Dietary L-carnitine Improves Pulmonary Hypertensive Response in Broiler Chickens Subjected to Hypobaric Hypoxia

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The present study was conducted to examine the effects of L-carnitine on pulmonary hypertensive response in broiler chickens reared at high altitude and exposed to hypobaric hypoxia. A total of 192 day-old male broilers (Cobb 500) were randomly assigned to 4 treatments and 4 replicates of 12 chicks. A basal diet composed of mainly corn and soybean meal was formulated and served as a control. Three additional treatments were made by supplementing graded levels of L-carnitine (50, 100, and 150mg/kg). Chicks received dietary treatments at free access from 1 to 42 days of age. Results indicated that feeding L-carnitine at 100mg/kg caused a significant increase in plasma nitric oxide (NO) with concomitant decrease in plasma malonedialdehyde (MDA). The Lead II electrocardiogram indicated reductions of S wave amplitude for all three doses of L-carnitine relative to the control and the difference between the birds that received L-carnitine at 50mg/kg and the control was significant \((P<0.05)\). The right ventricular weight ratio (RV/TV) tended to decline when L-carnitine supplemented to diets. In conclusion, L-carnitine reduced ascites mortality in broiler chickens by increased NO production, reduced MDA concentration, and reduced right ventricular hypertrophy.

Key words: ascites, carnitine, chicken, immunity

Introduction

Hypoxia is defined as reduced availability of atmospheric oxygen (reduced partial pressure of oxygen) that occurs as the altitude increases. The partial pressure of oxygen drops approximately 7mmHg for each 1,000m elevation in altitude, which is equal to a drop of approximately 2.5% in the air oxygen for each 1,000m increase in altitude (Julian, 2007). In broiler chickens that are raised at high altitude, pulmonary hypertension syndrome (PHS) is a common problem. ‘Pulmonary Hypertension Syndrome’ (PHS), ‘Pulmonary Arterial Hypertension’ (PAH), and ‘Ascites Syndrome’ commonly are used synonymously (Wideman et al., 2013). Ascites is serous fluid accumulation in the abdominal cavity of fast-growing broilers. The syndrome has been observed worldwide but the incidence is more dramatic in broilers reared at high altitudes (Khajali et al., 2007).

L-carnitine (\(\beta\)-hydroxy-\(\gamma\)-N-trimethylaminobutyric acid) is a quaternary ammonium compound with several cellular functions including the role in the transport of long-chain fatty acids into the mitochondrial matrix for oxidation to provide cellular energy (Arslan, 2006), the modulation of the cellular and especially intra-mitochondrial acyl-CoA:CoA ratio (Tanphaichitr and Leelahagul, 1993), the participation in scavenging reactive oxygen species (Liu et al., 2004), and immunomodulatory effect (Buyse et al., 2007). L-carnitine enhances myocardial function by improving fatty acid transport into the mitochondria (Ueland et al., 2012). Recently, Erbas et al. (2007) indicated that L-carnitine could increase plasma nitric oxide (NO) in human subjects. Nitric oxide is a potent vasodilator that opposes the onset of PHS in broiler chickens (Khajali and Wideman, 2010).

L-carnitine is widely distributed in feed from animal sources. In plant sources, however, the content and availability of carnitine is limited (Arslan, 2006). Diets of broiler chickens are mainly composed of corn and soybean meal. Therefore, 80 to 85% of the total body carnitine originates from endogenous synthesis. L-carnitine is synthesized from amino acids methionine and lysine in the presence of adequate amounts of iron, ascorbic acid, pyridoxine, and niacin (Steiber et al., 2004). In corn-soybean meal diets that are commonly fed to broiler chickens, methionine and lysine are the first and second limiting amino acids, respectively.
(Fernandez et al., 1994) and ascorbic acid has shown to be necessary although it is synthesized in bird’s body (Attia et al., 2011). Meanwhile, the requirements for amino acids in broilers grown at high altitude regions have been shown to be significantly higher than those published in feeding standards (Basoo et al., 2012; Khajali et al., 2013). In the present study, therefore, L-carnitine was supplemented to a corn-soybean meal diet of broilers exposed to hypobaric hypoxia and looked at beneficial and possible effects with respect to pulmonary hypertensive and immune responses.

Materials and Methods

Birds and Experimental Facility

The experiment was conducted in the experimental facility of Shahrekord University, Shahrekord, Iran, an area with an altitude of 2,100 m above sea level. The experimental animals were kept, maintained and treated according to accepted standards for the humane treatment of animals. The Institutional Animal Care and Use Committee of Shahrekord University approved all procedures used in this study.

A total of 192 day-old male broilers (Cobb 500) were randomized across 16 floor pens measuring 1.5 m² (12 birds per pen). Each pen was equipped with a bell drinker and a feed trough. Day-old chicks were allocated to pens so that all pens had equal initial body weights (456 g ± 10 g). The temperature of the experimental facility was maintained at about 32°C during Week 1, and set at 25°C for Week 2, 20°C for Week 3, and 15°C thereafter to predispose birds to ascites as previously described (Khajali et al., 2007). Birds were subjected to 23 hr light and 1 hr dark throughout the trial. Birds had free access to feed and water.

Treatments

A basal diet composed of mainly corn and soybean meal was formulated for the starting (1 to 3 wk of age; AME = 3000 kcal/kg; CP = 215 g/kg) and growing (3 to 6 wk of age; AME = 3100 kcal/kg; CP = 195 g/kg) stages and served as a control (Table 1). To make dietary treatments, supplement of L-carnitine (Carniking®, Lohmann Co. Ltd. Germany) at 50 mg/kg increments was fortified to the basal diet at the expense of washed builder’s sand. Treatments, therefore, consisted of four graded levels of L-carnitine (0, 50, 100, and 150 mg/kg).

Each dietary treatment had four replicates of 12 birds each. All diets had the same level of calculated metabolizable energy and crude protein and met the amino acid requirements advocated by NRC (1994). All diets offered ad libitum in mash feed.

Toe-Web Hypersensitivity

At 35 days of age, cutaneous basophils hypersensitivity response to phytohemaglutinin P (PHA-P) was determined, as an index of a T-cell-induced delayed-type hypersensitivity reaction, according to Corrier and Deloach (1990). In brief, the feet of 12 birds per treatment were cleaned with 70% ethanol and the thickness of the toe web between the third and fourth digits measured using a micrometer. One hundred microliters of a 100 μg/mL solution of phytohemaglutinin P (Sigma L-8754, St. Louis, MO) in sterile 0.85% saline was injected subcutaneously on the left toe. As a control, 100 μL of 0.85% saline was injected subcutaneously on the right toe web. After 4 and 24 hours, the toe webs were cleaned and measured again. The swelling reactions of toe web to PHA-P were calculated by the following swelling index: swelling index = [(thickness of left toe web after PHA-P injection — initial thickness of left toe web) — (thickness of right toe web after saline injection — initial thickness of right toe web)].

Blood and Plasma Measurements

At 42 days of age, two birds per pen (10 birds per treatment) were selected for blood collection. Blood samples (3 mL) were collected by heparinized syringes from the brachial vein and centrifuged at 2,500 × g for 10 min to obtain plasma. Plasma samples were used for the determination of nitric oxide (NO) and malonedialdehyde (MDA). Plasma NO (nitrate+nitrite) was measured according to Behrooj et al. (2012). This assay was based on the reduction of nitrate to nitrite by cadmium. Plasma samples were deproteinised by addition of ZnSO₄ (75 mmol/l) and NaOH (55 mmol/l) solutions. Upon centrifuging, the supernatants were recovered and diluted by glycine buffer (45 g/l; pH 9.7). Cadmium

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Starter (1 to 21 d)</th>
<th>Grower (21 to 42 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>520</td>
<td>597</td>
</tr>
<tr>
<td>Soybean meal (44%)</td>
<td>388</td>
<td>326</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Soy oil</td>
<td>40</td>
<td>39</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Salt</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin supplement*</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Trace mineral supplement**</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Sand***</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>AME (kcal/kg)</td>
<td>3000</td>
<td>3100</td>
</tr>
<tr>
<td>CP (g/kg)</td>
<td>216 (215)</td>
<td>197 (195)</td>
</tr>
<tr>
<td>Met+Cys (g/kg)</td>
<td>8.6 (8.51)</td>
<td>7.1 (7.05)</td>
</tr>
<tr>
<td>Lys (g/kg)</td>
<td>11.8 (11.73)</td>
<td>10.3 (10.33)</td>
</tr>
<tr>
<td>Thr (g/kg)</td>
<td>10 (9.94)</td>
<td>9 (8.92)</td>
</tr>
<tr>
<td>Ca (g/kg)</td>
<td>9.4</td>
<td>8.8</td>
</tr>
<tr>
<td>Available phosphorous (g/kg)</td>
<td>4.3</td>
<td>3.4</td>
</tr>
</tbody>
</table>

* Provided the following per kg of diet: vitamin A (trans-retinyl acetate), 3600 IU; vitamin D₃ (cholecalciferol), 800 IU; vitamin E (dl-tocopheryl acetate), 7.2 mg; vitamin K₃, 1.6 mg; vitamin B₁₂, 0.72 mg; vitamin B₂, 3.3 mg; vitamin B₆, 0.4 mg; vitamin B₉, 1.2 mg; vitamin B₁₂, 0.6 mg; folic acid, 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.
*** L-Carnitine was replaced for sand to provide the levels of 50, 100, and 150 mg/kg.

Values in parenthesis are obtained by analysis in duplicate samples.
granules (2–2.5 g) were rinsed three times in deionised water and swirled in a CuSO\textsubscript{4} solution (5 mmol/l) in glycine-NaOH buffer (15 g/l; pH 9.7) for 5 min to activate. Freshly activated cadmium granules were added to samples. Upon continuous stirring for 10 min, the samples were transferred to labelled tubes for nitrite determination by Griess reaction. Griess reagent 1 (1% sulfanilamide in 5% phosphoric acid) was added to the sample tubes and incubated for 10 min at room temperature while protected from light. Griess reagent 2 (N-naphthylethylenediamine dihydrochloride in water) was then dispensed to all samples and absorbance was measured at 540 nm within 10 min by a spectrophotometer (Corning 480, USA). MDA concentrations of the plasma samples were measured as an index of lipid peroxidation by the TBA method (Nair and Turner, 1984).

An aliquot of blood was collected in microhematocrit tubes for measuring hematocrit. The blood smears were also prepared on the glass slides for the determination of differential leukocyte count. The smears were stained using May-Grunwald and Giemsa stains (Lucas and Jamroz, 1961), approximately 2 to 4 h after methyl alcohol fixation. One hundred leukocytes, including granular (heterophils) and nongranular (lymphocytes) were counted and the heterophil to lymphocyte ratio (H:L) was calculated.

All chemical reagents were obtained from Sigma–Aldrich Co. (Sigma–Aldrich Co., St. Louis, MO, USA).

**Electrocardiographic Recording**

Ten chicks per treatment were randomly selected at day 40 and electrocardiograms (ECG) were recorded by an automatic instrument (Cardiomax FX-2111, Fukuda, Japan) while standardized at 10 mm=1 mV with a chart speed of 50 mm/s. Leads II was recorded for every chicken, and the amplitude of the T, R and S waves were measured.

**Carcass Measurements**

Final body weights were obtained at the end of feeding trial (42 days of age). At the same day, four birds per pen (16 birds per treatment) were euthanized for carcass processing. Data obtained at processing included hot eviscerated skinless carcass weight, breast weight (skinless and boneless, Pectoralis major and Pectorals minor), spleen weight, bursa weight, and abdominal fat weight. The hearts were also removed and the ventricles were dissected and weighted to calculate the ratio-to-total ventricular weight ratio (RV/TV ratio). The RV/TV is indicative of pulmonary hypertension. RV/TV values greater than 0.25 are considered as pulmonary hypertension (Izadinia et al., 2010; Khajali, F. and Fahimi, 2010).

**Mortality**

All mortalities throughout the experiment were necropsied to determine the cause of death. Nevertheless, mortalities from ascites (RV/TV greater than 0.29) were only considered for statistical analysis.

**Statistical Analysis**

Results were compared by ANOVA using SAS (1997) software in a completely randomized design. When there was sampling within pens, data were subjected to a nested design. The statistical model used for performance data was $Y_{ij} = \mu + T_i + e_{ij}$. For blood (hematocrit, H:L), plasma (NO, MDA) and carcass (carcass yield, breast yield, spleen percentage, bursa percentage, abdominal fat percentage, and RV:TV) data, the model was $Y_{ijk} = \mu + T_i + e_{ij} + \varepsilon_{ijk}$. In these models, $Y_{ij}$ and $Y_{ijk}$ are observations; $\mu$ is the general location parameter (i.e., the mean); $T_i$ is the effect for being in treatment $i$; $e_{ij}$ is random error; and $\varepsilon_{ijk}$ is subsampling error. All physiological data (carcass, blood and plasma) were checked to have a normal distribution and log transformed if necessary. Any data requiring log transformation were back-transformed for presentation of data. Means were separated by Duncan’s multiple range test.

**Results**

Table 2 indicates immune response of broilers fed different levels of L-carnitine. Though the toe web thickness index at 4 h postinjection was not influenced by dietary L-carnitine, the toe web thickness index at 24 h postinjection was significantly ($P<0.05$) influenced by dietary L-carnitine. The proportion of spleen to live body weight was not significantly changed between the control and L-carnitine supplemented groups. However, the proportion of bursa to live body weight was significantly ($P<0.05$) increased in 100 mg/kg L-carnitine diet compared to the control.

Table 3 depicts plasma and blood variables measured at the end of experiment (42d) in broilers receiving L-carnitine. Plasma concentration of NO was increased in birds received L-carnitine so that the difference between the control and L-carnitine group was sampling within pens, data were subjected to a nested design. Data obtained at processing included hot eviscerated skinless carcass weight, breast weight (skinless and boneless, Pectoralis major and Pectorals minor), spleen weight, bursa weight, and abdominal fat weight. The hearts were also removed and the ventricles were dissected and weighted to calculate the ratio-to-total ventricular weight ratio (RV/TV ratio). The RV/TV is indicative of pulmonary hypertension. RV/TV values greater than 0.25 are considered as pulmonary hypertension (Izadinia et al., 2010; Khajali, F. and Fahimi, 2010).

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<table>
<thead>
<tr>
<th>Variables</th>
<th>L-carnitine (mg/kg)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>50</td>
<td>100</td>
<td>150</td>
<td>SEM</td>
</tr>
<tr>
<td>Toe web index 4h (mm) measured at 35 days of age**</td>
<td>0.14$^{a}$</td>
<td>0.16$^{a}$</td>
<td>0.15$^{a}$</td>
<td>0.16$^{a}$</td>
<td>0.021</td>
</tr>
<tr>
<td>Toe web index 24h (mm) measured at 35 days of age*</td>
<td>0.18$^{b}$</td>
<td>0.24$^{ab}$</td>
<td>0.24$^{ab}$</td>
<td>0.27$^{a}$</td>
<td>0.021</td>
</tr>
<tr>
<td>Spleen weight ratio (%) measured at 42d**</td>
<td>0.14$^{ab}$</td>
<td>0.14$^{ab}$</td>
<td>0.17$^{a}$</td>
<td>0.12$^{b}$</td>
<td>0.010</td>
</tr>
<tr>
<td>Bursa weight ratio (%) measured at 42d**</td>
<td>0.13$^{b}$</td>
<td>0.13$^{b}$</td>
<td>0.16$^{a}$</td>
<td>0.11$^{b}$</td>
<td>0.009</td>
</tr>
</tbody>
</table>

$^{a,b}$Means in the same row with different letters are significantly different ($P<0.05$).

* Each mean represents values from 12 replicates.

** Each mean represents values from 16 replicates.
in plasma MDA. Broilers fed L-carnitine at 50 and 150 mg/kg had hematocrit value that was significantly lower than that of the birds fed the control diet ($P<0.05$). Heterophil to lymphocyte ratio was not significantly affected by dietary L-carnitine level.

The effects of dietary L-carnitine on carcass characteristics of broiler chickens are presented in Table 4. Carcass and breast yields were not significantly influenced by dietary L-carnitine supplementation. Although the proportion of heart to live body weight remained unchanged, the right to total ventricular weight ratio was significantly reduced ($P<0.05$) in birds received L-carnitine at 50 mg/kg when compared to the control. A significant decline in abdominal fat deposition was observed in birds received L-carnitine compared to the control group.

There were reductions of S wave amplitude for all three doses of L-carnitine relative to the control and the difference between the birds received L-carnitine at 50 mg/kg was significantly lower than the control (Table 5). However, no significant difference was found for R and T wave amplitudes among the treatments.

As shown in Table 6, birds fed with L-carnitine supplement had lower rate of ascitic mortality so that the differences between the 50 and 100 mg/kg L-carnitine groups were significant ($P<0.05$) compared to the control group.

**Discussion**

Toe-web index reflects the change of cellular immunity in chicken (Corrier and Deloach., 1990). Toe-web thickness 24 h postinjection revealed numerically higher values in L-
carnitine groups, which implied that broilers fed L-carnitine had more cell-mediated immunity than those on the control diet. Nevertheless, the only significant difference was detected between the control diet and 150 mg/kg L-carnitine diet. We did not observe a consistent effect of L-carnitine on proportional weights of spleen. Geng and coworkers (2007), however, indicated a tendency of L-carnitine to increase spleen weight and they attributed this finding to reduced ascites mortality in broiler chickens. Increased Bursa weight ratio in 150 mg/kg L-carnitine diet compared to the control can be considered an advantage. According to Lister (2010), enhanced Bursa weight means the higher ability of birds to resist and react to a range of disease challenges. This finding reflects an immunomodulatory effect of L-carnitine.

Plasma NO tended to increase by L-carnitine supplementation so that the difference between the group received L-carnitine at 100 mg/kg and the control was significant. In line with our findings, Erbas et al. (2007) indicated that L-carnitine increased NO through reduction in the activity of arginase and elevation in the activity of NO synthase. Recent study (Sharifi et al., 2009) indicated that L-carnitine also could reduce the activity of angiotensin converting enzyme in aorta, heart and kidney in rat, which resulted in higher NO production. It is well documented that diminished NO production has been implicated in the pathogenesis of pulmonary hypertension in human (Shaul, 2002). Available data suggest that reduction of NO synthesis, in pulmonary arterioles, is associated with increased pulmonary vasomotor tone, vascular remodeling and impaired heart function in hypertensive broiler chickens (Tan et al., 2007; Hassanpour et al., 2009). Enhanced NO production can justify a significant decline in mortality from PHS observed in the present experiment when L-carnitine was supplemented to diets at 50 and 100 mg/kg.

Reducing effect of MDA by L-carnitine supplement observed herein accounts for the antioxidative role of L-carnitine. MDA is a marker for oxidative stress and indicates the degree of lipid peroxidation. Reduced MDA level might be attributed to antioxidative role of L-carnitine as a scavenger of reactive oxygen species (ROS). Reactive oxygen species cause damage to cell membranes. Higher plasma MDA content in the control group of this study explains the antioxidative role of L-carnitine. Previous reports (Geng et al., 2004; Tan et al., 2008) further support this explanation. Geng et al. (2004) demonstrated that MDA content was significantly decreased by dietary supplementation of L-carnitine in broiler liver. Tan et al. (2008) indicated that addition of 100 mg/kg of L-carnitine supplement resulted in significantly lower plasma MDA in broiler chickens. Inhibition of ROS generation or scavenging ROS has been shown to inhibit the hypoxia-induced proliferation in the pulmonary vasculature and subsequently pulmonary hypertension (Springer et al., 2000). Therefore, reduced plasma MDA observed in the L-carnitine treatments can be associated with reduced mortality from PHS.

Our finding on reducing hematocrit in birds fed L-carnitine is in agreement with those reported by Geng et al. (2007). The reason that how L-carnitine could reduce hematocrit remains to be determined. Nevertheless, this observation can also explain the reduction in mortality from PHS observed in birds received L-carnitine. The ratio of heterophils to lymphocytes (H/L) is an index of stress in the chicken (Khajali et al., 2008). There was, however, no significant difference among the treatments for H/L.

There was no significant change in carcass, breast and heart yields between the L-carnitine supplemented diets and the control. In agreement with our study, Arslan (2006) in a review of literature reported that carcass and breast yields were not significantly affected by dietary L-carnitine supplement. Though the proportion of heart remained unchanged, the RV/TV ratio tended to lower values when L-carnitine administered. In line with our finding, Tan et al. (2008) reported a lower RV/TV in broilers fed with L-carnitine at 100 mg/kg. The RV/TV ratio is indicative of increased pulmonary arterial pressure. Therefore, it is reasonable to postulate that L-carnitine supplemented from the first day posthatch in a cool temperature may beneficially help the cardio-pulmonary function and postpone the occurrence of PHS in broiler chickens. A significant decline in deposition of fat in abdominal cavity was in accordance with previous reports (Rabie and Szilagyi, 1998). Abdominal fat deposition is a non-profitable conversion of dietary energy, which is less desirable for the processors, because it will increase the waste-disposal problems during the processing procedures. From nutritional viewpoint, it is also undesirable for consumers who are increasingly concerned about the health aspects of their food. Accordingly, the fat-reducing effect of L-carnitine supplement is an advantage in practical nutrition. Fat-reducing effect of L-carnitine is attributed to the formation of acylcarnitines that enables the transfer of activated acyl groups into the matrix of mitochondria via the carnitine-acylcarnitine translocase located within the mitochondrial inner membrane in order to enter β-oxidation process (Arslan, 2006).

Table 6. Effect of dietary L-carnitine on cumulative mortality from ascites of broiler chickens reared up to 42 days of age

<table>
<thead>
<tr>
<th>Variables (%)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascites mortality</td>
<td>22.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.75&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.66</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means in the same row with different letters are significantly different (P<0.05).
Electrocardiogram of birds in the present study indicates that the amplitude of the S waves is decreased during administration of L-carnitine. Kirby et al. (1999) indicated that the most important Lead II ECG parameter associated with the development of ascites was increasingly negative S wave amplitude. Kirby et al. (1999) used ECG data to develop a regression equation to estimate RV/TV. They found a relatively high correlation ($R^2=0.79$). Reduction of S wave amplitude in birds received L-carnitine further supports RV/TV data. Negatively lower S wave amplitudes in broilers received L-carnitine suggest a lower rate of ventricular hypertrophy and dilatation. Mortality from PHS was remarkably lower in birds fed with L-carnitine at 50 and 100 mg/kg. However, the highest dose of L-carnitine (150 mg/kg) could not significantly reduce the ascites mortality compared to the control. It has been confirmed that high dose of dietary carnitine reduces its biosynthesis in the tissues. Such an intracellular reduction in carnitine biosynthesis may portend possible pathologic changes in long term (Rebouche, 1983). Considering the fact that more than 80% of the total body carnitine originates from endogenous synthesis, our data also show that high dose of carnitine (i.e. 150 mg/kg) was not able to confer beneficial effects such as NO production, and reduction of lipid peroxidation and ascites mortality as much as observed in lower doses of carnitine (i.e. 50 and 100 mg/kg).

**Conclusion**

Mortality from PHS was remarkably lower in birds fed with L-carnitine. In deed, reduced hematocrit and MDA concentration, increased NO production, and reduced RV/TV in birds received L-carnitine reflects in lower rate of ascites. It is concluded that L-carnitine supplementation has beneficial effects in the prevention of pulmonary hypertension and ventricular hypertrophy in broilers reared at high altitude.

**Acknowledgments**

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