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Changes in biochemical parameters of a broiler chicken line with cold-induced ascites

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Abstract Ascites syndrome (AS), caused by an imbalance between high oxygen demand and its supply by cardiopulmonary system in broiler chickens, is a major problem in the broiler industry worldwide. An experiment was designed to investigate the differences in biochemical parameters between the ascitic and healthy chickens in a pure broiler sire line under cold conditions. A total of 817 one-day-old mixed-sex chickens from 71 half-sib families were grown and AS was induced on day 21 using a cold temperature model. On the 32nd day of the trial, blood samples from five birds per sire family were collected and blood parameters (hematocrit percentage and serum metabolites including, glucose, total protein, albumin, triglycerides, cholesterol, high-density lipoprotein cholesterol (HDL), calcium, magnesium, phosphorus, alanine aminotransferase, aspartate aminotransferase, malonedialdehyde and total antioxidant status) were measured. The results showed that the male birds were more sensitive to AS than the female birds. Hematocrit percentage of the ascitic chickens was higher than the healthy chickens (P<0.0001). In both sexes, the ascitic birds showed higher right ventricle/total ventricle ratio (RV/TV) than the healthy birds (P<0.0001). The concentrations of serum HDL and cholesterol in the ascitic male birds were significantly lower than that of the non-ascitic male birds. Reduction of serum cholesterol in ascitic birds may be due to its use for the synthesis of corticosterone under cold stress conditions and ascites. In this work, the traits were measured after the occurrence of ascites. Therefore, traits such as RV/TV, hematocrit, HDL and cholesterol could be useful only in diagnosis of birds that are developing AS. Since it is shown that the values of these traits are different between the healthy and ascitic chickens before the start of AS, these could be potential predictors of AS. Thus, they may be useful in designing breeding programs to produce more AS-resistant birds.

Keywords: cold stress, broiler line, HDL, hematocrit, pulmonary hypertension syndrome

Introduction

Ascites syndrome (AS) (serous fluid accumulation in the abdominal cavity), also known as pulmonary hypertension syndrome, is a costly and main metabolic disease in the broiler industry worldwide (Maxwell and Robertson, 1997; Julian, 2000). Ascites is a multifactorial syndrome caused by interactions between environmental, physiological, genetic and management factors (Baghbanzadeh and Decuypere, 2008). This metabolic disorder is developed due to a failure to fully supply the increasing demand for oxygen (Wideman, 2001) incurred by fast growth, cold temperatures or high-energy diets (Wideman et al., 2007). The failure is caused by an inconformity between cardiopulmonary system output and the demands of the body (Decuypere et al., 2000). It begins with insufficiency of oxygen for the metabolism (Julian, 2000) leading to hypoxemia and tissue hypoxia, pulmonary hypertension, proliferation of immature red blood cells and increasing hematocrit values and blood viscosity (Shlosberg et al., 1998), right ventricle hypertrophy and a drop in cardiac output (Wideman and French, 1999), which eventually lead to liver harm and leakage of yellow fluid with fibrin clots into the abdominal cavity and death (Balog et al., 2003). Therefore, several organs (such as heart, lung, liver, kidney, etc.) are involved in the occurrence of AS. Decuypere et al. (2005) suggested that the syndrome is not created by an increased oxygen demand of rapid growth rate per se at low altitude, but by an impaired oxygen supply to maintain a fast growth rate. Because the peak of the incidence of AS is between 4 and 6 weeks of age (Maxwell and Robertson, 1997), the economic loss due to AS is high and significant, so that 5% of the broilers and 20% of the roaster birds die due to AS (Balog, 2003; Baghbanzadeh and Decuypere, 2008).

In recent decades, intense genetic selection of modern chickens for rapid growth has produced fast growing chickens with a high metabolic rate (Decuypere et al., 2005). The higher metabolic rate leads to enormous oxygen demand (Decuypere et al., 2000) which in turn causes AS to develop. Gleeson (1986) reported that oxygen demand is increased by 185% in low ambient temperature. It is believed that there is a high correlation between AS syndrome and cold temperature (Ipek and Sahan, 2006). Previous reports have shown that cold temperature can stimulate AS (e. g., Daneshyar et al., 2009; Van As et al., 2010). Cold temperature causes increasing AS susceptibility by increasing the metabolic rate and, consequently, producing metabolic hypoxia (Stolz, 1993) or hypercapnia (Scheele et al., 2003) in broiler chickens.

In spite of intense research on AS for many years, its pathogenesis and underlying mechanisms have remained unclear (Wang et al., 2012). It has been reported that AS causes abnormal metabolism of corticosteroids and triiodothyronine (Luger et al., 2003). Significant changes in the level of liver glucose and lipids metabolism are related to metabolic abnormalities, and this is due to the difference in expression of proteins (Shimomura et al., 2000). In addition, it is suggested that AS might be related to oxidative stress influenced by the reactive oxygen species (Ruiz-Feria, 2009). There are few reports concerning the changes of biochemical parameters such as fasting blood glucose, serum protein, triglyceride (Zhang et al., 2010; Kheiri et al., 2011), hepatic Malondialdehyde concentration, serum glucose and cholesterol level, hepatic lactic acid (Wang et al., 2012), serum albumin, liver metabolic enzymes activities of broiler chickens grown under a cold temperature environment (Yersin et al., 1992; Biswas et al., 1995).

Moderate heritability for AS suggests that selection against AS is possible in broiler chickens (Druyan et al., 2007). Therefore, by selecting against AS in poultry breeding programs, the frequency of susceptible individuals can be reduced. This requires suitable indicators to be included in the selection index. For this reason, the current work was conducted to investigate the effect of cold temperature on the biochemical parameters and identify the most appropriate biomarker as a selection criterion.

Materials and methods

A total of 817 one-day-old chicks of both sexes (420 males and 397 females from 71 half-sib families) from a pure sire line were raised. Due to the fact that the health status of the chickens under test was not known at the start of the trial, a total of 817 birds were raised as an experimental group until the end of the trial. AS was induced using a cold temperature model suggested by Daneshyar et al. (2009). In order to enhance the probability of incidence of pulmonary hypertension in almost all of the susceptible chickens, cold temperatures were combined with high energy diets to encourage a rapid growth rate (Wideman et al., 1995). The rearing environment temperature was kept at 32-34°C during the first and second days of age and then was gradually lowered by 0.5°C every other day until 24°C was reached on day 21 at which point the cold temperature challenge was started. The temperature of the house was maintained at 18°C from 22 days of age to the end of the experiment (48 days). In the trial, signs of AS were noted after 3 weeks of age. The diets were formulated to meet nutritional requirements for broiler chickens in the starter (days 1–10), grower (days 11-24), and finisher stages (days 25 upward).

Animal ethics statement

All of the experimental procedures involving animals used in this study were approved by the ethics committee of the Department of Animal Science, Tabriz University, Iran (Permission number: 357/2017).

Measurement of body weight, growth rate and AS heart index

Body weight (BW) was measured at days 1 (BW_1) and 42 (BW_{42}), and growth rate (GR) between BW_1 and BW_{42} was calculated per chicken using the following formula:

$$GR=(BW_m-BW_n/d_m-d_n) \quad (1)$$

where, BW_m and BW_n are the measured BWs at day m (d_m) and day n (d_n) of age, respectively.

Hearts were collected from all chickens; from those that died during the experiment and also from the slaughtered birds at the day 48 of age. The AS heart index (AHI) or the RV/TV ratio (right ventricle/total ventricle) was calculated for each bird. Birds that showed RV/TV > 0.27 and signs of AS (ascetic fluid or hydropericardium, or both) were assigned a health status (HS) of 1 (ascitic) and 0 (healthy), otherwise.

Measurement of blood biochemical parameters

On the 32nd day of the trial (due to the incidence of AS peaks between 4 and 6 weeks of age), five birds per sire family (including 3 males and 2 females) were designated for blood collection from the wing vein. One mL blood samples were collected by venipuncture into EDTA-K3 anticoagulation tubes for hematocrit (HCT) measurement. A Hettich centrifuge model D-7200 (Tuttlingen, Germany) was used to centrifuge the whole blood for HCT preparation. Another set of blood samples was immediately collected into non-anticoagulant tubes to obtain serum by centrifuging the blood samples at 2500 rpm for 10 min. Concentration of the serum fasting blood glucose (GLU), cholesterol (CHO), triglyceride (TG), calcium (CAL), magnesium (MAG), phosphorus (PHO), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), total antioxidant status (TAS) and HDL were measured using an ABBOTT ALCYON 300/300I ANALYZER. Malondialdehyde (MDA) concentration in serum was measured as an index of lipid peroxidation using the thiobarbituric acid method (Yagi, 1984). After determining the health status of the birds at the end of trial (Through RV/TV ratio and the presence or absence of ascetic fluid), data from blood samples were divided into healthy and ascitic groups according to the health status of the birds. A number of the blood samples that were not suitable for obtaining serum and measuring of blood biochemical parameters were excluded, so the number of samples from some families was less than 5 birds.

Statistical analysis

The mixed procedure of SAS (SAS Institute, 2002) was used to analyze the differences of biochemical parameters between ascitic and healthy chickens using the following model:

$$y_{ijk} = \mu + Sex_i + HS_j + (Sex*HS)_{ij} + Sire + e_{ijk} \quad (2)$$

This model included fixed effects of sex (including two levels: male and female) and health status (HS) (including two levels: healthy and ascitic) and the random genetic effect of the sire. The Tukey multiple-range method was used for comparing the least squares means differences between the various levels of fixed effects. The difference in AS frequency between the two sexes was statistically tested for significance using the Chi-square **(χ^2)** test (df = 1 and P-value = 0.01). The CORR procedure of SAS (SAS Institute, 2002) was employed to estimate the Pearson correlation between the traits.

Results

AS-related mortality

In this trial, signs such as anorexia, high respiration rate and decreased mobility were observed in the ascitic birds. Excessive accumulation of fluid in the abdominal cavity and the pericardium, right ventricular hypertrophy, loose heart, lung edema and hypertrophy of the liver were also observed. The ascitic chickens remained smaller than their normal counterparts. Eighty three birds out of the 817 birds were excluded from the dataset by the 3rd week due to the lack of uniformity of weight. Of the 734 survivors (349 females and 385 males), 433 (58.99%) birds died as a result of AS (including 244 male and 189 female birds). In the third week of the experiment, the rate of mortality due to AS was relatively low (39 birds) and gently increased in weeks 4, 5, 6 and 7 (89, 89, 122 and 94 birds per week, respectively) (Figure 1). The mortality rates for the males and females was 56% and 44%, respectively. The difference of AS incidence between the two sexes was highly significant ($\chi2$ test; $P<0.01$).

RV/TV, biochemical factors and growth-related traits

Table 1 presents the least squares means of RV/TV and growth-related traits for the healthy and ascitic chickens. In both sexes, the ascitic birds showed higher RV/TV ratios than healthy birds ($P<0.0001$). Least squares means of the blood biochemical parameters are presented in Table 2. We observed higher hematocrit in

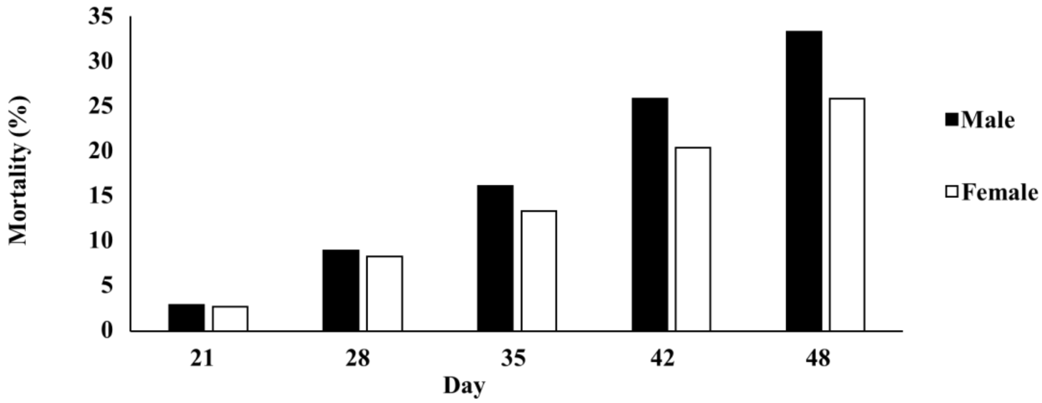

Figure 1. Mortality rate due to ascites for the male and female birds

Table 1. Least squares means of right ventricular weight/total ventricular and growth-related traits in healthy and ascitic chicks

	Males				Female			
Traits	Health	Ascites	Diff (%)	P- value	Health	Ascites	Diff (%)	P- value
RV/TV	0.24(117)	0.38(242)	58.3	<.0001	0.23(133)	0.37(180)	60.9	<.0001
BW_1	47.2(139)	47.7(242)	1.0	0.79	44.4(157)	44.3(183)	-0.2	0.99
BW_{42}	2160 (92)	2007 (53)	-7.1	0.12	1993 (95)	1870 (43)	-6.2	0.3
GR_{1-42}	51.5 (92)	47.8 (53)	-7.2	0.12	47.5 (95)	44.5 (43)	-6.3	0.35

The number in brackets represents the number of chicks in each group. Diff (%) indicates the difference in percentage between the means of healthy and ascitic chicks = Ascitic – health/health × 100. RV/TV: right ventricular weight/total ventricular; BW_1 and BW_{42}: Body weights at days 1 and 42 of age; GR_{1-42}: growth rate between BW_1 and BW_{42}.

Table 2. Least squares means of blood biochemical parameters in healthy and ascitic chicks

	Males				Females			
Traits	Health	Ascites	Diff (%)	P- value	Health	Ascites	Diff (%)	P- value
HCT_1	35.7 (80)	38.4 (91)	7.6	0.02	36.2 (104)	37.3 (76)	3.0	0.66
HCT_2	36.4 (17)	47.16 (14)	30.7	<.0001	39.7 (24)	48.8 (16)	22.9	<.0001
GLU	257 (69)	262 (70)	1.9	0.71	234 (32)	230 (19)	-1.7	0.97
CHO*	171 (69)	158 (70)	-7.7	0.03	141 (32)	139 (18)	-1.4	0.99
TG	52.6 (69)	50.3 (70)	-4.4	0.63	46 (32)	45.4 (19)	-1.3	0.99
CAL	7.83 (69)	7.52 (70)	-3.9	0.50	7.10 (32)	7.08 (19)	-0.3	1.00
PHO	4.82 (69)	5.02 (70)	4.1	0.26	4.82 (32)	4.80 (19)	-0.4	0.99
MAG	1.82 (65)	1.89 (67)	3.8	0.64	1.66 (26)	1.92 (18)	15.7	0.07
AST	183 (69)	193 (69)	5.5	0.46	203 (32)	206 (19)	1.5	0.99
ALT	5.10 (62)	6.83 (63)	33.9	0.41	9.44 (32)	6.11 (17)	-35.3	0.27
ALB	1.48 (69)	1.41 (69)	-4.7	0.35	1.36 (32)	1.42 (17)	4.4	0.83
TP	3.65 (69)	3.46 (69)	-5.2	0.26	3.36 (32)	3.52 (17)	4.8	0.81
TAS	1.18 (69)	1.14 (68)	-3.4	0.92	1.29 (31)	1.23 (17)	-4.9	0.97
HDL*	94.5(640)	77.4 (66)	-18.1	<.0001	80.7 (30)	73.7 (17)	-8.7	0.66
MDA	1.58 (68)	1.79 (68)	13.3	0.14	1.44 (27)	1.42 (14)	-1.4	0.99

The number in brackets represents the number of chicks in each group. Diff (%) indicates the difference percentage between the means of healthy and ascitic chicks = ascitic – health/health × 100. HTC_1: percentage of hematocrit in randomly selected birds; HTC_2: percentage of hematocrit in birds that health status had been diagnosed prior to slaughter through apparent symptoms; GLU: glucose (mg/dL); CHO: cholesterol (mg/dL); TG: triglyceride (mg/dL); CAL: calcium (mg/dL); MAG: magnesium (mg/dL); PHO: phosphorus (mg/dL); ALT: alanine aminotransferase (U/L); AST: aspartate aminotransferase (U/L); TP: total protein (g/dL); ALB: albumin (g/dL); TAS: total antioxidant status (mmol); HDL: High-density lipoprotein (mg/dL) and MDA: Malondialdehyde (μmol/L). * Interaction between sex and health status (HS) is significant.

ascitic chickens than in the healthy birds (P<0.0001). Cholesterol and HDL were significantly lower in the ascitic male chickens as compared with those of the healthy chickens (P<0.05). Least squares means of magnesium concentration was higher in ascitic female chickens than healthy female chickens (1.92 and 1.66, respectively; P=0.07).

Correlation between traits

Table 3 presents the correlations between RV/TV, BW_1,

Table 3. Estimates of the Pearson's correlation coefficient between right ventricular weight/total ventricular and growth-related traits

Traits	RV/TV	BW_1	BW_{42}
BW_1	-0.02		
BW_{42}	-0.24^{**}	0.25^{**}	
GR_{1-42}	-0.24^{**}	0.24^{**}	0.99^{**}

*P<0.05; ** P<0.01. RV/TV: right ventricular weigh/total ventricular; BW_1 and BW_{42}: Body weights at days 1 and 42 of age; GR_{1-42}: growth rate between BW_1 and BW_{42}.

BW_{42} and GR_{1-42}. RV/TV had a significant and weak correlation with both BW_{42} and GR_{1-42}. The correlations between RV/TV, BW_1, BW_{42} and GR_{1-42} with the biochemical parameters have been shown in Table 4. BW_1 was positively and weakly correlated with glucose (r=0.16; P<0.05) but negatively correlated with magnesium (r=-0.15; P<0.05). BW_1 was also weakly correlated with aspartate aminotransferase and malonedialdehyde (r=-0.15; P<0.05 and r=0.14; P<0.05, respectively). RV/TV was strongly correlated with hematocrit (r=0.64; P=0.0001) but weakly correlated with magnesium (r=0.17; P<0.05). The correlation of HDL and cholesterol was strong (r=0.75; P<0.0001). There was negative phenotypic correlation between HDL and RV/TV (r=-0.22; P=0.004) while correlation of HDL with BW_{42} and GR_{1-42} was positive (r=0.24; P=0.03 and r=0.24; P=0.01, respectively). Cholesterol also had a positive and weak correlation with BW_{42} and GR_{1-42} (r=0.20; P=0.04). Table 5 shows the correlations between biochemical parameters. The results indicated that both cholesterol and HDL had a positive correlation with glucose, triglyceride, calcium, phosphorus,

Table 4. Estimates of the Pearson's correlation coefficient between right ventricular weight/total ventricular and growth-related traits with biochemical factors

Traits	HCT_1	HCT_2	GLU	CHO	HDL	TG	CAL	PHO	MAG	TP	ALB	AST	ALT	TAS	MDA
RV/TV	0.16^{**}	0.64^{**}	0.08	-0.09	-0.22^{**}	0.03	0.01	0.04	0.17^{*}	-0.08	-0.07	0.05	-0.09	-0.04	0.11
BW_1	-0.009	0.12	0.16^{*}	0.12	0.06	0.023	0.03	0.08	-0.15^{*}	-0.002	-0.06	-0.15^{*}	-0.07	-0.1	0.14^{*}
BW_{42}	-0.03	-	0.13	0.20^{*}	0.24^{**}	-0.12	0.10	-0.11	-0.07	-0.0004	0.10	-0.02	-0.007	-0.16	-0.02
GR_{1-42}	-0.02	-	0.13	0.20^{*}	0.24^{**}	-0.12	0.10	-0.12	-0.07	-0.004	0.10	-0.01	-0.003	-0.16	-0.02

*P<0.05; ** P<0.01. HTC_1: percentage of hematocrit in randomly selected birds; HTC_2: percentage of hematocrit in birds that health status had been diagnosed prior to slaughter through apparent symptoms; GLU: glucose (mg/dL); CHO: cholesterol (mg/dL); HDL: High-density lipoprotein (mg/dL); TG: triglyceride (mg/dL); CAL: calcium (mg/dL); PHO: phosphorus (mg/dL); MAG: magnesium (mg/dL); TP: total protein (g/dL); ALB: albumin (g/dL); AST: aspartate aminotransferase (U/L); ALT: alanine aminotransferase (U/L); TAS: total antioxidant status (mmol); MDA: Malondialdehyde (µmol/L).; RV/TV: right ventricular weight/total ventricular; BW_1 and BW_{42}: Body weights at days 1 and 42 of age; GR_{1-42}: growth rate between BW_1 and BW_{42}.

Table 5. Estimates of the Pearson's correlation coefficient between biochemical factors

Traits	HCT_1	HCT_2	GLU	CHO	HDL	TG	CAL	PHO	MAG	TP	ALB	AST	ALT	TAS
HCT_2	0.12													
GLU	0.04	-0.15												
CHO	-0.02	-0.10	0.49^{**}											
HDL	-0.001	-0.28	0.38^{**}	0.75^{**}										
TG	-0.15	-0.18	0.26^{**}	0.41^{**}	0.22^{**}									
CAL	0.10	-0.27	0.40^{**}	0.53^{**}	0.39^{**}	0.32^{**}								
PHO	0.10	-0.44^{*}	0.22^{**}	0.32^{**}	0.15^{*}	0.28^{**}	0.33^{**}							
MAG	0.19^{*}	0.13	0.03	0.01	-0.007	0.001	0.10	0.31^{**}						
TP	0.03	-0.05	0.15^{*}	0.41^{**}	0.34^{**}	0.28^{**}	0.33^{**}	0.38^{**}	0.11					
ALB	0.03	-0.15	0.27^{**}	0.55^{**}	0.48^{**}	0.30^{**}	0.41^{**}	0.38^{**}	0.07	0.80^{**}				
AST	0.05	-0.08	0.10	-0.01	-0.03	0.01	0.10	-0.04	-0.004	-0.04	0.03			
ALT	0.19^{*}	0.30	-0.02	-0.10	-0.10	0.01	-0.04	0.11	0.06	0.003	0.04	0.25^{**}		
TAS	0.01	-0.06	-0.02	-0.07	-0.04	0.10	0.10	0.12	0.32^{**}	0.10	0.08	0.09	0.20^{**}	
MDA	0.02	0.028	0.07	0.12	-0.04	-0.02	-0.02	0.22^{**}	0.12	0.05	0.08	0.07	-0.03	0.09

*P<0.05; ** P<0.01. HTC_1: percentage of hematocrit in randomly selected birds; HTC_2: percentage of hematocrit in birds that health status had been diagnosed prior to slaughter through apparent symptoms; GLU: glucose (mg/dL); CHO: cholesterol (mg/dL); HDL: High-density lipoprotein (mg/dL); TG: triglyceride (mg/dL); CAL: calcium (mg/dL); PHO: phosphorus (mg/dL); MAG: magnesium (mg/dL); TP: total protein (g/dL); ALB: albumin (g/dL); AST: aspartate aminotransferase (U/L); ALT: alanine aminotransferase (U/L); TAS: total antioxidant status (mmol) and MDA: Malondialdehyde (µmol/L).

albumin and total protein. Also, hematocrit was significantly correlated with phosphorus, magnesium and alanine aminotransferase (r=- 0.44; P<0.05, r=0.19; P<0.05 and r=0.19; P<0.05, respectively).

Discussion

The high incidence of AS (58.99%) observed in our experiment may indicate a high susceptibility of the studied sire lines to AS. Dewil et al. (1996) studied the specification of embryos of two broiler lines (paternal line and maternal line) with a divergent susceptibility for the AS. Paternal lines are lines with a faster growth rate (ascites sensitive line) while maternal lines are lines with a slower growth rate (ascites resistant line). These researchers found that a high susceptibility to AS attained from genetic selection is connected to several physiological variables at the embryonic phase. They reported lower thyroid metabolism in the embryo stage as a disposing agent for later development of heart failure and occurrence of AS in sensitive birds, since this agent causes later hatching and development of a hypoxic condition in the embryonic stage. In the present study, 63.4% (244/385) of the males and 54.1% (189/349) of the females died due to AS. Therefore, it seems that males are more sensitive to AS than females. This result is in agreement with the previous studies (Hasanpur et al., 2015; Wideman and French, 2000). Such finding may be due to the higher metabolism rate of males than females. Furthermore, female chickens had a lower growth rate than the male chickens and this causes susceptible female chickens to develop AS symptoms later in life. If cold stress increases to a sufficient extent to increase the metabolism in the females, the AS rate in females may be equal to that in males.

The hypoxia that is created during the development of AS causes increasing cardiac output and hence leads to right ventricle hypertrophy. A ratio of RV/TV greater than 0.27-0.30 is considered as an indicator of AS incidence in many studies (Zerehdaran et al., 2006). Desirable heritability has been reported for RV/TV (Closter et al., 2009). Thus, it is believed that the occurrence of AS can be reduced by selection based on RV/TV, a quantitative trait which can be measured in both cold and normal conditions. In the present study, a significant difference in RV/TV ratio was observed between the ascitic and healthy chickens (P<0.0001). This finding is in accordance with the previous reports (Ipek and Sahan, 2006; Wang et al., 2012). Cold temperature, by increasing cardiac output, causes increased RV/TV value which consequently results in higher morbidity and mortality due to AS. We observed a significantly negative correlation between RV/TV and both BW_{42} and GR_{1-42} (r=-0.24; P<0.0001 and r=-0.24; P<0.0001, respectively). Pakdel et al. (2005) reported a negative genetic correlation between BW and RV/TV (r=-0.27) under cold conditions and a positive genetic correlation between BW and RV/TV (r=0.50) under normal temperature conditions. Pakdel et al. (2005) also estimated a negative phenotypic correlation between BW and RV/TV (r=-0.30) under cold conditions and reported that there is a genotype by environmental interaction for BW and ascites indicator traits. These results indicate that the correlation between BW and AS is influenced by the susceptibility to ascites and effect of environment temperature. Therefore, non-ascitic chickens under normal temperature conditions are not necessarily chickens resistant to AS under cold-stress conditions (this is also the case for BW). It means that susceptible birds with higher RV/TV have lower BW under cold conditions, so the birds with high BW under cold conditions are resistant. Thus, it can be concluded that in the susceptible birds, ascites-related genes may have higher expression levels than productivity-related genes under environmental conditions that induce ascites (cold stress or high altitudes). Therefore, the reaction of the body to AS and cold stress is an increased metabolic rate to maintain the thermal homeostasis by using carbohydrates, especially liver glycogen and mobilization of fat, resulting in a rapid decrease in body mass (Pakdel et al., 2005; Ahmed et al., 2016).

Hematological modifications including increased hematocrit and immature hemoglobin have been seen in ascitic broilers (Balog et al., 2003). Chickens possess a thicker breathing membrane than other birds, and the respiratory membrane of broiler chickens is thicker than that of laying hens. In broilers, high blood flow prevents full hemoglobin saturation from oxygen in the lungs (Julian, 2000). The continuation of hypoxemia induces the kidney to release erythropoietin. The effect of erythropoietin on bone marrow increases the activity of the hematopoiesis and increases the amount of red blood cell production (polycythemia) (Luger et al., 2003). Although high levels of hematocrit in the short term contribute to elimination of hypoxemia, higher hematocrit increases the viscosity of the blood and reduces the ability of the right ventricle to pump blood into lung veins, thereby exacerbating AS (Shlosberg et al., 1998). In the present study, we observed significant changes in the hematocrit between the ascitic (high hematocrit) and healthy (low hematocrit) broilers. This result is in agreement with the reports of Luger et al. (2003) and Yersin et al. (1992). Pakdel et al. (2005) reported positive phenotypic and genetic correlation between RV/TV and hematocrit. In the present study, there was also a phenotypic correlation between RV/TV and hematocrit (r=0.64; P=0.0001). Thus, chickens with higher genetic merit for hematocrit are probably susceptible to AS under cold conditions.

Due to the large metabolic changes that occur during the development of AS, various variables are expected to be affected by AS (Shimomura et al., 2000; Luger et al., 2003). Although there are several studies on hematological parameters and blood gases (Van As et al., 2010; Hassanzadeh et al., 2014; Hasanpur et al., 2016), there is little information about the changes in biochemical parameters in ascitic conditions.

Magnesium (Mg) is an essential mineral that is required for fundamental enzymatic reactions, carbohydrate metabolism, immune function and keeping heart rhythm steady etc. (Sahin et al., 2005). In chickens, magnesium supplementation causes decreased corticosterone and stress (D'Souza et al., 1998). In our study, we observed a high concentration of magnesium in ascitic females than healthy females (P=0.07). Moreover, magnesium was positively correlated with RV/TV (r=0.17; P=0.02), Therefore, it can be concluded that magnesium can help to keep heart rhythm steady and reduce cold-induced stress and AS.

Cholesterol is a waxy substance that is needed for the synthesis of cortisol, corticosterone and sex hormones, and production of bile, and it is an important lipid in some membranes (Ma, 2004). HDL, which is known as good cholesterol, is the main lipoprotein class in avian species (Mossab et al., 2001). Our results showed that cholesterol and HDL levels in ascitic birds were significantly lower than in healthy birds. In addition, we observed lower fat in the abdominal cavity of ascitic broilers (data not shown). This finding is in agreement with the results of Tankson et al. (2002). Daneshyar et al. (2009) found no significant difference between healthy and ascitic chickens for cholesterol level. Wang et al. (2012) reported that the cholesterol level in AS broilers is significantly higher than in healthy chickens.

Hyperthyroidism, the production of excessive amounts of thyroid hormones by the thyroid gland, causes increased energy expenditure, reduced cholesterol levels, increased lipolysis and gluconeogenesis and weight loss (Motomura and Brent, 1998). Also, cold stress causes the release of corticosteroids from adrenal cortical tissue (Zhao et al., 2009). The primary corticosteroid in birds is corticosterone (De Roos, 1960). One of the most important metabolic functions of the stress hormone, corticosterone, is to elevate gluconeogenesis for endogenous glucose production (Exton, 1979). It has been reported that THs concentration was reduced in the plasma of ascitic chickens especially after 5 weeks of age (Guo et al., 2007). This may be due to the metabolic disarrangement that is caused during the development of AS. Luger et al. (2003) reported that corticosterone concentration increases in ascitic chickens. Aarif et al. (2014) and Priya and Gomathy (2008) have reported that total cholesterol levels were decreased under cold stress (P<0.01), whereas cortisol and corticosterone concentration were increased under the same conditions. It might be concluded that cholesterol is used to synthesis of corticosteroids that results in reduced cholesterol concentration in AS birds. Thus, reduction of serum cholesterol may well be an adaptive physiological response to cold stress and ascites.

We did not observe any significant differences in glucose levels between the healthy and ascitic birds. Previous studies have reported that fasting blood sugar was significantly higher in the chickens raised under cold temperature than the chickens raised under normal temperature and attributed this to high levels of gluconeogenesis in ascitic birds (Daneshyar et al., 2009; Wang et al., 2012). There are reports that the concentration of serum albumin (Yersin et al., 1992; Biswas et al., 1995) and total protein (Tankson et al., 2002; Daneshyar et al., 2009) in ascitic chickens are lower than those in healthy chickens. In our study, the differences in serum total protein (P=0.26) and albumin (P=0.35) between the ascitic and healthy chicks were not significant.

Conclusions

The high frequency of AS observed in our studied population suggests that sire lines may be more susceptible to AS. This study was conducted to investigate the modification of biochemical parameters together with RV/TV and growth-related traits in ascitic and healthy chickens grown under cold temperature conditions. When birds are exposed to ascites-inducing challenge, ascitic birds exhibit significant changes compared with the healthy birds in traits such as RV/TV, hematocrit, HDL and cholesterol that were measured after the start of the ascites inducing challenge. It may be concluded that these traits indicate secondary manifestations of AS and therefore could be useful only in diagnosis of birds that are developing AS, but not in prediction of AS susceptibility. We measured hematocrit value, cholesterol and HDL after the occurrence of AS (5 weeks of age). Since it is shown that the values of these traits is different between the healthy and ascitic chickens before the occurrence of AS, these can be potential predictors of

AS (according to correlations between these traits with RV/TV). Therefore, breeders can use these phenotypic traits in their genetic selection programs to reduce the incidence of AS.

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