Total RNA from hindbrain, lung, and heart (right ventricle) tissues was extracted using RNX-Plus solution (Sinaclon Bioscience, Karaj, Iran) and the acid guanidinium thiocyanate-phenol-chloroform single-step method [30]. The resulting RNA pellet was resuspended in 20 µl of diethyl pyrocarbonate (DEPC)-treated water and treated with RNAase-free DNAase (Sinaclon) to remove contaminating genomic DNA. Spectrophotometry was used to assess the quality and integrity of the RNA samples. Only RNA samples with an A260/A280 ratio between 1.8 and 2.2 were used for cDNA synthesis. cDNA synthesis was performed using the Easy cDNA Synthesis Kit (Parstous Co., Mashhad, Iran) according to the manufacturer's instructions, and the resulting cDNA was stored at -20 °C until real-time quantitative PCR (RT-qPCR) was performed.

Gene expression assay by RT-qPCR

To investigate potential changes in the transcriptional levels of *iNOS*, *CPT1*, *CPT2*, *SOD1*, *GPX*, *CAT*, and *tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ)* across three experimental groups, RT-qPCR was performed using SYBR Green Master Mix-Plus (Amplicon, Odense, Denmark). *YWHAZ* served as a stable control gene for normalizing cDNA input and quantifying relative target gene expression. Specific primers for each gene are detailed in Table 2. Each PCR reaction was performed in triplicate with 10 ng of cDNA and 400 nM of each specific primer in a total volume of 10 µl. The PCR amplification program consisted of an initial denaturation at 94 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 15 s, annealing at 60–62 °C for 10–20 s, and extension at 72 °C for 15 s. No-template and no-reverse transcriptase controls were included in each PCR reaction. Threshold cycle numbers and mean efficiency values were recorded and calculated using LinRegPCR software. Relative gene

Table 2 Details of the primers used for quantitative real-time PCR analysis for chickens

Target	Primers (5′-3′)	PCR product	Accession no.
YWHAZ	AGGAGCCGAGCTGTCCAATG	84 bp	NM_001031343.1
	CTCCAAGATGACCTACGGGCTC		
SOD1	CACTGCATCATTGGCCGTACCA	224 bp	NM_205064.1
	GCTTGCACACGGAAGAGCAAGT		
GPX1	GCTGTTCGCCTTCCTGAGAG	118 bp	NM_001277853.1
	GTTCCAGGAGACGTCGTTGC		
CAT	TGGCGGTAGGAGTCTGGTCT	112 bp	NM_001031215.1
	GTCCCGTCCGTCAGCCATTT		
iNOS	AGGCCAAACATCCTGGAGGTC	371 bp	U46504
	TCATAGAGACGCTGCTGCCAG		
CPT1	TGTGAGTGATTGGTGGGAAGAG	117 bp	NM_001012898.1
	GCTGCCTGTATGGTTGTGGG		
CPT2	GGGTCGTGTTGGGCTGTT	106 bp	NM_001031287.3
	CTGGGCAGGCTCTTCTGGTA		

YWHAZ, tyrosine 3-monooxygenase/tryptopHan 5-monooxygenase activation protein zeta; SOD1, Superoxide dismutase 1; GPX, Glutathione peroxidase; iNOS, Inducible nitric oxide synthase; CAT, Catalase, CPT1, Carnitine palmitoyltransferase 1; CPT2, Carnitine palmitoyltransferase 2; bp, base pair

Table 3 Effect of L-carnitine on plasma-blood and heart variables in broiler chickens (42 days of age)

Variables	Control	LC-50	LC-100	SEM	*p*-value
Malondialdehyde (μmol/L)	3.39[a]	2.51[b]	1.93[b]	0.235	0.003
Nitric oxide (μmol/L)	2.52[b]	5.61[a]	5.93[a]	0.871	0.031
Hematocrit	47.4[a]	38.2[b]	36.8[b]	2.60	0.028
RV: TV	0.29[a]	0.22[b]	0.21[b]	0.017	0.006
Ascites mortality (%)	18.0	14.0	8.0	2.58	0.284

[a, b,c] Means in the same raw with different letter superscripts are significantly different. LC-50, L-carnitine supplementation at 50 mg/kg; LC-100, L-carnitine supplementation at 100 mg/kg; RV: TV, right ventricle to total ventricle weight ratio

Table 4 Effects of L-carnitine on expression of genes in different tissues of broiler chickens (42 days of age)

Genes	Control	LC-50	LC-100	SEM	*p*-value
CPT1					
Lung	0.005	0.006	0.011	0.002	0.498
Brain (Hindbrain)	0.005[b]	0.011[b]	0.076[a]	0.009	0.046
Heart	0.011[b]	0.037[ab]	0.061[a]	0.005	0.014
CPT2					
Lung	0.002	0.073	0.094	0.015	0.177
Brain (Hindbrain)	0.023[b]	0.142[ab]	0.379[a]	0.029	0.007
Heart	0.008[b]	0.314[a]	0.401[a]	0.021	< 0.001
GPX					
Lung	0.013[c]	0.174[b]	0.470[a]	0.021	< 0.001
Brain (Hindbrain)	0.066[b]	0.168[ab]	0.269[a]	0.016	0.007
Heart	0.030[b]	0.119[b]	0.348[a]	0.015	< 0.001
CAT					
Lung	0.007[b]	0.026[b]	0.090[a]	0.005	0.001
Brain (Hindbrain)	0.006[b]	0.036[b]	0.134[a]	0.008	0.001
Heart	0.009[b]	0.024[b]	0.086[a]	0.003	< 0.001
SOD1					
Lung	0.001[b]	0.009[b]	0.052[a]	0.001	< 0.001
Brain (Hindbrain)	0.007[c]	0.036[b]	0.091[a]	0.004	< 0.001
Heart	0.059[b]	0.062[b]	0.176[a]	0.014	0.037
iNOS					
Lung	0.015[b]	0.015[b]	0.088[a]	0.003	< 0.001
Brain (Hindbrain)	0.017[b]	0.020[b]	0.083[a]	0.004	< 0.001
Heart	0.024[b]	0.038[b]	0.146[a]	0.009	0.003

[a, b,c] Means in the same raw with different superscripts are significantly different. LC-50, L-carnitine supplementation at 50 mg/kg; LC-100, L-carnitine supplementation at 100 mg/kg

expression (target/*YWHAZ*) was then calculated as described previously [31].

Statistical analysis

Data were offered as mean ± standard error of the mean (SEM). Differences between the mean values of the three experimental groups were analysed by one-way ANOVA using SAS (1997) software in a completely randomized design.

Results

Effect of L-carnitine on mortality rate, plasma-blood variables, and PHS index

Table 3 indicates the effect of two concentrations of L-carnitine (50 and 100 mg/kg) on the mortality rate, blood malondialdehyde (MDA), nitric oxide (NO), and hematocrit levels, and RV: TV ratios of broiler chickens at high altitude under cold stress. Mortality rates did not differ significantly among treatments ($P > 0.05$).

The right ventricular to total ventricular weight (RV: TV, as PHS index), MDA (the indicator of lipid peroxidation) levels, and hematocrit (%) decreased in the LC-50 and LC-100 groups compared to the control group while nitrite (the indicator of nitric oxide) levels increased ($P < 0.05$). The difference between L-carnitine groups was not significant ($P > 0.05$).

Effect of L-carnitine on gene expression

Table 4 indicates the effect of two concentrations of L-carnitine (50 and 100 mg/kg) on the relative expression of *iNOS*, *CPT1*, *CPT2*, *SOD1*, *GPX*, and *CAT* genes in the

heart, hindbrain, and lung of broiler chickens at high-altitude under cold stress.

The analysis reveals no statistically significant differences in the expression of *CPT1* and *CPT2* levels in the lung across the groups ($P > 0.05$). In the hindbrain, the expression of CPT1 and CPT2 genes increased in the LC-100 group compared to the control group ($P < 0.05$), while the expression of these genes did not change in the LC-50 group compared to the control group ($P > 0.05$). In the heart, the expression of *CPT1* and *CPT2* genes increased in the LC-100 group compared to the control group ($P < 0.05$). The expression of CPT2 also increased in LC-50 compared to the control ($P < 0.05$), while the expression of *CPT1* gene did not change in the LC-50 group compared to the control and LC-100 groups ($P > 0.05$).

The expression of *GPX* gene was upregulated in the lung of the LC-50 group compared to the control, and further upregulated in the LC-100 group compared to both the LC-50 and control groups ($P < 0.05$). While the *GPX* gene expression was upregulated in the hindbrain and heart of the LC-100 group compared to the control group ($P < 0.05$), no such change was observed in the LC-50 group compared to the control group ($P > 0.05$).

The expression of *CAT*, *SOD1*, and *iNOS* genes was increased in the heart, lung, and hindbrain of the LC-100 group compared to the LC-50 and control groups ($P < 0.05$), while the expression of *CAT* and *iNOS* genes did not significantly change in the LC-50 group compared to the control group. The expression of *SOD1* also increased in the hindbrain of the LC-50 group compared to the control group ($P < 0.05$).

Discussion

L-Carnitine (3-hydroxy-4-N-trimethylaminobutyrate) is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine. Its structure consists of a polar head group (trimethylammonium) and a hydrophobic tail (carboxylate and hydroxyl groups), which enables it to function as a molecular shuttle for fatty acids across biological membranes [16]. The unique molecular structure of L-carnitine enables its involvement in diverse metabolic and antioxidant processes. It combats PHS in broilers by enhancing fatty acid oxidation, scavenging ROS, and promoting NO-dependent vasodilation, key mechanisms that counteract the effects of high-altitude hypoxia and cold stress [16].

The significant reduction in the RV: TV ratio, a key index of PHS, in the LC-50 and LC-100 groups highlights the cardioprotective effects of L-carnitine. This finding is consistent with previous studies that demonstrated that L-carnitine supplementation decreases the incidence of PHS in broiler chickens by improving cardiac function and reducing pulmonary vascular resistance [32]. The reduction in hematocrit levels in the L-carnitine-supplemented groups further supports this observation, as elevated hematocrit is a common feature of PHS due to increased blood viscosity and pulmonary hypertension [33].

The study demonstrated that L-carnitine supplementation significantly reduced MDA levels, a marker of lipid peroxidation, in the plasma of broiler chickens. This finding is consistent with previous studies that have highlighted L-carnitine's role in reducing oxidative stress and the activity of antioxidant enzymes [19, 34]. Our data indicated an upregulation of *GPX*, *CAT*, and *SOD1* gene expression in the heart, lung, and hindbrain of chickens in the LC-100 group, suggesting that L-carnitine enhances the endogenous antioxidant defense system. It has been reported that oxidants play a fundamental role in the pathophysiology of both cardiac and pulmonary tissues concerning heart failure. Their role in promoting oxidative stress leads to cellular damage, vascular remodeling, and ultimately contributes to the progression of congestive heart failure [35]. A previous study found a significant increase in oxidants within the hindbrain of chickens with pulmonary hypertension [27]. The hindbrain is composed of the medulla, pons, and cerebellum, with the rostral ventrolateral medulla (RVLM) serving as a key center for regulating the sympathetic nervous system (SNS), which is essential in the development of hypertension. The RVLM controls the basal central sympathetic outflow and integrates inputs from baroreceptors, chemoreceptors, and visceral receptors via the nucleus of the solitary tract. Research has demonstrated that the accumulation of oxidants in the RVLM contributes to the neural mechanisms associated with hypertension [27, 36]. It was also reported that L-carnitine supplementation may reduce the sympathetic vasomotor tone, leading to modulated hypertension [37]. However, the antioxidant effects of L-carnitine may be helpful in the improvement of chicken PHS. The lack of significant changes in *CAT*, *GPX*, and *SOD1* gene expression in the LC-50 group indicates a dose-dependent effect of L-carnitine. The differential response of antioxidant genes across tissues (heart, lung, and hindbrain) also suggests that the protective effects of L-carnitine may be tissue-specific.

The upregulation of *CPT1* and *CPT2* gene expression in the hindbrain and heart of the LC-100 group indicates that L-carnitine enhances fatty acid oxidation, a critical process for energy production under stress conditions. Previous research has identified the suppression of energy metabolism as a fundamental factor in cardiac dysfunction associated with sepsis and endotoxemia. Additionally, the inhibition of CPT enzymes has been shown to worsen cardiac dysfunction and inflammation [38, 39].

The CPT system is predominantly found in astrocytes and neural progenitor cells, while it is absent in neurons, microglia, and oligodendrocytes. This system not only meets the bioenergetic requirements of astrocytes but also supports neuronal metabolism by supplying ketones [40]. However, the increase in CPT levels due to L-carnitine supplementation may enhance brain metabolism, resulting in improved regulation of hypertension in chickens.

The lack of significant changes in *CPT1 and CPT2* gene expression in the lung across all groups suggests that the lung may rely less on fatty acid oxidation for energy.

The increase in nitric oxide (NO) levels in the LC-50 and LC-100 groups suggests that L-carnitine may improve vascular homeostasis by enhancing NO bioavailability. This is consistent with the previous findings that L-carnitine supplementation increased NO production, leading to vasodilation and improved PHS [17, 32]. The upregulation of iNOS gene expression in the LC-100 group further supports this mechanism in the different tissues. In a previous study, it was demonstrated that the expression of the iNOS gene in the hindbrain of pulmonary hypertensive chickens remained unchanged [41]. However, our data revealed that L-carnitine could stimulate the expression of this gene in those chickens.

Conclusion

This study demonstrates that dietary L-carnitine, particularly at 100 mg/kg, effectively reduces PHS in broiler chickens under high-altitude hypoxia and cold stress by improving cardiac function, enhancing antioxidant capacity, upregulating antioxidant and fatty acid metabolism genes in key tissues (heart, lung, hindbrain), and improving vascular homeostasis.Together, these results highlight L-carnitine's dual role in optimizing energy metabolism and oxidative stress responses, making it a valuable dietary intervention for improving broiler health under environmental stress conditions.

Acknowledgements
The authors would like to acknowledge the invaluable contributions of the technicians at the poultry farm of Shahrekord University.

Author contributions
B.A. was the supervisor, designed the study, and analyzed data. M.H.I. and S.A. performed the rearing and conducted the experiments, collected the samples, and assembled data. H.H. B.A. and F.K. contributed to writing, reviewing, and editing the final manuscript. All authors read and approved the final manuscript.

Funding
This research was supported by the funds granted for a student thesis via Vice Chancellor for Research of Shahrekord University.

Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval
The project underwent ethical review and was approved by the local Ethics Committee of Shahrekord University (IR.SKU. REC.1403.001). The care and use of experimental animals complied with local animal welfare laws, guidelines, and policies. The study was also carried out in compliance with the ARRIVE guidelines [42].

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 8 March 2025 / Accepted: 6 May 2025
Published online: 22 May 2025

References

1. Khajali F. Managing broiler production challenges at high altitude. Vet Med Sci. 2022;8(4):1519–27.
2. Gaur P, Prasad S, Kumar B, Sharma SK, Vats P. High-altitude hypoxia induced reactive oxygen species generation, signaling, and mitigation approaches. Int J Biometeorol. 2021;65:601–15.
3. Sharifi M, Khajali F, Hassanpour H. Antioxidant supplementation of low-protein diets reduced susceptibility to pulmonary hypertension in broiler chickens Raised at high altitude. J Anim Physiol Anim Nutr. 2016;100(1):69–76.
4. Krishnan G, Devaraj C, Silpa M, Sejian V. Thermoregulation in birds. In: Textbook of veterinary physiology. edn.: Springer;2023:751–64.
5. Ahmadipour B, Kalantar M, Hassanpour H. Effect of Securigera securidaca seed on the gene expression of pulmonary hypertensive broiler chickens induced by cool temperature and high-altitude. Poult Sci J. 2019;7(2):163–9.
6. Hossain ME, Akter N. Further insights into the prevention of pulmonary hypertension syndrome (ascites) in broiler: a 65-year review. World's Poult Sci J. 2022;78(3):641–88.
7. Hassanpour H, Yazdani A, Khabir Soreshjani K, Asgharzadeh S. Evaluation of endothelial and inducible nitric oxide synthase genes expression in the heart of broiler chickens with experimental pulmonary hypertension. Br Poult Sci. 2009;50(6):725–32.
8. Tan X, Hu S-H, Wang X-L. Possible role of nitric oxide in the pathogenesis of pulmonary hypertension in broilers: a synopsis. Avian Pathol. 2007;36(4):261–7.
9. Li Y, Wang K, Li C. Oxidative stress in poultry and the therapeutic role of herbal medicine in intestinal health. Antioxidants. 2024;13(11):1375.
10. Oke O, Akosile O, Oni A, Opowoye I, Ishola C, Adebiyi J, Odeyemi A, Adjei-Mensah B, Uyanga V, Abioja M. Oxidative stress in poultry production. Poult Sci. 2024;104003.
11. Netzer N, Gatterer H, Faulhaber M, Burtscher M, Pramsohler S, Pesta D. Hypoxia, oxidative stress and fat. Biomolecules. 2015;5(2):1143–50.
12. Mylonis I, Simos G, Paraskeva E. Hypoxia-inducible factors and the regulation of lipid metabolism. Cells. 2019;8(3):214.
13. Schlaepfer IR, Joshi M. CPT1A-mediated fat oxidation, mechanisms, and therapeutic potential. Endocrinology. 2020;161(2):bqz046.
14. Adeva-Andany MM, Carneiro-Freire N, Seco-Filgueira M, Fernández-Fernández C, Mouriño-Bayolo D. Mitochondrial β-oxidation of saturated fatty acids in humans. Mitochondrion. 2019;46:73–90.
15. Chang N. Functional analysis of the oncogene carnitine palmitoyltransferase 1 C and its regulation under hypoxia. University of Zurich; 2017.
16. Azizi-Chekosari M, Bouye M, Seidavi A. Effects of L-carnitine supplementation in diets of broiler chickens. J Hell Vet Med Soc. 2021;72(1):2611–28.
17. Yousefi A, Khajali F, Hassanpour H, Khajali Z. Dietary L-carnitine improves pulmonary hypertensive response in broiler chickens subjected to hypobaric hypoxia. J Poult Sci. 2013;50(2):143–9.
18. Tan X, Hu S, Wang X. The effect of dietary l-carnitine supplementation on pulmonary hypertension syndrome mortality in broilers exposed to low temperatures. J Anim Physiol Anim Nutr. 2008;92(2):203–10.
19. Çetin E, Güçlü BK. Effect of dietary l-carnitine supplementation and energy level on oxidant/antioxidant balance in laying hens subjected to high stocking density. J Anim Physiol Anim Nutr. 2020;104(1):136–43.

20. Ahmadipour B, Sharifi M, Khajali F. Pulmonary hypertensive response of broiler chickens to arginine and guanidinoacetic acid under high-altitude hypoxia. Acta Vet Hung. 2018;66(1):116–24.
21. Aviagen R. Ross 308 nutrition specifications. Scotland (UK), Aviagen. 2019.
22. Hasani FF, Ahmadipour B, Hassanpour H, Khajali F. Effect of L-carnitine, emulsifier, and their combination on lipid peroxidation, nitric oxide production, and heterophil/lymphocyte ratio in chickens under high altitude. J Basic Clin Pathophysiol. 2025;13(1):30–5.
23. Ahmadipour B, Hasani FF, Hassanpour H, Khajali F. Effect of L-carnitine and emulsifier on growth performance, lipid profile, lipogenesis, and oxidative stress of broiler chickens Raised at high altitude. Poult Sci. 2025;105157.
24. Ringseis R, Keller J, Eder K. Basic mechanisms of the regulation of L-carnitine status in monogastrics and efficacy of L-carnitine as a feed additive in pigs and poultry. J Anim Physiol Anim Nutr. 2018;102(6):1686–719.
25. Ahmadipour B, Naeini SZ, Sharifi M, Khajali F. Growth performance and right ventricular hypertrophy responses of broiler chickens to guanidinoacetic acid supplementation under hypobaric hypoxia. J Poult Sci. 2018;55(1):60–4.
26. Hassanpour H, Farhadi N, Bahadoran S, Akbari M. Cardiac telomere attrition following changes in the expression of shelterin genes in pulmonary hypertensive chickens. Br Poult Sci. 2023;64(3):370–6.
27. Hassanpour H, Khalaji-Pirbalouty V, Nasiri L, Mohebbi A, Bahadoran S. Oxidant and enzymatic antioxidant status (gene expression and activity) in the brain of chickens with cold-induced pulmonary hypertension. Int J Biometeorol. 2015;59:1615–21.
28. Navarro-Gonzalvez JA, García-Benayas C, Arenas J. Semiautomated measurement of nitrate in biological fluids. Clin Chem. 1998;44(3):679–81.
29. De Leon JAD, Borges CR. Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. J Visualized Experiments: JoVE. 2020;159. https://doi.org/10.3791/61122.
30. Ahmadipour B, Pat S, Abaszadeh S, Hassanpour H, Khajali F. Pomegranate Peel as a phytogenic in broiler chickens: influence upon antioxidant, lipogenesis and hypotensive response. Vet Med Sci. 2021;7(5):1907–13.
31. Pirany N, Bakrani Balani A, Hassanpour H, Mehraban H. Differential expression of genes implicated in liver lipid metabolism in broiler chickens differing in weight. Br Poult Sci. 2020;61(1):10–6.
32. Khajali F, Khajali Z. L-carnitine supplementation decreases lipid peroxidation and improves cardiopulmonary function in broiler chickens reared at high altitude. Acta Vet Hung. 2014;62(4):489–99.
33. Janwari AQ, Mir MS, Amin U, Ahmad S, Shah MS, Khan HM, Kamil SA, Darzi MM. Prevalence and epidemiology of pulmonary hypertension syndrome in broiler chicken reared under temperate Climatic conditions of Northern Himalayas. J Entomol Zool Stud. 2018;6:250–7.
34. Surai PF. Antioxidant action of carnitine: molecular mechanisms and practical applications. EC Vet Sci. 2015;2(1):66–84.
35. Shah AK, Bhullar SK, Elimban V, Dhalla NS. Oxidative stress as a mechanism for functional alterations in cardiac hypertrophy and heart failure. Antioxidants. 2021;10(6):931.
36. Hirooka Y. Sympathetic activation in hypertension: importance of the central nervous system. Am J Hypertens. 2020;33(10):914–26.
37. Hart EC, Joyner MJ, Wallin BG, Johnson CP, Curry TB, Eisenach JH, Charkoudian N. Age-related differences in the sympathetic-hemodynamic balance in men. Hypertension. 2009;54(1):127–33.
38. Makrecka-Kuka M, Korzh S, Videja M, Vilskersts R, Sevostjanovs E, Zharkova-Malkova O, Arsenyan P, Kuka J, Dambrova M, Liepinsh E. Inhibition of CPT2 exacerbates cardiac dysfunction and inflammation in experimental endotoxaemia. J Cell Mol Med. 2020;24(20):11903–11.
39. He L, Kim T, Long Q, Liu J, Wang P, Zhou Y, Ding Y, Prasain J, Wood PA, Yang Q. Carnitine palmitoyltransferase-1b deficiency aggravates pressure overload–induced cardiac hypertrophy caused by lipotoxicity. Circulation. 2012;126(14):1705–16.
40. Jernberg JN, Bowman CE, Wolfgang MJ, Scafidi S. Developmental regulation and localization of carnitine palmitoyltransferases (CPT s) in rat brain. J Neurochem. 2017;142(3):407–19.
41. Hassanpour H, Nikoukar Z, Nasiri L, Bahadoran S. Differential gene expression of three nitric oxide synthases is consistent with increased nitric oxide in the hindbrain of broilers with cold-induced pulmonary hypertension. Br Poult Sci. 2015;56(4):436–42.
42. Du Sert NP, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M. Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. 2020;18(7):e3000411.

Publisher's note