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Original

# Effects of L-carnitine supplementation on ascites syndrome in the broilers grown at high altitude

Efectos de la suplementación con L-carnitina en el síndrome de ascitis en los pollos de engorde criados a gran altura

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## Abstract:

**Objective**. Investigate the effects of *L-carnitine* as a potential means of reducing the incidence of ascites in broilers and its relationship with physiological and biochemical paramaters. **Material and methods.** One-day-old 300 male broiler chicks (Ross 308) were used in the trial. The group without *L-carnitine* supplementation (0) was assigned as control and the groups that received 100, 150, 200 and 250 mg/L *L-carnitine* supplementation in water were assigned as treatment groups. The trial was completed in 35 days. **Results.** *L-carnitine* supplementation did not have any significant effect on live weight gain, feed consumption, water consumption and feed conversion ratio. Levels of blood plasma and hemogram parameters HDL, Triglyceride, CK, RBC and MCH were significantly affected by *L-carnitine* ($p<0.05$). Blood gas parameter pH value was significantly affected by *L-carnitine* supplementation in the broilers with ascites. Blood gas pH value significantly increased with 100 mg/L *L-carnitine* supplementation compared to that of control ($p<0.05$). While blood pH was 7.21 in the animals with ascites, it was determined as 7.48 in healthy animals. Concentrations of $SO_2$ and $ctO_2$ were higher in healthy animals, while ctCO2P and hemoglobin concentrations were higher in ascitic animals ($p<0.05$). **Conclusions.** Ascites mortality rates starting from the control group were calculated respectively as %; 20.00, 18.33, 26.67, 28.33 and 28.33%. 76.71% of total ascites deaths were in the 5th week. It was concluded that low doses of *L-carnitine* supplementation may have positive effects in the broilers grown at high altitude.

Keywords: Blood gas, broiler, L-carnitine, performance parameters, pulmonary hypertension .

## Resumen:

**Objetivo.** Investigar los efectos de la *L-carnitina* como un medio potencial para reducir la incidencia de ascitis en pollos de engorde y su relación con parámetros fisiológicos y bioquímicos. **Material y métodos.** Se utilizaron 300 pollos de engorde machos de un día de edad (Ross 308) en el ensayo. El grupo sin suplementación de *L-carnitina* (0) se asignó como control y los grupos que recibieron suplementos de 100, 150, 200 y 250 mg/L de *L-carnitina* en agua se asignaron como grupos de tratamiento. La prueba se completó en 35 días. **Resultados.** La suplementación de *L-carnitina* no tuvo ningún efecto significativo sobre el aumento de peso vivo, consumo de alimento, consumo de agua y tasa de conversión alimenticia. Los niveles de plasma sanguíneo y los parámetros del hemograma HDL, triglicéridos, CK RBC y MCH se vieron afectados significativamente por *L-carnitina* ($p<0.05$). El valor del pH del parámetro del gas en sangre se vio significativamente afectado por la suplementación con *L-carnitina* en los pollos de engorde con ascitis. El valor del pH del gas en la sangre aumentó significativamente con la suplementación de 100 mg/L de *L-carnitina* en comparación con la del control ($p<0.05$). Mientras que el pH de la sangre fue de 7.21 en los animales con ascitis, se determinó como 7.48 en animales sanos. Las concentraciones de $SO_2$ y $ctO_2$ fueron mayores en animales sanos, mientras que las concentraciones de ctCO2P y hemoglobina fueron mayores en animales ascíticos ($p<0.05$). **Conclusiones.** Las tasas de mortalidad por ascitis a partir del grupo control se calcularon respectivamente como %; 20.00, 18.33, 26.67 y 28.33. 76.71% de las muertes totales de ascitis fueron en la quinta semana. Se concluyó que dosis bajas de suplementos de *L-carnitina* pueden tener efectos positivos en los pollos de engorde criados a gran altitude.

Palabras clave: L-carnitina, gas en sangre, hipertensión pulmonar, parámertos de rendimento, pollos de engorde.

## INTRODUCTION

Carnitine is chemically in D- and L-form and only L-form exists in nature and is important for human and animal nutrition (1). *L-carnitine* is a white, highly water-soluble powder characterized by high thermal stability (up to 200 °C). Its chemical name is β-hydroxy-# -trimethyl-amino butyrate. *L-carnitine* is also referred to as Vitamin BT (2). *L carnitine* has an important effect on energy production and fat metabolism in the body by providing mitochondrial transport of long chain fatty acids and mitochondria removal of short and medium chain fatty acids (3). In addition to its use in animal feeding (4), *L-carnitine* has also found use in a number of health sectors such as prevention of heart attacks and other heart conditions (5), hemodialysis (6) and infertility treatment (7).

Ascites (pulmonary hypertension) is a multifactorial syndrome resulted from interactions among genetic, environmental, dietary and physiological factors (8). Ascites is a cardiovascular disorder predominantly observed in fast-growing chickens rather than in chicks. In fast-growing chickens, hypobaric hypoxia (lack of oxygen) causes right ventricular hypertrophy (abnormal enlargement) and expansion. As a result, fluid accumulates in the abdominal region death causes due to oxygen deficiency (9). There are two hypotheses regarding the syndrome of ascites: Hypothesis 1: In the development of ascites syndrome, primary lesions occur in the lungs (pulmonary hypertension) and consequently the heart is affected (right ventricular hypertrophy). Hypothesis 2: Primary lesion in the development of ascites syndrome is caused by systemic hypertension-induced left ventricular insufficiency leading to right ventricular hypertrophy and acidosis (10). The prevalence of ascites has been reported to increase in the recent years. Selections made to achieve faster growth in chicks have improved genetic potential, but also caused some anatomical and physiological limitations and the same improvement led to negative impacts on the health of animals. A possible deterioration in oxygen resources (altitude, ventilation) will stimulate the development of many mechanisms in the cardiopulmonary system, which will trigger ascites syndrome.

*L-carnitine* supplementation is considered to relate to ascites syndrome due to its effect on energy metabolism. This study aimed to investigate the effects of *L-carnitine* as a potential means of reducing the incidence of ascites in broilers triggered by high altitude and its relationship with physiological and biochemical paramaters.

## MATERIAL AND METHODS

**Animal, feeds and feeding.** One-day-old 300 male broiler chicks (Ross 308) with the same body weight average were used in the trial. At the trial, animals were separated into 5 groups each of which were divided into 3 subgroups. The group without *L-carnitine* supplementation was the control, and the groups supplemented with 100, 150, 200 and 250 mg/l *L-carnitine* in drinking water were the treatment groups. *L-carnitine* was used in its white, pure powder form and was obtained from Vimar Food Agriculture and Livestock Industry Trade Inc. / Turkey. L-carnitine was added to the drinking water on a daily basis. The trial was carried out in November 2014 and January 2015 at altitude of 1727 meters in Van-Turkey.

Ingredients and nutrient analysis results of the feeds used in the trial are given in table 1. Broiler chicks were fed with starter feed for days 1-10, followed by grower feed for days 10-21, and finisher feed for days 21-35. A corn-soybean meal basal diet was formulated according to NRC (1994). The broilers received pellet feeds throughout the whole period. Feed and water were given freely to all groups. The trial lasted 35 days. A 24-hour lighting program was applied throughout the trial.

TABLE 1

**Table 1.** Composition (g/kg) and nutrient contents (%) of mixed feeds used in the trial.

| Ingredients | Broiler starter diet (Days 1-10) | Broiler grower diet (Days 10-21) | Broiler finisher diet (Days 21-35) |
|---|---|---|---|
| Corn | 411.07 | 450.70 | 460.74 |
| Soybean meal (%46HP) | 197.90 | 120.31 | 119.71 |
| Full-fat soybean | 140.00 | 160.00 | 160.00 |
| Corn gluten flour (%60HP) | 86.75 | 64.26 | 20.00 |
| Rice (Broken) | 40.00 | 40.00 | 40.00 |
| Fish Flour (%53-60) | 40.00 | 40.00 | 60.00 |
| Sunflower meal (%36) | 30.00 | 40.00 | 40.00 |
| DCP (%18 P) | 10.44 | 5.73 | 2.69 |
| Chicken feather flour | 10.00 | 10.00 | 10.00 |
| Meat-bone flour (%35HP) | 10.00 | 28.38 | 30.00 |
| Corn extract (Full-fat) | - | 20.00 | 20.00 |
| Soybean oil | - | - | 19.45 |
| Marble powder | 9.43 | 6.83 | 5.73 |
| L-Lysine | 4.12 | 4.20 | 2.99 |
| Sodium bicarbonate | 3.20 | 2.65 | 1.82 |
| DL-methionine | 2.21 | 2.09 | 2.01 |
| Salt | 1.40 | 1.21 | 1.60 |
| Vitamin premixture1 | 1.00 | 1.00 | 1.00 |
| Mineral premixture2 | 1.00 | 1.00 | 1.00 |
| L-Threonine | 0.88 | 1.04 | 0.66 |
| Anticoccidial | 0.60 | 0.60 | 0.60 |
| Total | 1000.00 | 1000.00 | 1000.00 |
| Analysed nutrients (%) | | | |
| Dry matter | 89.44 | 89.19 | 89.26 |
| Crude protein | 25.70 | 23.74 | 20.95 |
| Crude Fat | 4.42 | 5.12 | 7.65 |
| Crude cellulose | 3.11 | 2.48 | 3.11 |
| Crude cinder | 5.81 | 5.45 | 5.07 |
| Calculated nutrients (%) | | | |
| ME (kcal/kg) | 3020 | 3120 | 3220 |
| Lysine | 1.44 | 1.34 | 1.21 |
| Methionine | 0.68 | 0.64 | 0.57 |
| Calcium | 1.05 | 0.95 | 0.85 |
| Phosphorus | 0.81 | 0.74 | 0.70 |
| Sodium | 0.18 | 0.17 | 0.16 |

[1]: Per 1 kg diet: 12.500.000 IU Vitamin A, 3.500.000 IU Vitamin D3, 100.000 mg Vitamin E, 4.000 mg Vitamin K3, 3.000 mg Vitamin B1, 8.000 mg Vitamin B2, 5.000 mg Vitamin B6, 20 mg Vitamin B12, 70.000 mg Niacin, 20.000 mg Pantothenic Acid, 2.000 mg Folic Acid, 250 mg Biotin, 100.000 mg Vitamin C, 125.000 mg Antioxidant. [2]: Per 1 kg diet: 120.00 mg Manganese, 40.000 mg Iron, 110.00 mg Zinc, 16.00 mg Copper, 500 mg Cobalt, 1.250 mg Iodine, 300 mg Selenium.

**Blood biochemistry, mineral, hormone and blood gas values.** For blood biochemical analyzes, blood samples were collected during the slaughter of 6 animals from each subgroup. Thirty minutes after collection of the blood samples, serums separated by centrifugation for 5 minutes at 4000 rpm were stored in ependorfs at -20°C until analysis. Blood biochemical analysis was performed by Swelab Alfa (Sweden) brand complete blood count device in whole blood samples. All samples were analyzed in a single study in one day. T3 and T4 hormones were detected in the plasma with Roche Diagnostic (United States) Elecsys e-411 Hormone Autoanalyzer using Roche Brand Kits. On the 35$^{th}$ day of trial, blood samples taken from the wing veins of 3 ascitic and 3 healthy broilers from each subgroup were collected in heparin containing syringes to avoid clot formation. Blood gas, oximetry, electrolyte and metabolite parameters were measured by Radiometer ABL-700 Blood Gas Analyser.

**Statistics**. Variance analysis was employed based on General Linear Model (PROC GLM) procedure in accordance with the trial design (random parcels experimental design) and Duncan's Multiple-Range Test was used to compare group averages. Statistical analyses were performed by SAS (version 9.2) statistical package software.

## RESULTS

**Performance parameters.** Live weights of the treatment groups on weekly basis are given in table 2. When the groups were compared in terms of live weight gains, the differences between the group averages only at 7$^{th}$, 14$^{th}$ and 28$^{th}$ days were found to be statistically significant ($p<0.05$). By 35$^{th}$ days, the end of trial, no significant differences were recorded between groups with respect to live weight ($p>0.05$).

TABLE 2

**Table 2.** The effects of L-carnitine supplementation in water on performance parameters and water consumption values (ml) of broilers.

| | Treatment groups | | | | | | |
|---|---|---|---|---|---|---|---|
| Days | Control | 100mg/L L-carnitine | 150mg/L L-carnitine | 200mg/L L-carnitine | 250mg/L L-carnitine | SEM | p= |
| Live Weight, g | | | | | | | |
| Day 0 | 46.61 | 46.53 | 46.34 | 46.63 | 46.59 | 0.235 | 0.995 |
| Day 7 | 207.63[ab] | 210.84[a] | 209.93[a] | 200.51[b] | 213.95[a] | 1.396 | 0.005 |
| Day 14 | 568.00[a] | 532.22[b] | 558.63[a] | 519.54[b] | 575.78[a] | 3.443 | 0.001 |
| Day 21 | 1095.84 | 1088.81 | 1087.84 | 1065.59 | 1090.39 | 7.033 | 0.699 |
| Day 28 | 1809.95[a] | 1808.51[a] | 1699.46[b] | 1737.04[ab] | 1715.75[ab] | 14.975 | 0.042 |
| Day 35 | 2475.56 | 2484.30 | 2486.94 | 2456.42 | 2386.84 | 23.470 | 0.689 |
| Feed consumption, g | | | | | | | |
| Day 7 | 211.25 | 213.05 | 211.15 | 206.75 | 211.93 | 1.027 | 0.239 |
| Day 14 | 458.82 | 510.28 | 478.78 | 465.85 | 497.79 | 9.417 | 0.256 |
| Day 21 | 789.93 | 785.33 | 753.93 | 803.07 | 797.24 | 11.357 | 0.525 |
| Day 28 | 1045.90 | 1050.59 | 968.00 | 999.40 | 961.50 | 16.481 | 0.179 |
| Day 35 | 1181.19 | 1184.35 | 1151.04 | 1140.43 | 1240.48 | 36.835 | 0.847 |
| Total | 3687.08 | 3743.59 | 3562.95 | 3615.49 | 3708.93 | 44.302 | 0.541 |
| Feed efficiency ratio, kg feed consumption/kg live weight gain | | | | | | | |
| Day 7 | 1.31 | 1.31 | 1.29 | 1.35 | 1.27 | 0.019 | 0.577 |
| Day 14 | 1.27 | 1.59 | 1.37 | 1.47 | 1.38 | 0.041 | 0.116 |
| Day 21 | 1.51 | 1.41 | 1.41 | 1.46 | 1.54 | 0.031 | 0.392 |
| Day 28 | 1.45 | 1.45 | 1.48 | 1.51 | 1.48 | 0.040 | 0.978 |
| Day 35 | 1.60 | 1.67 | 1.49 | 1.38 | 1.58 | 0.068 | 0.525 |
| Water consumption (ml) | | | | | | | |
| Day 7 | 390.33 | 381.33 | 377.33 | 372.10 | 377.67 | 5.388 | 0.754 |
| Day 14 | 1050.33 | 1074.67 | 1006.33 | 1018.33 | 1036.67 | 25.279 | 0.845 |
| Day 21 | 1703.27 | 1847.93 | 1736.27 | 1715.47 | 1638.03 | 58.234 | 0.762 |
| Day 28 | 2751.00 | 2915.00 | 2844.63 | 2601.70 | 2500.20 | 87.828 | 0.393 |
| Day 35 | 3571.07 | 3940.40 | 3721.73 | 3248.40 | 3150.87 | 113.085 | 0.107 |
| Total | 9465.67 | 10159.33 | 9686.37 | 8956.03 | 8703.43 | 265.058 | 0.292 |

*: Differences between group averages denoted by different letters in the same row are statistically significant ($p<0.05$). SEM: Standard error of difference between means.

*L-carnitine* supplementation in water did not have any significant effects on cumulative feed consumption of broilers by weeks and at the end of the trial ($p>0.05$). However, at the end of the trial, the highest amount of feed consumption was observed in the group receiving 100 mg/l L-carnitine, while the lowest was observed in the group receiving 150 mg/l *L-carnitine.*

As seen in table 2, no significant differences were detected in feed conversion ratios between groups throughout the trial ($p>0.05$). However, in the group receiving 200 mg/l *L-carnitine* supplementation, the feed efficiency ratio improved (1.38) numerically.

As indicated by table 2, no significant differences were recorded in water consumption values between groups throughout the trial ($p>0.05$). On the other hand, though not statistically significant, total water intake was higher in the group supplemented with 100 mg/ l *L-carnitine* compared to the other groups by the end of the trial.

**Mortality rates.** The effects of *L-carnitine* supplementation on mortality rates of broilers are presented in table 3. The lowest mortality rate (18.33%) was recorded in the group supplemented with 100 mg/l *L-*

*carnitine* while the highest mortality rate (28.33%) was recorded in the groups supplemented with 200 and 250 mg/l *L-carnitine.*

TABLE 3

**Table 3.** The effects of L-carnitine supplementation on ascites mortality rates in broilers.

| Treatment groups | Number of deaths from sscites | Mortality rate (%) |
|---|---|---|
| Control group | 12 | 20.00 |
| 100 mg/l L-carnitine<br>150 mg/l L-carnitine | 11 16 | 18.33 26.67 |
| 200 mg/l L-carnitine<br>250 mg/l L-carnitine | 17 17 | 28.3328.33 |

**Blood plasma and hemogram parameters.** The effects of *L-carnitine* supplementation in water on selected blood plasma and hemogram parameters are shown in table 4. By the end of the trial, *L-carnitine* supplementation did not result in any significant differences between groups in blood plasma parameters Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), total protein, cholesterol, Low density lipoproteins (LDL), Triiodothyronine (T3) concentration and hemogram parameters Mean corpuscular volume (MCV), Red cell distribution width (RDW), Red cell distribution with actually (RDWa), hematocrit, platelet, hemoglobin concentrations ($p>0.05$). On the other hand, significant differences were recorded in High density lipoproteins (HDL), triglyceride, creatine kinase, glucose, Thyroxine (T4), Red blood cell count (RBC), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHc) values between groups ($p<0.05$).

TABLE 4

**Table 4.** The effects of *L-carnitine* supplementation in water on selected blood plasma and hemogram parameters of broilers

| Parameters | Control | 100 mg/L L-carnitine | 150 mg/L L-carnitine | 200 mg/L L-carnitine | 250 mg/L L-carnitine | SEM | p= |
|---|---|---|---|---|---|---|---|
| | Treatment groups | | | | | | |
| Blood plasma parameters | | | | | | | |
| AST ( U/L) | 292.68 | 238.90 | 275.28 | 290.03 | 249.06 | 12.788 | 0.456 |
| ALT ( U/L) | 7.10 | 4.14 | 5.68 | 5.30 | 5.88 | 0.392 | 0.091 |
| ALP ( U/L) | 2913.18 | 2875.25 | 3681.17 | 3012.12 | 2525.14 | 225.836 | 0.745 |
| Total Protein(g/dl) | 3.35 | 3.10 | 2.98 | 3.01 | 3.03 | 0.061 | 0.242 |
| Cholesterol (mg/dl) | 133.50 | 119.36 | 108.67 | 111.56 | 114.13 | 3.029 | 0.065 |
| LDL(mg/dl) | 14.49 | 10.40 | 9.60 | 12.31 | 10.80 | 1.187 | 0.696 |
| HDL(mg/dl) | 118.15[a] | 95.10[b] | 92.68[b] | 93.60[b] | 91.34[b] | 2.902 | 0.006 |
| Triglyceride (mg/dl) | 51.47a[b] | 37.14[b] | 41.00[b] | 34.33[b] | 60.38[a] | 2.616 | 0.012 |
| Creatine Kinase (CK) (U/L) | 15744.58[ab] | 9893.33[b] | 20395.20[ab] | 24622.83[a] | 8605.25[b] | 585.238 | 0.057 |
| Glucose (mg/dl) | 298.67[ab] | 283.57[b] | 286.50[b] | 285.44b | 313.63[a] | 3.208 | 0.021 |
| T3(pg/ml) | 6.85 | 6.22 | 4.27 | 6.45 | 7.99 | 0.400 | 0.151 |
| T4(ng/dl) | 0.86[a] | 0.86[a] | 0.91[a] | 0.66[a] | 0.40[b] | 0.041 | 0.001 |
| Hemogram parameters | | | | | | | |
| RBC (1012/l) | 2.98a | 2.83ab | 2.71b | 2.89ab | 2.71b | 0.030 | 0.021 |
| MCV (fl) | 134.55 | 135.38 | 134.02 | 136.82 | 139.60 | 0.636 | 0.163 |
| RDW ( %) | 9.79 | 9.65 | 9.30 | 9.76 | 10.15 | 0.118 | 0.438 |
| RDWa (fl) | 77.55 | 77.39 | 74.22 | 79.16 | 82.65 | 1.041 | 0.339 |
| Hematocrit (%) | 40.19 | 38.34 | 36.30 | 39.64 | 37.78 | 0.475 | 0.083 |
| Platelet (Thrombocyte) (109/l) | 3.77 | 4.38 | 3.17 | 4.20 | 4.50 | 0.207 | 0.295 |
| Hemoglobin (g/dl) | 17.68 | 16.96 | 17.65 | 17.38 | 17.28 | 0.283 | 0.851 |
| MCH (pg) | 59.22b | 59.89b | 65.20a | 60.02b | 63.85ab | 0.706 | 0.041 |
| MCHC(g/dl) | 43.97b | 44.24b | 48.70a | 43.84b | 45.68ab | 0.496 | 0.025 |

*: Differences between group averages denoted by different letters in the same column are statistically significant ($p<0.05$). SEM: Standard error of difference between means.

Blood gas parameters. Blood gas, oximetry, electrolyte and metabolite were measured in the blood samples taken from 3 ascitic broilers and 3 healthy broilers from each subgroup. The effects of *L-carnitine* supplementation on blood gas paramaters of ascitic and healthy broilers are presented in table 5. *L-carnitine* supplementation in water did not have any significant effects on hematocrit, Sodium (Na), Potassium (K), Calcium (Ca), Plasma bicarbonate ($HCO_3$ p), Standart bicarbonate ($HCO_3$ pst), Base excess (C Base B) blood gas, electrolyte and metabolite values of ascitic and healthy broilers ($p>0.05$). On the other hand, Pouvoir hydrogen (pH), Partial pressure of carbon dioxide (pCO2), Partial pressure of oxygen ($pO_2$), Chlorine (Cl), Hemoglobin (Hb), Breathe carbon dioxide concentration ($ctCO_2B$), Partial pressure of carbon dioxide concentration ($ctCO_2P$), Anion K, Anion Gap, Oxygen saturation ($SO_2$) and Oxygen content ($ctO_2$) values were significantly affected by treatments ($p<0.05$). Blood pH value significantly decreased in ascitic broilers compared to healthy broilers.

TABLE 5.

**Table 5.** The effects of *L-carnitine* supplementation in water on blood gas paramaters of ascitic and healthy broilers.

| Blood gas parameters | | Treatment groups | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Control | 100 mg/l L-carnitine | 150 mg/l L-carnitine | 200 mg/l L-carnitine | 250 mg/l L-carnitine | SEM | p= |
| pH | Healthy | $7.48^{A}$ | 7.41 | $7.46^{A}$ | $7.44^{A}$ | $7.37^{A}$ | 0.013 | 0.070 |
| | Ascitic | $7.21^{bB}$ | $7.42^{a}$ | $7.32^{abB}$ | $7.32^{abB}$ | $7.29^{bB}$ | 0.016 | 0.032 |
| | SEM | 0.034 | 0.030 | 0.017 | 0.021 | 0.041 | | |
| | P | 0.009 | 0.837 | 0.008 | 0.036 | 0.361 | | |
| $pCO_2$ | Healthy | 37.00B | 40.00 | 38.50B | 40.00B | 39.00B | 0.880 | 0.904 |
| | Ascitic | 66.00A | 45.00 | 60.20A | 61.86A | 69.80A | 2.472 | 0.122 |
| | SEM | 4.195 | 2.915 | 3.425 | 3.431 | 6.088 | | |
| | P | 0.014 | 0.349 | 0.019 | 0.019 | 0.042 | | |
| $pO_2$ | Healthy | 38.50A | 42.00 | 40.00 | $35.50^{A}$ | $43.50^{A}$ | 2.199 | 0.488 |
| | Ascitic | $6.00^{B}$ | 27.50 | 13.80 | $4.43^{B}$ | $11.60^{B}$ | 2.473 | 0.115 |
| | SEM | 2.140 | 4.257 | 5.581 | 2.348 | 6.541 | | |
| | P | 0.001 | 0.137 | 0.053 | 0.001 | 0.047 | | |
| Hematokrit | Healthy | 31.00 | 31.00 | 40.00 | 31.50 | 29.00 | 2.604 | 0.406 |
| | Ascitic | 41.67 | 34.00 | 45.80 | 43.57 | 39.00 | 3.215 | 0.946 |
| | SEM | 2.273 | 12.083 | 1.706 | 3.130 | 8.804 | | |
| | P | 0.102 | 0.651 | 0.128 | 0.111 | 0.570 | | |
| Na | Healthy | 154.00 | 150.50 | 152.00 | 151.00 | 150.00 | 0.810 | 0.419 |
| | Ascitic | 144.40 | 151.50 | 143.80 | 143.00 | 148.80 | 1.332 | 0.268 |
| | SEM | 1.788 | 1.118 | 2.699 | 2.740 | 1.480 | | |
| | P | 0.070 | 0.591 | 0.156 | 0.211 | 0.682 | | |
| K | Healthy | 6.75 | 4.85 | 5.10 | 5.15 | 5.50 | 0.531 | 0.549 |
| | Ascitic | 8.20 | 3.95 | 7.02 | 7.29 | 6.50 | 0.399 | 0.098 |
| | SEM | 0.779 | 0.471 | 0.864 | 0.705 | 0.446 | | |
| | P | 0.365 | 0.309 | 0.288 | 0.196 | 0.284 | | |
| Ca | Healthy | 1.30 | 1.23 | 1.37 | 1.37 | 1.26 | 0.038 | 0.440 |
| | Ascitic | 1.25 | 1.18 | 1.22 | 1.26 | 1.18 | 0.041 | 0.933 |
| | SEM | 0.059 | 0.049 | 0.101 | 0.057 | 0.077 | | |
| | P | 0.669 | 0.547 | 0.491 | 0.390 | 0.597 | | |
| Cl | Healthy | $109.00^{A}$ | $109.50^{A}$ | 110.50 | 109.00 | 109.50 | 1.349 | 0.984 |
| | Ascitic | $100.80^{B}$ | $107.00^{B}$ | 100.60 | 99.86 | 103.60 | 1.172 | 0.416 |
| | SEM | 1.658 | 0.353 | 2.414 | 2.419 | 1.260 | | |
| | P | 0.045 | 0.037 | 0.079 | 0.118 | 0.054 | | |
| Hb | Healthy | 10.00 | 11.00 | 13.00 | 10.20 | $9.20^{B}$ | 0.842 | 0.369 |
| | Ascitic | 13.53 | 14.00 | 14.94 | 14.19 | $15.93^{A}$ | 0.683 | 0.783 |
| | SEM | 0.762 | 4.005 | 0.127 | 1.040 | 0.457 | | |
| | P | 0.105 | 0.649 | 0.568 | 0.114 | 0.001 | | |
| HCO3 p | Healthy | 27.10 | 24.45 | 27.00 | 26.75 | 23.35 | 1.316 | 0.633 |
| | Ascitic | 25.52 | 28.40 | 30.12 | 31.10 | 31.88 | 0.811 | 0.073 |
| | SEM | 1.532 | 1.645 | 1.085 | 1.451 | 1.774 | | |
| | P | 0.605 | 0.231 | 0.185 | 0.200 | 0.050 | | |
| HCO3 pst | Healthy | 24.20 | 24.20 | 27.05 | 26.50 | 23.00 | 0.756 | 0.199 |
| | Ascitic | 21.43 | 26.55 | 24.88 | 25.36 | 26.20 | 0.728 | 0.260 |
| | SEM | 2.184 | 1.151 | 0.768 | 1.161 | 1.579 | | |
| | P | 0.519 | 0.285 | 0.191 | 0.656 | 0.307 | | |
| C BaseB | Healthy | 0.10 | 0.50 | 3.50 | 2.90 | -3.10 | 1.176 | 0.248 |
| | Ascitic | -1.85 | 3.65 | 3.45 | 3.50 | 4.20 | 0.972 | 0.412 |
| | SEM | 5.444 | 3.040 | 0.800 | 1.335 | 2.453 | | |
| | p | 0.818 | 0.553 | 0.973 | 0.838 | 0.222 | | |
| ctCO2B | Healthy | 21.50 | $22.55^{B}$ | 23.95 | 24.75 | 22.00 | 0.863 | 0.483 |
| | Ascitic | 24.63 | $25.10^{A}$ | 27.48 | 28.70 | 29.60 | 0.795 | 0.231 |
| | SEM | 2.017 | 0.145 | 1.037 | 1.156 | 2.014 | | |
| | P | 0.442 | 0.006 | 0.128 | 0.151 | 0.094 | | |
| ctCO2P | Healthy | 28.20 | 25.65 | 28.20 | 28.00 | $24.35^{B}$ | 1.329 | 0.616 |
| | Ascitic | 27.56 | 29.75 | 31.96 | 33.01 | $34.02^{A}$ | 0.840 | 0.079 |
| | SEM | 1.540 | 1.691 | 1.151 | 1.510 | 1.827 | | |
| | P | 0.833 | 0.228 | 0.141 | 0.161 | 0.036 | | |
| cCa | Healthy | 1.35 | 1.23 | 1.41 | 1.40 | 1.24 | 0.037 | 0.208 |
| | Ascitic | 1.20 | 1.18 | 1.17 | 1.20 | 1.16 | 0.020 | 0.991 |
| | SEM | 0.072 | 0.035 | 0.091 | 0.046 | 0.072 | | |
| | P | 0.271 | 0.422 | 0.223 | 0.086 | 0.571 | | |
| Anion K | Healthy | 22.80 | 21.40 | 19.60 | 20.40 | 22.90 | 0.961 | 0.533 |
| | Ascitic | $26.28^{a}$ | $20.05^{b}$ | $20.10^{b}$ | $19.33^{b}$ | $19.82^{b}$ | 0.689 | 0.007 |
| | SEM | 2.137 | 0.506 | 0.698 | 0.988 | 1.463 | | |
| | P | 0.498 | 0.200 | 0.717 | 0.625 | 0.311 | | |
| Anion Gap | Healthy | 17.70 | 16.55 | 14.50 | 15.25 | 18.30 | 0.649 | 0.172 |
| | Ascitic | $18.08^{a}$ | $16.10^{ab}$ | $13.08^{ab}$ | $12.04^{b}$ | $13.32^{ab}$ | 0.755 | 0.050 |
| | SEM | 1.403 | 0.617 | 1.314 | 1.366 | 1.340 | | |
| | P | 0.907 | 0.657 | 0.588 | 0.305 | 0.103 | | |
| SO2 | Healthy | $76.95^{A}$ | 76.10 | $77.85^{A}$ | $70.10^{A}$ | $78.20^{A}$ | 3.091 | 0.764 |
| | Ascitic | $4.90^{bB}$ | $52.00^{a}$ | $21.10^{bB}$ | $5.26^{bB}$ | $18.98^{bB}$ | 4.106 | 0.034 |
| | SEM | 2.316 | 6.692 | 9.784 | 3.958 | 10.682 | | |
| | P | <.001 | 0.125 | 0.026 | 0.001 | 0.031 | | |
| ctO2 | Healthy | $10.80^{A}$ | 11.50 | $14.20^{A}$ | $10.05^{A}$ | 10.35 | 0.779 | 0.225 |
| | Ascitic | $1.47^{B}$ | 10.40 | $4.14^{B}$ | $0.84^{B}$ | 5.28 | 1.003 | 0.070 |
| | SEM | 0.889 | 3.080 | 1.888 | 0.598 | 2.736 | | |
| | P | 0.023 | 0.824 | 0.035 | 0.000 | 0.344 | | |

*: Differences between group averages denoted by different small letters in the same row are statistically significant (p<0.05). **: Differences between group averages denoted by different capital letters in the same column are statistically significant (p<0.05). SEM: Standard error of difference between means.

## DICUSSION

Similar to our live weight findings, 100 mg/kg*L-carnitine* supplementation to broiler rations with different energy levels (11); 900 mg/kg L-carnitine supplementation to 5% animal fat containing broiler rations (4); and 400, 600 and 800 mg/kg liquid and power *L-carnitine* supplementation to broiler rations (12) has been reported that *L-carnitine* additive has no significant effect on live weight gain at the end of the trial ($p<0.05$). Contrary to current research, there are studies reporting that *L-carnitine* contributes significantly to live weight gain. Parsaeimehr et al (13) reported that the addition of 300 mg/kg *L-carnitine* to rations containing different levels of animal fat significantly improved live weight gain. Jalali et al (14) reported significant increases in live weight at the end of the 35-day trial of *L-carnitine* addition (120 mg/kg) to rations containing soybean oil and sunflower oil. The differences observed among these research findings may be attributed to differences in rations, dose levels used, and combinations of other additives used along with *L-carnitine* supplementation. In summary, the fact that there is no statistical difference between the groups as revealed by the live weight data obtained at the end of the trial indicates that the doses of the *L-carnitine* supplementations used are not in the concentrations that will change the live weight value positively or negatively.

Murali et al (4) and Corduk et al (11) reported that *L-carnitine* supplementation in broiler rations had no significant effect on feed consumption. The results of these researchers are in line with our findings indicating that addition of different levels of *L-carnitine* in drinking water does not significantly affect feed consumption of broilers. On the other hand, there are also studies showing that *L-carnitine* supplement significantly affects feed consumption. Wang et al (15) reported that *L-carnitine* (100 mg/kg) supplement significantly reduced feed consumption in broilers raised at low ambient temperatures. Such differences between researches findings can be attributed to the dose levels of the *L-carnitine* supplement used, the ration energy levels and the animal species used. The fact that there is no statistical difference between the groups referred from the feed intake values obtained at the end of the study indicates that the *L-carnitine* dose levels used do not have a significant effect on appetite in broilers.

Murali et al (4); Taklimi et al (12) and Wang et al (15) reported that *L-carnitine* supplement had no significant effect on feed efficiency ratio in broilers. Likewise, Geng et al (16) conducted a study to determine the effects of *L-carnitine* and coenzyme Q10 additives on the immune system and growth performance of the broilers against ascites. *L-carnitine* was used at doses of 0, 75 and 100 mg/kg and coenzyme Q10 was used at doses of 0 and 40 mg/kg, respectively. As a result of the trial, the feed efficiency ratio in the group supplemented only with *L-carnitine* improved numerically. Michalczuk et al (17) reported that *L-carnitine* supplemented with drinking water (62.5 g/100 liters) improves the feed efficiency ratio numerically. The findings of these researchers are in agreement with the findings of this study. This consistency can be attributed to the fact that the levels of *L-carnitine* additives used do not affect feed consumption. Contrary to these findings, studies have shown that L-carnitine significantly contributes to the feed efficiency ratio. Sharifi et al (18) found that *L-carnitine* supplement (100 mg/kg) with reduced protein (3%) significantly improved feed efficiency. The differences observed between these research findings can be attributed to the differences in animal species and ration used.

Total water intake was numerically higher in the group supplemented with 100 mg/ L *L-carnitine* compared to the other groups by the end of the trial. This can be attributed to the fact that the group receiving 100 mg/l *L-carnitine* supplementation consumed higher amount of feed compared to the other groups. Although no statistically significant difference was found between the groups in terms of water consumption, it was noticed that water consumption decreased numerically with increasing levels of *L-carnitine* supplementation. This conclusion suggests that broilers will be of high importance in determining *L-carnitine* requirements.

In the current study, mortality rate in the group receiving 100 mg/l *L-carnitine* supplementation was found to be lower than that of the control group. The increase in ascites mortality rates in the 5th week towards the end of the trial can be attributed to the increased oxygen demand due to the increased live weights of the animals and the inability to meet them adequately, as well as the use of feed in pellet form. Zohair et al (19) reported that compared to powder feed, pellet feed triggers ascites in broilers raised at high altitudes. Ascites death rate in the current study is lower. This can be attributed to the fact that the animals were not subjected to cold stress, which triggers the ascites. In contrast to the present study, the rate of death due to ascites was identified as 3.75% for the group grown in normal temperature conditions; 12.5% for the cold stress group and 8.75% for the group exposed to cold stress and received L-carnitine (100 mg/kg) in the study conducted by Tan et al (20). These ascites death rates are lower than those obtained in the current trial.

Çördük et al (11) reported that *L-carnitine* supplementation in broiler rations did not significantly affect blood glucose, cholesterol and triglyceride levels. Likewise, total cholesterol, LDL and triglyceride levels (21); hemoglobin and MCV levels (22); serum cholesterol and LDL levels (23); blood glucose, LDL, cholesterol, triglyceride, and total protein levels (13) and serum hematocrit value (18) were not significantly affected by *L-carnitine* additive in broiler rations. These results are consistent with the findings of the present study.

Contrary to existing findings, Çördük et al (11) reported a significant increase in AST levels of broilers fed with high-energy rations. Şimşek et al (24) reported that WBC, RBC, hemoglobin and hematocrit values were higher in *L-carnitine* supplemented rats. Elgazzar et al (25) revealed that L-carnitine supplementation significantly reduced cholesterol, triglyceride, LDL, AST, ALT and ALP levels in New Zealand rabbits. Jali et al (14) found that *L-carnitine* supplement to rations containing soybean oil and sunflower oil significantly reduced triglyceride levels while significantly increasing blood total protein, cholesterol, HDL and LDL levels. The differences between these findings can be attributed to differences in the energy levels of the rations used, energy sources and animal material.

Geng et al (16) and Yousefi et al (26) reported that *L-carnitine* supplement significantly reduced the hematocrit value in broilers. In the current study, with *L-carnitine* supplement, the blood hematocrit value tended to decrease numerically though not statistically. The thyroid gland hormones (T3, T4) are directly related to the development of the animals as they increase the basal metabolism. When the thyroid gland is running poorly, there is a slow-down in the development of the animals due to the disorder that occurs in the basal metabolism (27). In the present study, plasma T4 levels were significantly reduced with high doses of *L-carnitine* supplement (250 mg/L). This may be related to the fact that the live weight gain is also found to be the lowest in this group.

While blood pH value was measured as 7.21 in ascitic broilers, it was measured as 7.48 in healthy broilers. Low dose of *L-carnitine* supplementation (200 mg/l) was observed to increase blood pH value in ascitic broilers ($p<0.05$). As expected, the $pCO_2$ value was found higher in ascitic animals compared to healthy animals, while the $pO_2$ value was found to be lower. However, these values were not resulted by statistically significant effects of *L-carnitine* supplementation. The lowest $pCO_2$ value for healthy animals was found to be 37.00 and the highest value for ascitic animals was 69.80. The highest $pO_2$ value for the healthy animals was 43.50 and the lowest value for the ascitic animals was 4.43. Van As et al (28) reported that while $pCO_2$ levels were significantly increased in ascites, $pO_2$ levels were significantly reduced. Hafshejani et al (29) reported a significant decrease in pH value in ascitic animals. The findings of these researchers are consistent with the findings of the present study. Although chlorine concentration value was not significant for all groups among all healthy and ascitic animals, it was statistically significant in control and the group receiving 100 mg/l *L-carnitine* supplementation. There was no statistical difference between the other treatment groups which can be attributed to the large intra-group variance. Concentrations of $SO_2$ and $ctO_2$ were higher in healthy animals, while $ctCO_2P$ and hemoglobin concentrations were higher in ascitic animals ($p<0.05$). Blood hematocrit value was also increased numerically in ascitic animals. These results are in agreement with the findings of Tekeli (30). In broilers at high altitudes, $SO_2$ and $FO_2Hb$ decrease oxygen saturation in blood and increase cTHb and Hctc (hematocrit) values, leading to ascites.

In conclusion, blood pH value significantly decreased in ascitic broilers compared to healthy broilers. *L-carnitine* supplementation was found to be beneficial in decreasing ascites (pulmonary hypertension) mortality rates. These positive effects can be achieved by using low doses of *L-carnitine* supplements. It is expected that *L-carnitine* supplementation in water of broilers which are fed on diets with lowered energy and protein contents can be more effective in reducing ascites mortality rates. More research is needed to confirm these benefits.

## Conflict of interests

The authors reported no potential conflict of interest

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