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Evaluation of Thyroid Hormones, Blood Gases, Body Antioxidant Status, the Activity of Blood Enzymes and Bone Characteristics in Broiler Chickens with Cold Induced Ascites

Research Article

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ABSTRACT

A total of 150 day old female chickens (Ross 308) were randomly allocated to 2 groups with 5 replicate and 15 chicks in each replicate to determine the effects of cold induced ascites on performance, antioxidant status, blood enzyme activities and bone metabolism. The two experimental treatments were: 1) chicks that reared under normal temperature (NT) and 2) chicks that reared under cold temperature (CT). The experiment was terminated at 42 day of chicken age. Feed intake was reduced significantly by ascites during the starter period. Weight gain of NT birds was higher than CT birds during the starter, grower, finisher and the whole period ($P<0.05$). Feed conversion ratio was greater for CT birds during the grower, finisher and the whole period ($P<0.05$). Total mortality was greater in CT birds than NT ones during the whole period ($P<0.05$). Cold-induced ascites increased the right ventricular, total ventricular and ventricular septum weights and right ventricular (RV)/total ventricular (TV) ratio at day 42 ($P<0.05$). Blood pO_2, O_2 saturation, pH and T4 level were lower and blood pCO_2, T3 and calcium level was higher in CT birds than NT birds ($P<0.05$). The birds of both treatments had the same tibia length but femur length was shorter in CT birds ($P<0.05$). The diameter of both tibia and femur was smaller in CT birds ($P<0.05$). CT birds had a higher incidence of leg problems than NT ones during the whole period of the experimental phase ($P<0.05$). In conclusion, cold induced ascites reduced the performance, increased mortality and caused leg problems in broiler chickens.

KEY WORDS ascites, broiler, leg problem, performance.

INTRODUCTION

Pulmonary Hypertension Syndrome (PHS) or ascites is one of the considerable causes of high mortality in modern broilers. The economic losses due to ascites are about 1 billion US $ annually around the world (Maxwell and Robertson, 1997). It is estimated that 5% of broilers and 20% of roaster birds are dying due to ascites (Balog, 2003). Nowadays, the fast growing broiler chickens do not always have enough lung capacity to provide their oxygen demands. This inadequacy results in impaired ability to regulate the energy balance under extreme conditions, such as low ambient temperature or high altitude (Luger *et al.* 2001) that leads to increase the cardiac output, pulmonary hypertension, right ventricle and ventricular hypertrophy, cardiopulmonary dysfunction and finally ascites and death (Julian, 1993; Julian, 1998; Wideman and Bottje, 1993; Maxwell *et al.* 1995). Many changes in heart tissue (Daneshyar *et al.* 2009), blood gases (Olkowski *et al.* 1999; Daneshyar *et al.* 2007), antioxidant status (Han *et al.* 2005;

Wang *et al* . 2012), blood enzymes (Fathi *et al*. 2011) and thyroid hormones (Scheele *et al*. 1992; Moayyedian *et al*. 2011) can be occurred during the ascites process. Tekeli (2014) revealed that the level of oxygen saturation decreased and inversely blood hemoglobin, hematocrit and right ventricular diameter significantly increased in ascitic broilers under high altitude.

Moayyedian *et al*. (2011) detected that broilers under cold temperature had a higher mortality, RV/TV ratio and venous pCO_2 and lower pO_2 than control birds. In other studies, Han *et al*. (2005) and Fathi *et al*. (2011) indicated that lung and liver ROS, plasma nitric oxide (NO) level, super oxide dismutase (SOD) activity and total antioxidant capacity (TAC) were lower and plasma malondialdehyde (MDA) were higher in cold-induced ascitic birds. Ipek and Sahan, (2006) detected that ascites mortality, right ventricular (RV) and RV/TV ratio was higher in cold stress birds. Daneshyar *et al*. (2009) did not detect any changes of lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in ascetic birds but mortality and RV/TV value was higher. In an experiment, Camacho-Escobar *et al*. (2011) indicated that the treatments with high ascites mortality accompanied with higher incidence of leg disorders level during the experiment. Leg problems is a major economic and welfare problem (Bradshaw *et al*. 2002) with incidence of typically less than 2 or 3% in broilers flocks and can affects the diet accessibility, feed conversion ratio and growth rate. Leg problems and developmental disorders of long bones can be affected by genetics, breeder nutrition, incubation, infectious diseases, and environmental stressors (Bradshaw *et al*. 2002; Oviedo Rondón *et al*. 2006a; Oviedo Rondón *et al*. 2006b).

It has been distinguished that environmental conditions such as low ambient temperature might negatively change the bone deformities or leg problems in modern strain with fast growing rate.

Some information is available about performance, mortality, heart tissue, blood indices, blood gases, thyroid hormones, blood enzyme activity of LDH, AST, alkaline phosphatase (ALP), NO and antioxidant status, but more information about leg disorder with ascites syndrome are needed.

The first aim of this study was to evaluate the possible effects of ascites on performance, mortality, heart tissue, blood indices, blood gases, thyroid hormones, blood enzyme activity and antioxidant status in female broilers. Since, there is no report regarding the effects of cold induced ascites on bone characteristics and related mineral metabolism in broiler chickens, the possible changes of bone structure and leg problem were investigated as the second aim of the current experiment.

MATERIALS AND METHODS

A total of one hundred and fifty day old female chicks (Ross 308, initial weight: 45±3 g) were obtained from a commercial hatchery randomly allocated to 2 groups; a control and ascitic groups with 5 replicates of 15 chicks each. Water and feed were provided *ad libitum*. All the chickens were fed the similar starter (day 1-10 of age), grower (day 11-24 of age) and finisher (day 25-41 of age) diets in mash form (Table 1).

Rations were formulated by amino feed[1] software according to the Ross requirement recommendation (Aviagen, 2014). The ascitic birds were reared in a house with 1670 m altitude under cold temperature condition. The temperature was 33 ˚C during the first week of the experiment and reduced to 26.0 ± 1 ˚C, 20.0 ± 1 ˚C and 15.0 ± 1 ˚C at 7, 14 and 21 day of age, respectively, and was maintained at 15.0 ± 1 ˚C until the end of the experiment (Luger *et al*. 2001). The control group were raised under normal temperature (NT birds) with 33 ˚C during the first week of the experiment. The temperature for NT birds was regularly reduced by 3 ˚C per week up to 3 wk of age and then was kept constant at 24.0 ± 1 ˚C until the end of the experiment. The average feed intake (FI, g/bird/day), average body weight gain (BWG, g/bird/day) and feed conversion ratio (FCR) were determined during the starter, grower, finisher and the whole periods. During the experiment, mortality was recorded daily and all the dead chickens were diagnosed for ascites according to ratio of right ventricle to total ventricle weight, amber-colored fluid in the abdominal cavity and pericardium. Leg problems were estimated visually on chicks with arthritis, tibial dyschondroplasia, rickets, osteochondrosis, prosis, prostrate status, or difficulty to walk (Williams *et al*. 2000). At day 42 of age, 2 birds/pen (ten for each treatment) were selected, weighed and killed by decapitation to obtain the heart tissue, blood and bone samples. The heart was dissected and proportional weight of total ventricular (TV) and right ventricular (RV) was determined and ventricular septum thickens (VS) measured by digital caliper. The heart ratio was calculated by the weight of the right ventricle as a percentage of the total ventricle weight (RV/TV). Blood samples were collected in heparinized syringes (1 mL) and kept on ice and instantly moved to laboratory less than two hours for blood gas measurements by pH/blood gas Analyzer (ABL50, ABL995, France; Daneshyar *et al*. 2012). Some blood samples collected in non heparinized syringes (2 mL) and allowed to clot for 2 h at 37 ˚C. The serum was then decanted and stored at 20 ˚C for later analyses (Daneshyar *et al*. 2012). Serum samples were thawed and serum malondialdehyde (MDA) content were determined by thiobarbituric acid method.

Table 1 Composition of experimental diets

Ingredients (%)	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn grain	44.029	52.05	58.445
Corn gluten	9.409	-	-
Soybean meal	39.369	40.078	34.319
Soybean oil	2.392	3.815	3.397
Dicalcium phosphate	2.223	1.956	1.811
Carbonate calcium	1.219	0.969	0.955
L-lysine HCl	0.242	0.029	0.02
DL-methionine	0.214	0.245	0.207
Vitamin and mineral premix[1]	0.5	0.5	0.5
Salt	0.255	0.326	0.326
Bicarbonate sodium	0.149	0.031	0.02
Total	100	100	100
Calculated analysis			
Dry matter (%)	89.801	89.535	89.17
ME (kcal/kg)	2950	3000	3050
Crude fat (%)	4.294	5.799	5.581
Calcium (%)	1.027	0.88	0.831
Available phosphorus (%)	0.491	0.44	0.411
Chloride (%)	0.23	0.23	0.23
Sodium (%)	0.167	0.162	0.158
Methionine (%)	0.643	0.569	0.506
Lysine (%)	1.478	1.256	1.104
Arginine (%)	1.632	1.512	1.347
Methionine + cystine (%)	1.083	0.924	0.835
Threonine (%)	1.004	0.856	0.771
Tryptophan (%)	0.298	0.278	0.246

[1] Supplied per kilogram of diet: Retinol: 9000 IU; Alpha tochopherol acetate: 36 IU; Cholecaciferol: 2000 IU; Cyanocobalamin: 15 mg; Riboflavin: 6.6 mg; Calcium pantothenate: 9.8 mg; Niacin: 30 mg; Choline chloride: 625 mg; Biotin: 0.1 mg; Thiamine: 1.75 mg; Pyridoxine: 3 mg; Folic acid: 1 mg; Menadione: 2 mg; Antioxidant (ethoxy queen): 100 mg; Manganese: 248 mg; Zinc: 211 mg; Copper: 25 mg; Iron: 125 mg; Iodine: 2.5 mg and Selenium: 0.5 mg.

The plasma activities of glutathione peroxidase (GPX), superoxide dismutase (SOD) and total antioxidant capacity (TAC) were measured by Randox Kits (Randox Labs, Crumlin, UK). Uric acid were determined colorimetrically (Pars Azmon kit, Tehran, Iran) and plasma thyroid hormones (T3, T4) were measured by ELISA method (Pishtaz Tab Zaman Company Kite, Iran). Plasma LDH, AST and ALP enzyme activities and blood Ca and P levels were determined by spectrophotometric device (Alcyon 300, USA). Plasma nitric oxide (NO) assayed according to Katrina *et al*. (2001) method (UV-visa array, spectrophotometer photonix, AR 2015). After carcass separation, tibia bone for each bird was removed and length and diameter measured by digital caliper. The bone-dry matter and ash contents were determined by AOAC method (1990) and percent of calcium (Ca) and phosphorus (P) measured spectrophometrically.

The data were analyzed based on a completely randomized design using the GLM procedure of SAS (SAS, 2002). When the overall model was statistically different ($P<0.05$), the Tukey-Kramer multiple comparison test was used to compare the mean values of healthy and ascitic birds ($P<0.05$).

RESULTS AND DISCUSSION

The results of performance traits are shown in Table 2. Feed intake (FI) was affected significantly by ascites during the starter period and NT birds had a higher FI than CT birds ($P<0.05$). There was a significant difference between the treatments for WG, NT birds had higher WG than CT ones during the whole rearing periods ($P<0.05$). Ascetics increased the FCR during all of the periods except starter. Total rate of mortality due to ascites was significantly greater in CT treated birds than that of NT ones (46.6% *vs*. 4%) during the whole period (Figure 1; $P<0.05$). Cold environment significantly increased the weights of heart parts such as right ventricular (RV), total ventricular (TV), RV/TV ratio and ventricular septum thickness (VS). (Table 3; $P<0.05$).

The RV (0.1 *vs*. 0.04), TV (0.43 *vs*. 0.29), RV/TV ratio (27.9% *vs*. 15.3%) and VS (3.08 *vs*. 1.86) of CT exposed birds was greater than those of NT exposed ones. The results of thyroid hormones, blood gases and blood minerals are shown in Table 4. The blood pO2, O_2 saturation and pH were lower and pCO_2 was higher in CT exposed birds as compared to NT exposed ones ($P<0.05$).

Table 2 Feed intake (g/d/bird), weight gain (g/d/birds) and feed conservation ratio of female broiler chicks reared under normal (NT) and cold (CT) environmental temperatures

Treatments	NT	CT	P-value	Pooled SEM
Feed intake				
Starter	26.31[a]	23.98[b]	0.04	0.6
Grower	64.04	69.8	0.08	1.68
Finisher	142.8	140.2	0.82	5.5
Total	77.7	77.9	0.95	2.07
Weight gain				
Starter	18.68[a]	15.96[b]	0.003	0.55
Grower	36.9[a]	31.1[b]	0.009	1.25
Finisher	75.9[a]	52.9[b]	0.001	4.4
Total	43.8[a]	33.3[b]	0.004	1.94
Feed conversion ratio (FCR)				
Starter	1.38	1.5	0.054	0.03
Grower	1.73[b]	2.25[a]	0.004	0.11
Finisher	1.89[b]	2.65[a]	0.0001	0.13
Total	1.66[a]	2.13[b]	0.001	0.08

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Proportional weights of total ventricular (TV), right ventricular (RV) and ventricular septum (VS) and RV/TV ratio of broiler chicks reared under normal (NT) and cold (CT) environmental temperatures

Treatments	TV	RV	VS	RV/TV
NT	0.29[b]	0.04[b]	1.86[b]	0.15.3[b]
CT	0.43[a]	0.1[a]	3.08[a]	0.27.9
P-value	0.005	0.001	0.003	0.0003
SEM	0.03	0.01	0.25	0.02

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Thyroid hormones (ng/mL), blood gases (mmHg) levels and blood mineral (mg/dL) of broiler chicks reared under normal (NT) and cold (CT) environmental temperatures

Treatments	Thyroid hormone		Blood gases				Blood mineral	
	T3	T4	pCo_2	pO_2	pH	O_2 saturation (%)	Calcium	Phosphorous
NT	1.64[b]	16.48[a]	53.6[b]	65.5[a]	7.39[a]	63.5[a]	8.7	6.2
CT	1.92[a]	10.94[b]	58.7[a]	55.4[b]	7.15[b]	51.6[b]	9.9	6.3
P-value	0.0001	0.0001	0.003	0.0008	0.04	0.0001	0.02	0.8
SEM	0.05	0.96	1.01	1.91	0.06	2.03	0.29	0.3

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

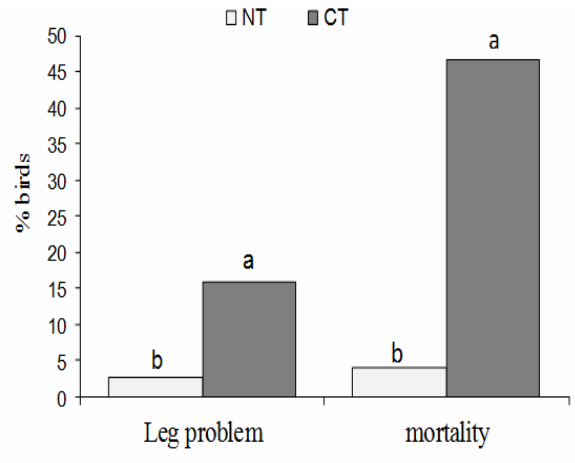

Figure 1 Leg problem and mortality of broiler chickens reared under normal (NT) and cold (CT) environmental temperatures

Plasma T3 level was higher and plasma T4 level was lower in CT exposed birds when compared to NT exposed birds. Blood phosphorous did not changed as effect of treatments but blood Ca level was significantly higher in CT exposed birds than NT exposed ones (P<0.05). According to the results of antioxidant status in Table 5, there were no significant differences for plasma MDA, total antioxidant capacity (TAC), SOD, GPX and uric acid levels between the treatments at day 42 of age (P<0.05).

The results of blood enzymes in both treatments showed similar activity for ALP, LDH, AST and NO enzymes (Table 6). The bone contents are shown in Table 7. There were no significant differences between the treatments for ash, Ca, P and moister in both the tibia and femur (P<0.05).

The birds of both treatments had the same tibia length but femur length was shorter in CT exposed birds (Figure 2; P<0.05).

Table 5 Plasma antioxidant activity of female broiler chicks reared under normal (NT) and cold (CT) environmental temperature

Treatments	SOD (U/g Hb)	GPX (U/g Hb)	MDA (nmol/mL)	TAC (nmol/L)	Uric acid (mg/dL)
NT	1078.1	33.14	1.82	1.86	3.16
CT	617.1	23.57	2.46	2.25	4.98
P-value	0.1	0.1	0.1	0.18	0.15
SEM	142.9	2.96	1.17	0.14	0.62

SOD: super oxide dismutase; GPX: glutathione peroxidase; TAC: total antioxidant capacity and MDA: malondialdehyde.
SEM: standard error of the means.

Table 6 Blood enzyme activity (U/L) of broiler chicks reared under normal (NT) and cold (CT) environmental temperatures

Treatments	ALP (U/L)	LDH (U/L)	AST (U/L)	NO (µm)
NT	1840	3770	297.5	19.34
CT	1375	1918	256.6	20.97
P-value	0.14	0.09	0.36	0.49
SEM	154.2	557.7	31.9	38.7

ALP: alkaline phosphatase; LDH: lactate dehydrogenase; AST: aspartate amino transferas and NO: nitric oxidase.
SEM: standard error of the means.

Table 7 Tibia and femur contents of broiler chicks reared under normal (NT) and cold (CT) environmental temperature

Treatments	Ash (%)		Calcium (%)		Phosphorous (%)		Moister (%)	
	Femur	Tibia	Femur	Tibia	Femur	Tibia	Femur	Tibia
NT	45.6	47.2	18.2	31.9	16.7	18.9	16.8	31.9
CT	41.2	53.2	21.8	30.6	17.8	15.5	22.2	30.6
P-value	0.34	0.51	0.13	0.59	0.69	0.10	0.12	0.13
SEM	2.2	186	1.1	13.6	1.3	9.1	1.7	71.3

SEM: standard error of the means.

The diameter of both tibia and femur was smaller in CT exposed birds as compared to NT exposed birds (Figure 3; P<0.05). Furthermore, CT birds had a higher incidence of leg problems than NT ones (15.99% *vs*. 2.67%) during the whole period (Figure 1; P<0.05).

The high ascites mortality in present experiment was in consistent with that of Daneshyar *et al*. (2012) and Fathi *et al*. (2011). Cold temperature together with high altitude (1670 m) in present experiment is the reason of high ascites mortality.

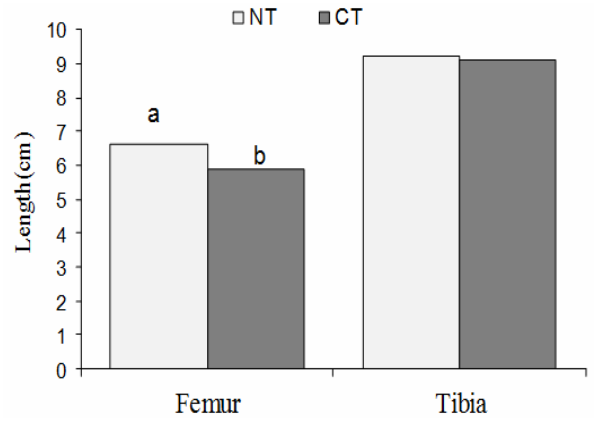

Figure 2 Femur and tibia length of broiler chickens reared under normal (NT) and cold (CT) environmental temperatures

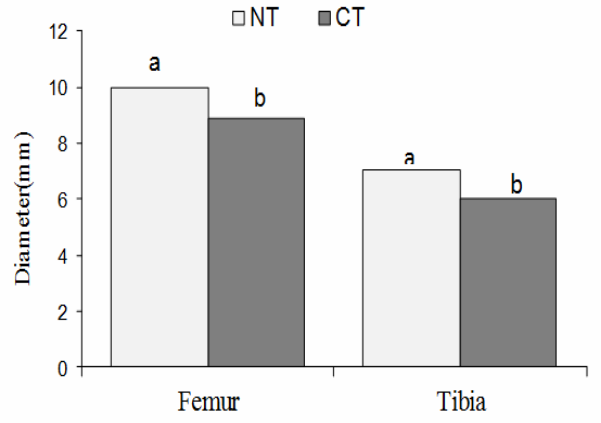

Figure 3 Femur and tibia diameter of broiler chickens reared under normal (NT) and cold (CT) environmental temperatures

In the experiment, ascites symptoms were noticeable from day 20 onward. During wk 4, ascites signs were observed as the abdominal cavity fluid, heart enlargement and mortality in cold exposed group, which was approved by high mortality rate (46.6%) and low grow rate (1752 g). The above mentioned negative effects of ascites supported by the results of many previous researches (Ipek and Sahan, 2006; Guo *et al*. 2007; Daneshyar *et al*. 2009).

Oxygen pressure and atmospheric pressure is lower at high altitude and this phenomenon along with cold environment increases the oxygen needs in order to maintain their body temperature (Wideman and Tackett, 2000). Lower feed efficiency and body weight gain in CT birds closely associated with cold temperature and utilization of energy to heat production (Wideman, 1988). This means that energy partitioning is different between the CT and NT

birds and the amount of energy that contribute to grow as the protein and fat mas in NT birds was used for heat production in CT birds.

The greater RV/TV ratio and ventricular septum thickness of CT birds indicates the ascites development in recent experiment. Our results are supported by the results of previous studies which were showed significant difference between healthy and ascitic birds in heart parameter and hematology indexes (Daneshyar *et al*. 2009; Daneshyar *et al*. 2007; Guo *et al*. 2007; Ipek and Sahan, 2006). The right ventricle: total ventricle ratio above 0.27 also strongly indicates the start of ascites development, as proposed by Balog (2003). In agreement with our finding, failure function, hypertrophy and enlargement of right ventricle was shown in ascetic birds and therefore RV/TV ratio enhanced consequently (Daneshyar *et al*. 2007; Daneshyar *et al*. 2012; Huchzermeyer, 2012; Ocak, 2006). Our finding supported by previous researchers who reported that cold induced ascitic birds had significantly higher blood pCO2 and lower pO2 (Moayyedian *et al*. 2011; Van As *et al*. 2010), lower blood pH (Hafshejani *et al*. 2012; Closter *et al*. 2009) lower O2 saturation (Daneshyar *et al*. 2007), higher plasma T3 and lower plasma T4 level (Scheele *et al*. 1992; Moayyedian *et al*. 2011) than control birds. Blood gases and thyroid hormones can be used for ascetic detection whereas pCO2 and pO2 can predict the ascites development. Oxygen demands for cold exposed broilers increased in order to maintain their body temperature (Wideman and Tackett, 2000) or because of high metabolic rate, which leads to hypoxemia and subsequently development of pulmonary hypertension.

In ascetic birds, body tissue forced with decreased blood pO2 and increased blood pCO2 levels are the signs of hypoxemia condition that appeared in response to high growth rate, hypoxemia and subsequently development of pulmonary hypertension (Gupta, 2011). Furthermore, the low blood pH of ascetic birds declines the affinity of hemoglobin for oxygen in the lung and release of oxygen to the tissues as known Bohr effect (David *et al*. 1966). Lower blood pH in CT birds might be related to high metabolic rate and consequently decreased oxygen affinity to hemoglobin and hemoglobin saturation in the lung (Issacks *et al*. 1986).

Thyroid hormones have important role for in controlling metabolic rate, (Decuypere *et al*. 2000; Lin *et al*. 2008) and are linked with ascites susceptibility in broiler chickens under adverse environmental conditions (Hassanzadeh *et al*. 2004; De Smit *et al*. 2005). The T3 is the main hormone that is related to body temperature regulation and stimulates the body metabolism and increases the chickens growth (Yahav, 2000; Hafshejani *et al*. 2012). Our results are supported by Scheele *et al*. (1992) and Moayyedian *et al*. (2011) that indicated the birds which were exposed to cold temperature (CT) showed lower plasma T4 levels and higher plasma T3 levels compared with the birds that reared at normal temperature (NT). In different results, Luger *et al*. (2001) detected that plasma T3 and T4 levels decreased while broilers exposed to low ambient temperature. The plasma T3 concentration is positively correlated with oxygen consumption in broilers (Bobek *et al*. 1977; Gabarrou *et al*. 1997). However, T3 concentration in the ascetic birds, which were exposed to cold temperature was significantly higher because of high oxygen demands and increase of metabolic rate (Stojevi *et al*. 2000). The decreased plasma T4 content can be explained by a negative feedback of T3 on the hypothalamus resulting in a decreased thyroid releasing hormone secretion, therefore lower T4 release (Moayyedian *et al*. 2011) and increase in shifting of T4 to T3 conversion, in cold exposing chickens.

MDA concentration is an important index for lipid peroxidation and oxidative damage caused by ROS in the cell (Iqbal *et al*. 2002) and plays an important role in ascites incidence. Some researchers suggested that heart failure in broilers with hypoxia and subsequent ascites syndrome can be associated with ROS production during oxidative stress (Fathi *et al*. 2011). Cold stress is one of the effective factors for inducing hypoxemic conditions in tissue of cold exposed birds that can generate ROS (Park and Kehre, 1991; Dawson *et al*. 1993; Han *et al*. 2004b) which may cause lipid peroxidation in the membrane of the cells resulting in injury of organs such as lung, heart and liver (Arab *et al*. 2006). MDA level is the main index of lipid peroxidation and therefore numerically higher plasma MDA content in CT-exposed birds can indicates the mild ROS production and lipid peroxidation in ascetic birds.

In the health body, protective enzymatic systems such as SOD and GPX are the natural body antioxidants that play important role to neutralize free radicals function (Duthie *et al*. 1989). SOD catalyzes the conversion of superoxide to hydrogen peroxide that is in turn reduced by GPX to water (Iqbal *et al*. 2002). Although plasma SOD and GPX activities have not been affected by ascites in present study, some researchers have reported the changes of the mentioned enzymes in ascitic birds. For example, Han *et al*. (2005) and Wang *et al*. (2012) detected the lower plasma SOD and GPX activity in cold exposed birds. Fathi *et al*. (2011) reported the higher GPX and SOD activity in plasma and liver tissue of cold induced ascites. Iqbal *et al*. (2002), indicated the lower reduced glutathione (GSH) level and higher GSSG/GSH ratio, as consequence of increased in GPX activity in lung mitochondria of ascetic birds. The possible inconsistency could be related to mild peroxidation (numerically lower plasma MDA content) of CT birds in present study, which had not affected the antioxidant enzyme activities.

None of the plasma enzymes was affected by ascites which was consistent with the results of previous literature that detected no significant changes for LDH (Daneshyar *et al*. 2009; Khajali and Qujeq, 2005) or AST (Daneshyar *et al*. 2009; Tankson *et al*. 2002) activity under cold temperature.

Although Han *et al*. (2005) indicated decreased nitric oxide synthase activity in cold temperature. We observed no changes of calcium and phosphorous contents in the tibia and femur in our study but blood calcium levels and leg problems were higher in ascetic birds. Moreover, the diameter and length of both tibia and femur bones were shorter in cold reared birds.

Although there is no report for the bone metabolism of broiler chickens under cold induced ascites and according to our knowledge this study was the first in this field, these changes could have two reasons. One possible reason is the high blood calcium content in CT exposed birds which might be related to low calcium needs for lower growth rate and shorter bones.

Second possible reason is the low calcium deposition to bone structures in CT exposed birds which might had led to smaller length and diameter of bones and consequently leg disorder incidence. Camacho-Escobar *et al*. (2011) who indicated high rate of leg disorders in ascetic birds confirm high percentage of leg problem. Low size of tibia and femur in ascetic birds could be related to lower calcium absorption.

The results of Wolfenson *et al*. (1987) approve namely the reduction of calcium absorption in cold-stressed birds. Lower size of bones in ascetic birds can be results of mineral imbalance that caused the high leg disorder in ascetic birds. Other reason can be discussed by high metabolism rate in cold temperature, which may leads to lower plasma pH and acidosis in CT-exposed birds. Acidosis may affect negatively the availability of 1,25-dihydroxycholecalciferol (Julian, 1998) and causes slow growth plate by chondrocytes proliferation, tibia and femur disorder and in final leg disorder in ascetic birds.

CONCLUSION

According to the results of present study, cold-induced ascites changes the thyroid hormone metabolism, blood gases and heart parts indices and reduces the performance and increase the mortality in broiler chickens. Furthermore, it leads to lower plasma pH, acidosis and possibly low availability of 1,25-dihydroxycholecalciferol which causes lower calcium absorption and deposition to bones and consequently smaller length and diameter of bones and leg problems in female broiler chickens.

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