



ABSTRACT

Ascites syndrome, as one of the most common metabolic disorders in fast growing broiler chickens, has received considerable critical attention. Understanding the complexity and key factors in occurrence of ascites is vitally important for control of economic losses. Specifically, the hypothesis that will be tested is that higher ascites incidence can be observed in fast growing commercial strains. To confirm this hypothesis, the overall goal of this report was to pursue and compare growth measurements between two groups of birds: a healthy (control) group reared under standard breeding conditions and an ascites syndrome group reared under ascites inducing conditions. Two populations of broilers were used and differences between male and female broilers were recorded. For this purpose, growth characteristics data were collected from seven body weights between the ages of one and 42 days and nine growth rate parameters were studied in two populations that originated from 47 and 71 paternal half-sib families of meat-type sire chicken line. Statistical analyses for association study and estimating the heritability for particular traits were performed according to appropriate models using SAS, WOMBAT and Thrgibbs1F90 software. Our results showed no statistically significant difference in body weight and growth rate traits between healthy and ascitic broilers (P>0.05). Moreover, the results demonstrated moderate to high heritability (0.19 to 0.62) for ascites indicator traits (AITs). This finding highlights that selection for AITs may genetically improve resistance to ascites in this investigated chicken line. A further novel finding is that there were no consistent direct genetic correlations between AITs and growth traits (GTs). This evidence provides the opportunity to grow birds to heavier live bodyweight because high growth rate and body weight are not the main cause of susceptibility to ascites syndrome.

KEY WORDS ascites, broiler chickens, growth traits, rapid growth rate.

INTRODUCTION

Research on ascites syndrome, also called hypertension syndrome, in commercial chickens has a long history since the 1960 s (Hall and Machicao, 1968; Julian, 1993; Das and Deka, 2019). Unfortunately, this well-known problem is reported to affect approximately 5-8% of broilers worldwide and was reported mostly in fast growing chicken strains (Maxwell and Robertson, 1998; Pavlidis *et al.* 2007); with an estimated 20% mortality rate (Afolayan *et al.* 2016). Most previous literatures highlight that ascites metabolic disorder causes several negative consequences including increased mortality (Druyan *et al.* 2007a), reduced body weight (Closter *et al.* 2009), and reduced meat quality such as reddish color of the breast fillet (Pakdel *et al.* 2005a), causing economic damage to the poultry indus-

try (Kamely et al. 2015; Tarrant et al. 2017). Numerous studies have provided a description in the early stages of occurrence of this syndrome, in chickens with normal body size. However, typically chicken growth showed a significant reduction during the development of ascites (Wideman and Kirby, 1995; Kamely et al. 2015; Afolayan et al. 2016). Many authors have suggested an association of incidence of ascites with high altitude. However, the same incidence of ascites has been reported in low altitude chicken farms (Scheele et al. 2005; Ahmadpanah et al. 2017). Despite numerous studies and the fact that the key factors causing ascites have been heavily researched, information on how growth rate and bodyweight of chickens is related to susceptibility to ascites are still limited. Therefore, further studies are required to differentiate these complexities in the ascites syndrome issue (Wang et al. 2007).

Several theories have been proposed to clarify this syndrome. For instance, deficient oxygen supply is often a primary cause of ascites, particularly when this factor is associated with rapid growth and high feed efficiency in birds. Also, previous studies have emphasized that lack of oxygen for metabolism initiated several cascades of events such as heart and lung failure during ascites in chickens (Kamely et al. 2015; Tarrant et al. 2017). Studies by Druyan et al. (2007a), and Girmachew et al. (2020) concluded that higher-growth rate chicks had a tendency to higher ascites incidence and mortality rate than slow growth chicks. A closer look at the literature on the association between body growth rate and ascites incidence, however, reveals a number of gaps and shortcomings. Therefore, the purpose of the present study being to test association of ascites incidence with growth characteristics in two commercial chicken strains.

MATERIALS AND METHODS

Birds and experiment conditions

Two separate trials were designed for this study using two pure broiler lines, namely S1 and S2. The experiments were performed using two different generations of the paternal line of a pure meat-type line and in each generation twice hatched. Prior to the main experiment, a population of 1608 chickens of the first generation was established using 67 paternal half-sib families and wing tag banding was used for individual identification and pedigree identification at hatchery.

This experimental generation was used to create average of 20 offspring from each paternal family (47 families) with similar genetic background to allow for logical interpretation of the main ascites resistance and the susceptibility situation in line herds.

Criteria for selecting the birds were as follows: live body weight and growth characteristics and additional data related to ascites incidence. Subsequently, overall, 461 birds originating from 47 paternal half-sib families of a pure meat-type parental were then used for further investigation and were reared under ascites inducing conditions (AICs). We utilize secondary data from 994 birds with the same genetic background which were kept under both different induced high and low ascites stress conditional treatments. We cannot deny the presence of natural cold season in experimental geographical location don't allow create induced cold stress condition to all birds and therefore, for all experimental birds provided standard breeding conditions (SBCs). Data were collected from two comparative ascites occurrences under SBCs and AICs. Parental family was the main criterion for genetic background evaluation and we ignored the role of the maternal effect. Similarly, 1550 chickens belonging to the second generation originated from 71 paternal half-sib families (an average of 15 offspring from each paternal family) and wing tags were used for individual identification. Similar pattern was done for this sample size too. Next, to combine and summarize this approach, 823 birds created from both first and second parental chicken line populations under two low-ascitic and high-ascitic conditions were chosen for further research. Sexing of chickens was performed by feather sexing and broilers were reared in litter floor housing.

In the current research, ascites 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.* 1998).

The chicken house was divided into 42 separate pens of the same dimensions (1.8 meter length, 1.2 meter width and 90 cm height), and then litter floor cover was provided using a 3 cm layer of straw material to ensure the welfare of the experimental chickens. Cross ventilation systems were incorporated at the poultry farms to control environmental conditions and a central heating system plus lighting based on ordinary lamps were provided in the poultry rearing system. House temperature was similar at then beginning of rearing for both first and second regeneration of parental chicken lines.

Ascites-inducing conditions

During the initial 21 days of the rearing period, all birds experienced standard normal temperature housing conditions. For both SBC and AIC bird groups, indoor temperature of the poultry house was kept at 32-34 °C during the first and second days of age. The conditions for the AIC bird group were an intentionally induced cold stress condition produced by turning off the central heater during 21-42 days of rearing and maintaining the indoor temperature around 15-18 °C during the day and 10-15 °C during the night to guarantee occurrence of ascites in the chicken house. For the first two days, 24 hour light was provided and subsequently the intensity of the light was gradually decreased and changed to 23 hours light and 1 hour darkness. Feed and water were provided *ad libitum* during the entire trial. A three-phase diet was formulated to meet nutritional requirements for broiler chickens in the starter (days 1-10), grower (days 11-24), and finisher stages (days 25 upward). Both male and female birds used one type of diet.

Diet ingredients used in the preparation of this diet include: corn, soybean meal, wheat bran, soybean oil, dicalcium phosphate, NaCl, calcium carbonate, lysine, methionine, vitamin and mineral supplements, enzymes and permitted additives. The energy (metaboizable energy (ME), kcal/kg of diet) and crude protein (CP) (%) contents of the starter, grower, and finisher diets were 2986.9 and 20.71, 3047.3 and 18.69, and 3083.5 and 17.60, respectively. The ingredients and chemical composition of the basal diets were listed in Table1.

Diagnosis of ascites and monitoring of mortality rate

In this trial, mortality in the SBC and AIC groups was recorded along with the symptoms and presumed cause of death. Birds with ascetic fluid in the abdominal cavity or in the hydropericardium and a right/total ventricular weight ratio (RV/TV) above 0.30 were considered as ascetic and given code 1 after autopsy and birds indicating normal and healthy phenotype and appearance were assigned the code of zero.

Growth characteristics and related parameters

In order to investigate the effects of SBCs and AICs on growth related parameters, birds were weighed individually once a week after 2 h of starvation. Growth rate (GR) or average daily weight gain was calculated individually as shown below:

 $GR = (W_i - W_i) / (d_i - d_i)$

Where:

 W_j and W_i : estimated body weights at day j (d_j) and day i (d_i) of age, respectively.

To study the relationship between GTs (body weight and growth rate) and ascites incidence, records of weekly weights from 1 to 42 days were used separately for the SBC and AIC groups.

Growth characteristics data were collected from seven body weights (BW₁, BW₇, BW₁₄, BW₂₁, BW₂₈, BW₃₅, BW₄₂) between the age of one to 42 days and nine growth rate parameters (GR₁₋₇, GR₇₋₁₄, GR₁₄₋₂₁, GR₁₋₂₁, GR₂₁₋₂₈, GR₂₈₋₃₅, GR₃₅₋₄₂, GR₂₁₋₄₂, GR₁₋₄₂) in same period in the population of birds that originated from 47 and 71 paternal half-sib families of meat-type sire chicken line.

Statistical analysis

Comparisons between the two groups (AICs and SBCs) separated into male and female were made using proc FREQ and proc CHISQ and a Pearson correlation analysis was conducted in order to assess AICs and SBCs experimental group using proc CORR using SAS statistical software version 9.4 and also significance levels were set at the and 1% level SAS (2004).

FOXPRO software was used to record and edit collected phenotypic data. Mixed procedure of SAS was used to analyze the mentioned data using the following model:

 $y = \mu + \text{Sex} + \text{HS} + \text{Sex} \times \text{HS} + \text{Sire} + \text{e}$

Where:

Sex and HS (health status): considered as fixed effects. Sire and residual: considered as random effects.

Tukey multiple-range method was chosen for comparing the least square mean differences of the ascitic and healthy chicks. Comparing the least square means between different levels of sex effect and other effects were not reported. However, if the interaction between health and sex was significant, comparing the least square means between healthy and ascetic birds in males and females was reported separately. Water belly, also known as ascites, and ratios of right/left ventricular weight (RV/LV) and RV/TV are the most significant AITs. To estimate the heritability of RV/LV and RV/TV as well as genetic correlations with GTs, WOMBAT software (Meyer, 2007) based on the maximum likelihood method was used and to estimate the heritability of water belly and its genetic correlations with GTs, Thrgibbs1F90 software (Tsuruta and Misztal, 2006) based on Bayesian and Gibbs sampling was used. In the study of the genetic relationship between GTs and the incidence of ascites, the data of live body weight traits and RV/LV, RV/TV of both SBCs and AICs and the data of water belly were used only from AICs.

The heritability of AITs was estimated by univariate analysis and the correlation between GTs and AITs was assessed by bivariate analyzes. The statistical model was used as follows:

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y = \mu + A + Sex + e
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Where:

A: considered as random effects (additive genetic effects). Sex (male and female): considered as fixed-effect.

In the maximum likelihood algorithm for both univariate and bivariate analysis, the convergence criterion for stopping replications was 10^{-8} . In the analysis of water belly with other traits, the relevant files were made using software RenumF90 and after the analysis, software postgibbsF90 was used to calculate the final values of the components of variance. After estimating these components, genetic correlations of different traits were calculated. The number of Gibbs samples was 100000 and the number of samples removed at the beginning of the analysis was considered to be 30000 samples. Out of every 10 Gibbs samples, only one was used to estimate the final values of (co) variance components.

RESULTS AND DISCUSSION

In this study we investigated the genetic pattern and variability of incidence of ascites in paternal chicken line and correlation between the incidence of ascites and growth characteristics. We hypothesized that the individual bird response variability would be different from that of the induced ascites rearing conditions. In the present study, the incidence of ascites began in all four rearing periods after three weeks, which intensified as the end of the rearing period approached, which confirm that cold temperatures after 21 day of rearing are able to artificially mimic ascites occurrence and associated ascites symptoms within individuals of a chicken flock.

Our results are consistent with McGovern *et al.* (1999) and Squires and Julian (2001) who exposed birds to cold temperatures to induce experimental ascites after three weeks and also Luger *et al.* (2003) and Scheele *et al.* (2005) who examined different lines of broilers and stated that temperature fluctuations during the day and night increase the weight of the right ventricle of the heart and the incidence of ascites and death. When comparing our results to those of older studies, it must be pointed out some researchers used a hypobaric chamber (simulated 3000 m above sea level) as discussed by Pavlidis *et al.* (2007) and Wideman *et al.* (2013).

A similar pattern of results was obtained by Shlosberg *et al.* (1996) who reported that ascites generally occurs below the temperature of comfort zone and cold stress is one of main factors which stimulates ascites in a poultry house (Wang *et al.* 2007; Daneshyar *et al.* 2009).

In the present study, we used a cold stress protocol to induce ascited and to identify birds genetically susceptible and resistance to ascites.

Ingredient (%)	Starter (1-8 days)	Grower (11-24 days)	Finisher (25-42 days)
Corn	60.00	66.5	70.00
Soybean meal (44% CP)	33.5	28.3	25.00
Wheat bran	0.95	-	-
Soybean oil	0.90	0.73	0.77
DL-methionine	0.33	0.27	0.26
L-lysine, HCL	0.22	0.19	0.17
Threonine	0.08	0.07	0.07
Di calcium phosphate	2.02	2.00	1.83
Calcium carbonate	1.11	-	-
Oyster mount	-	1.00	0.95
Common salt	0.22	0.24	0.25
Sodium bicarbonate	0.17	0.15	0.15
Vitamin premix ¹	0.25	0.25	0.25
Mineral premix ²	0.25	0.25	0.25
Coccidiostat	-	0.05	0.05
Total	100.00	100.00	100.00
Calculated composition			
Metabolizable energy, kcal/kg	2986.9	3047.3	3083.5
Crude protein, %	20.71	18.69	17.60
Available P, %	0.520	0.450	0.440
Ca, %	1.072	0.900	0.880
Cl, %	0.187	0.187	0.186
Na, %	0.166	0.166	0.166
K, %	1.086	0.969	0.902
Lys, %	1.228	1.069	1.014
Met + Cys, %	0.090	0.810	0.797

 Table 1 Percentage inclusion and calculated composition of basal diet in starter, grower and finisher period

¹ Supplied per kilogram of diet: vitamin A: 12000 IU; vitamin D₃: 5000 IU; vitamin E: 80 IU; vitamin K₃: 3.5 mg; vitamin B₁: 4 mg; vitamin B₂: 8.00 mg; vitamin B₆: 4.00 mg; vitamin B₁₂: 0.018 mg; Biotin: 0.020 mg; Folic acid: 2.20 mg; Pantothenic acid: 20 mg; Choline: 1700 mg and Nicotinic acid: 70 mg.
 ² Supplied per kilogram of diet: Cu: 16 ppm; Zn: 100 ppm; Fe: 50 ppm; Mn: 120 ppm; Se: 0.30 ppm and I: 1.25 ppm.

From the observed results of the first generation birds, 11.2% (164 out of 1485 chickens) illustrated during whole twice period of rearing. The additional outcomes demonstrate two things. First, the frequency of ascites was significantly (χ^2 test; P<0.0001) higher in the AICs group (28.4%) in comparison to the SBCs group (3.11%). Second, the difference between males and females for the incidence of ascites during the research experiment were not significant (χ^2 test; P>0.05).

For the birds of the second generation, 36.7% (569 out of a 1550 chickens) illustrated during whole twice period of rearing. The statistical analysis found evidence for significantly higher ascites occurrence frequency (χ^2 test; P<0.0001) in the AICs group (65.9%) versus the SBCs (9.3%) group. Furthermore, the incidence of ascites in females (47.9%) was lower than in males (52.1%) under the AICs rearing condition in the poultry house.

Together, the present findings confirm the incidence of ascites was more frequent in certain paternal families in the first generation. The study results provided some interesting findings regarding to ascites, for example, in almost 3 investigated paternal families surveyed, under AICs or SBCs, no ascites birds were observed, however in the other families, more than 50% developed ascites symptoms under the AICs rearing condition during the experiment.

Some complicated observation also generated during study. For example, some paternal families expressed the syndrome only under the AICs and did not indicate any ascites symptoms under the SBCs. In contrast, some families expressed ascites in both rearing conditions as well in the first generation group. Moreover, in the response to simulated ascites-inducing conditions, almost all parental families in the second generation expressed a higher incidence of ascites symptoms with high variability within and between investigated families which produced suitable raw material for further study.

Figure 1 presents a graphical distribution of ascites occurrence in different investigated paternal chicken families. From the data presented in Figure 1, it is apparent that genetic background of investigated families could create different response to ascites-inducing conditions during this experiment. Therefore, selection against this metabolic syndrome could produce some genetic improvement to control its incidence in commercial herds.

Figure 2 illustrates the frequency of families with different incidences of ascites. A zero number indicates that all members of the family are healthy, and the non-zero numbers indicate the proportion of family members expressing ascites symptoms. The normal level of ascites incidence expected in commercial broilers is estimated around 3% and can be up to 5%; however, the majority of these studies describe that more than 5% causes significant economic damage to the poultry farm (Maxwell and Robertson, 1998). We did not find the same pattern and the frequency of this abnormality in this chicken line was higher than similar reported works.

The findings of the present study are directly in line with previous findings regarding to incidence of ascites reported by Naghous et al. (2012a) and Naghous et al. (2012b) (16.6%) and Closter et al. (2012) (9.5%) in a Netherlands pure maternal line. However, these basic findings are inconsistent with research showing lower incidence of ascites in studies reported by Druyan et al. (2007b), Druyan et al. (2008), Druyan et al. (2009), Gou et al. (2007) and Pakdel et al. (2002). By comparing these results, it seems that there is a tendency for females to express lower incidence of ascites than males. It is generally accepted that variability of response pattern to ascites-inducing conditions depends on the genetic background of investigated commercial chickens. Druyan et al. (2007a) demonstrated that genetic selection against ascites can create offspring with significant relative resistance to this metabolic disorder. Researchers at the University of Arkansas have verified that breeding strategies and divergent selection can result in the production of birds that are either resistant or susceptible to ascites.5% and 60% correlation coefficient in first and second gen. Wideman et al. (2013) reported 7% resistant birds and 98% susceptible birds in the 14th generation of genetic selection against ascites. In the present study, the results of the correlational analysis of ascites incidence between different families under AICs with SBCs are set out high significant a positive trend. From the statistical analysis, it can be seen 32erations, respectively. The incidence of ascites under SBCs was higher for fast-growing birds than for slow-growing birds (Druyan et al. 2008).

Table 2 provides statistical description of the traits measured under AICs and SBCs in the first and second generations.

The means and standard deviation for the weight of birds at 35 days reared under SBCs and AICs were 1670.04 g, (± 250.54) and 1660.04 g (± 220.14) , respectively. Also, the same ratios for the weight of birds at 42 days were 2324.58 g (±313.21) in SBCs and 2255.24 g (±297.21) in AICs. It can be seen from the age of 28 days until the end of the rearing period that body weight values and growth rates in bird reared under SBCs were higher than birds reared under AICs. A significant reduction of growth occurs in the AICs during the 42 day period due to ascites symptoms and low feed intake. These results are in agreement with findings reported by Roush and Wideman (2000), Closter et al. (2009), Closter et al. (2012), Pakdel et al. (2005a) and Pakdel et al. (2005b) who stated that ascitic birds after the age of 28 days had lower growth rates than their healthy counterparts due to disease.

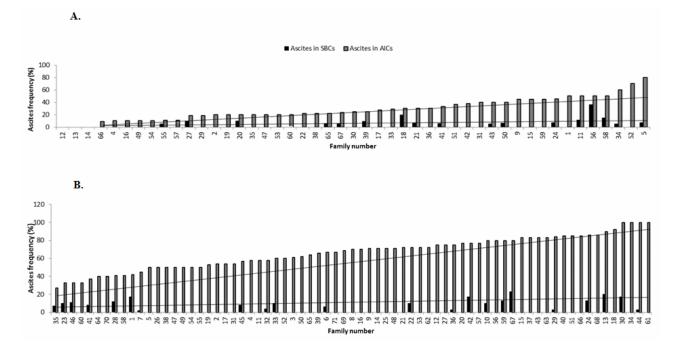


Figure 1 The ratio of the frequency of ascites to the total number of famili members (vertical axis) in different families (horizontal axis) A: the first generation includes offspring of 67 sire and B: the second generation includes offspring of 71 sire The stripe graph shows this ratio in ascites inducing conditions (AICs) and the black graph shows this ratio in standard breeding conditions (SBCs)

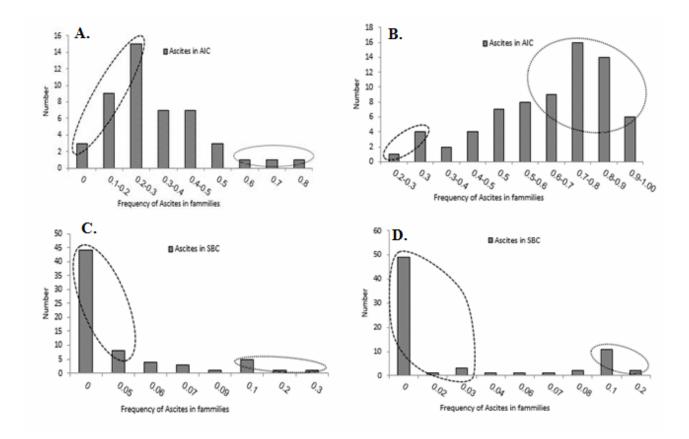


Figure 2 Frequency of families with different ascites: A: first generation includes of offspring to 47 sire reared under ascites inducing conditions (AICs); B: second generation includes of offspring to 71 sire reared under ascites inducing conditions (AICs); C: first generation includes of offspring to 47 sire reared under standard breeding conditions (SBCs) and D: second generation includes of offspring of 71 sire reared under standard breeding conditions (SBCs)

Traits			First ge	neration			Second generation						
TTaits	AICs				SBCs			AICs SBCs			SBCs	BCs	
	Number	Mean	SD^2	Number	Mean	SD	Number	Mean	SD	Number	Mean	SD	
BW_1	-	-	-	1104	60	5.7	739	45.99	4.32	823	45.13	3.99	
BW_7	-	-	-	1058	128.70	19.2	723	135.99	20.55	811	127.71	18.75	
BW_{14}	-	-	-	1020	290.94	48.4	725	290.33	49.07	793	274.92	53.44	
BW_{21}	-	-	-	1194	602.85	102.5	709	577.07	97.84	785	547.98	98.72	
BW_{28}	411	1077.09	163.15	590	1076.33	171	598	953.04	159.87	777	972.87	164.14	
BW35	414	1660.04	220.14	617	1670.04	250.54	493	1435.73	262.59	765	1519.61	242.83	
BW_{42}	342	2255.24	297.21	800	2324.58	313.21	309	2006.20	316.45	735	2133.63	301.71	
GR ₁₋₇	-	-	-	1025	11.45	2.84	723	15.11	3.26	811	13.76	2.94	
GR ₇₋₁₄	-	-	-	987	23.15	5.20	725	22.22	5.21	790	21.06	5.62	
GR ₁₄₋₂₁	-	-	-	949	44.74	9.32	709	41.23	9.37	785	38.95	8.73	
GR ₁₋₂₁	-	-	-	954	27.20	4.99	709	26.58	4.80	785	25.14	4.87	
GR21-28	396	67.09	11.68	523	69.02	11.89	595	53.85	14.12	773	61.02	11.56	
GR ₂₈₋₃₅	380	83.32	14.67	553	84.56	17.20	485	69.34	24.93	762	78.24	15.85	
GR ₃₅₋₄₂	330	84.96	21.77	579	92.77	21.60	306	76.93	34.15	733	86.65	18.95	
GR ₂₁₋₄₂	328	78.38	11.25	501	82.66	11.66	309	67.84	12.70	735	75.55	11.46	
GR ₁₋₄₂	330	53.64	7.09	672	55.02	7.71	309	47.82	7.68	735	50.93	7.33	

Table 2 Statistical description of the traits measured under ascites inducing conditions (AICs) and standard breeding conditions (SBCs)

BW1 to BW42: body weight traits between the ages of one and 42 days and GR1-7 to GR1-42: growth rate traits at these intervals.

SD: standard deviation

Table 3 Least square means of growth traits (GTs) in healthy and ascitic chickens in the first generation (reared under standard breeding conditions (SBCs))

			Male (n=433) ¹				Female (1	n=561)
Traits	Healthy	Ascetic	Difference ²	Significance level	Healthy	Ascetic	Difference	Significance level
	(n=418)	(n=15)	(%)	(P>F)	(n=544)	(n=17)	(%)	(P>F)
BW_1	60.0	59.2	1.4	NS	60.1	60.4	-0.5	NS
BW_7	129.8	129.9	-0.1	NS	125.6	119.1	5.5	NS
BW_{14}	294.2	286.3	2.8	NS	279.6	261.6	6.9	NS
BW_{21}	616.2	629.4	-2.1	NS	584.5	578.3	1.1	NS
BW_{28}	1113.6	1162.6	-4.2	NS	1043.0	1022.3	2.0	NS
BW35	1754.0	1831.3	-4.2	NS	1598.6	1496.7	6.8	NS
BW_{42}	2485.7	2447.0	1.6	NS	2202.5	2014.7	9.3	NS
GR1-7	11.64	11.83	-1.5	NS	10.89	9.65	12.9	NS
GR ₇₋₁₄	23.67	22.32	6.1	NS	21.78	20.40	6.8	NS
GR ₁₄₋₂₁	46.08	46.29	-0.4	NS	44.05	44.77	-1.6	NS
GR ₁₋₂₁	27.74	27.68	0.2	NS	26.18	25.98	0.8	NS
GR ₂₁₋₂₈	72.41	76.62	-5.5	NS	66.18	63.56	4.1	NS
GR ₂₈₋₃₅	91.45	96.23	-5.0	NS	78.44	64.45	21.7	**
GR ₃₅₋₄₂	102.51	89.75	14.2	NS	86.38	75.16	14.9	NS
GR ₂₁₋₄₂	89.54	87.61	2.2	NS	77.84	67.08	16.0	***
GR ₁₋₄₂	58.89	57.93	1.7	NS	52.19	48.18	8.3	NS

¹ The number of birds for each class were in parentheses.

² The difference between means of healthy and ascitic chickens [((healthy-ascitic)/ascitic) \times 100].

BW₁ to BW₄: body weight traits between the ages of one and 42 days and GR₁₋₇ to GR₁₋₄₂: growth rate traits at these intervals. * (P ≤ 0.05); ** (P ≤ 0.01) and *** (P ≤ 0.001).

NS: non significant.

Mean values and standard deviation of the weight of birds at 35 days reared under SBCs and AICs were 1519.61 g. (±242.83) and 1435.73 g. (±262.59), respectively. Also, the same ratios of the weight of broilers at 42 days were 2133.63 g (±301.71) in SBCs and 2006.20 g (±316.45) in AICs. Body related traits expressed lower values in AICs than for the SBCs during the 1-28 days period of rearing in the second generation. This trend continued during days 35-42 of the rearing period due to the incidence of ascites symptoms at this specific bird age.

Overall these findings are in accordance with findings reported by Closter et al. (2012) and Varmaghany et al. (2021) who stated that the highest mortality of broilers due to ascites was after 30 days of the rearing period and the mortality peaked between six and seven weeks.

Tables 3, 4, 5 and 6 show the least square mean of GTs in healthy and ascitic birds measured under SBCs and AICs conditions.

There was a significant effect of gender (P<0.05) on growth related traits in all rearing situations.

		Μ	ale (n=223) ¹			Fer	nale (n=241)	
Traits	Healthy (n=152)	Ascitic (n=71)	Difference ² (%)	Significance level (P>F)	Healthy (n=180)	Ascitic (n=61)	Difference (%)	Significance level (P>F)
BW_1	60.2	61.5	-2.2	NS	59.6	59.6	-0.1	NS
BW ₇	133.4	128.50	3.8	NS	128.0	130.3	-1.8	NS
BW_{14}	305.5	306.23	-0.3	NS	286.1	297.8	-3.9	NS
BW ₂₁	625.2	638.5	-2.1	NS	585.1	598.6	-2.3	NS
BW ₂₈	1114.0	1143.9	-2.6	NS	1024.3	1063.1	-3.6	NS
BW35	1730.7	1747.1	-0.9	NS	1593.5	1589.9	0.2	NS
BW_{42}	2377.4	2308.7	2.9	NS	2189.0	2053.7	6.6	*
GR ₁₋₇	12.19	11.22	8.6	NS	11.38	11.82	-3.7	NS
GR ₇₋₁₄	24.57	25.28	-2.8	NS	22.58	23.69	-4.7	NS
GR ₁₄₋₂₁	45.51	47.95	-5.1	NS	42.69	43.34	-1.5	NS
GR ₁₋₂₁	28.26	28.95	-2.4	NS	26.28	27.24	-3.5	NS
GR ₂₁₋₂₈	70.87	72.23	-1.9	NS	62.64	64.96	-2.4	NS
GR ₂₈₋₃₅	87.70	85.30	2.8	NS	81.15	76.79	5.7	NS
GR ₃₅₋₄₂	91.96	88.30	4.1	NS	83.15	68.74	20.9	***
GR ₂₁₋₄₂	83.55	80.00	4.4	NS	75.90	69.47	9.2	**
GR ₁₋₄₂	56.48	54.92	2.8	NS	51.92	49.47	5.0	NS

Table 4 Least square means of growth traits (GTs) in healthy and ascitic chickens in the first generation (reared under ascites inducing conditions (AICs))

The number of birds for each class were in parentheses.

² The difference between means of healthy and ascitic chickens [((healthy-ascitic)/ascitic) \times 100].

BW₁ to BW₄₂: body weight traits between the ages of one and 42 days and GR₁₋₇ to GR₁₋₄₂: growth rate traits at these intervals. * ($P \le 0.05$); ** ($P \le 0.01$) and *** ($P \le 0.001$).

NS: non significant.

Table 5 Least square means of growth traits (GTs) in healthy and ascitic chickens in the second generation (reared under standard breeding conditions (SBCs))

		Male (n	$=381)^{1}$				Female (n=442)
Traits	Healthy (n=317)	Ascitic (n=64)	Difference ² (%)	Significance level (P>F)	Healthy (n=424)	Ascitic (n=18)	Difference (%)	Significance level (P>F)
\mathbf{BW}_1	45.52	44.99	1.17	NS	45.08	45.26	-0.38	NS
BW_7	127.24	128.13	-0.69	NS	25.62	139.35	-9.85	***
BW_{14}	278.62	278.14	0.17	NS	272.52	283.79	-3.97	NS
BW_{21}	527.96	545.16	-3.15	NS	522.88	551.96	-5.27	NS
BW_{28}	942.31	961.22	-1.97	NS	909.88	883.92	2.94	NS
BW ₃₅	1490.04	1326.67	12.31	NS	1401.46	1021.09	37.25	NS
BW_{42}	2090.02	1216.47	71.81	NS	1940.94	836.40	132.06	NS
GR ₁₋₇	13.83	13.93	-0.67	NS	14.00	15.66	-10.55	NS
GR ₇₋₁₄	21.99	21.23	3.57	NS	21.15	20.79	1.74	NS
GR14-21	39.09	38.92	0.42	NS	38.19	38.53	-0.89	NS
GR1-21	25.92	25.37	2.15	NS	24.99	25.54	-2.15	NS
GR ₂₁₋₂₈	62.09	62.06	0.05	NS	56.93	52.87	7.67	NS
GR ₂₈₋₃₅	80.86	67.09	20.53	NS	74.52	59.53	25.20	NS
GR ₃₅₋₄₂	89.36	51.61	73.15	NS	80.08	37.70	112.42	NS
GR ₂₁₋₄₂	80.52	69.09	16.54	***	72.23	53.58	34.81	***
GR ₁₋₄₂	54.18	47.50	14.06	NS	49.25	39.23	25.55	NS

¹ The number of birds for each class were in parentheses. ² The difference between means of healthy and ascitic chickens [((healthy-ascitic)/ascitic) \times 100].

BW1 to BW42: body weight traits between the ages of one and 42 days and GR1-7 to GR1-42: growth rate traits at these intervals.

* (P≤0.05); ** (P≤0.01) and *** (P≤0.001).

NS: non significant.

From the results, it is clear that male express high rate value in case of growth measurement indicators than female birds. One possible explanation is that males have a higher metabolic rate than females due to different hormonal profiles and perhaps this factor caused a greater susceptibility to ascites in males.

A similar conclusion was reached during first generation rearing period as 35 days age male birds express significant higher body weight than female in SBCs group.

However, there was no statistically significant association between occurrence of ascites and growth rate in male birds.

		Male	$(n=381)^1$				Female (n=34	46)
T !4	Healthy	Ascitic	Difference ²	Significance level	Healthy	Ascitic	Difference	Significance level
Traits	(n=111)	(n=270)	(%)	(P>F)	(n=136)	(n=210)	(%)	(P>F)
BW_1	47.15	47.47	-0.67	NS	44.24	44.12	0.27	NS
BW_7	135.32	135.96	-0.47	NS	134.27	136.21	-1.42	NS
BW_{14}	300.51	304.22	-1.22	NS	274.56	276.23	-0.60	NS
BW_{21}	605.11	610.66	-0.91	NS	533.97	547.21	-2.42	NS
BW_{28}	997.01	1000.55	-0.35	NS	894.66	906.13	-1.26	NS
BW35	1544.01	1493.27	-3.26	NS	1364.71	1344.45	1.51	NS
BW_{42}	2104.74	2089.27	0.74	NS	1938.78	1929.37	0.49	NS
GR1-7	14.85	14.80	0.29	NS	15.12	15.50	-2.42	NS
GR ₇₋₁₄	23.64	24.17	-2.19	NS	20.19	20.23	-0.21	NS
GR ₁₄₋₂₁	43.67	43.83	-0.37	NS	37.88	38.87	-2.55	NS
GR ₁₋₂₁	27.92	28.18	-0.91	NS	24.63	25.20	-2.27	NS
GR ₂₁₋₂₈	57.00	55.34	3.00	NS	52.32	51.92	0.79	NS
GR ₂₈₋₃₅	77.05	70.60	9.14	NS	66.92	63.70	5.06	NS
GR ₃₅₋₄₂	81.19	74.72	8.66	NS	760.08	76.06	0.03	NS
GR ₂₁₋₄₂	71.18	69.85	1.90	NS	65.43	65.08	0.54	NS
GR ₁₋₄₂	43.67	43.83	-0.37	NS	37.88	38.87	-2.55	NS

Table 6 Least square means of growth traits (GTs) in healthy and ascitic chickens in the second generation (eared under ascites inducing conditions (AICs))

¹ The number of birds for each class were in parentheses.

² The difference between means of healthy and ascitic chickens [((healthy-ascitic)/ascitic) × 100].

 BW_1 to BW_{42} : body weight traits between the ages of one and 42 days and GR_{1-7} to GR_{1-42} : growth rate traits at these intervals.

* (P≤0.05); ** (P≤0.01) and *** (P≤0.001).

NS: non significant.

In contrast to the findings for male birds, at 28 days, healthy female birds expressed significantly higher growth related indicators compared to ascites-affected female birds (P<0.05) in all GTs period. Moreover, our observation illustrated ascites affected birds in both gender in 28 days age expressed zero to 5% higher weights under AICs. There was a significant reduction (P<0.05) in growth curve pattern occurred in 21 day old ascitic birds due to cold stress ascites pressure. The results for both sexes after 28 days showed that there was a significant reduction of growthrelated parameters during the rearing period. Hasanpur et al. (2015) showed that before the age of 28 days, despite a higher growth rate in ascitic birds than healthy birds, this difference in growth rate was not significant. However, with increasing age, growth in ascitic birds decreased and at the end of the rearing period, the weight of ascitic birds was significantly lower than healthy birds.

Table 7 illustrates an overview of estimates of heritability of AITs under AICs and SBCs. These data suggest that the heritability coefficient (posterior standard deviation) of water belly was high. Another heritability related indicator of AITs (i. e. RV/TV and RV/LV) indicates a similar high pattern. As in many studies, the heritability of AITs has been reported to be medium to high (De Greef *et al.* 2001; Ahmadpanah *et al.* 2017). This estimated heritability value for ascites is particularly important as it explains why a different range of susceptibility and resistance to ascites was observed in different paternal families. It is important to note that this value for heritability of AITs (i. e. RV/TV and RV/LV) creates the possibility for improvement of the genetic of composition of commercial chicken flocks.

Table 7 H	Estimates c	of heritabili	ty of indic	ator traits	(AITs) u	inder ascites
inducing c	conditions	(AICs) and	standard b	preeding c	onditions	(SBCs)

Generation	Trait	Number	h ² (SE or PSD)
	RV/LV _{SBCs}	295	0.51 (0.17)
	RV/TV_{SBCs}	295	0.58 (0.17)
First	RV/LV _{AICs}	327	0.19 (0.17)
	RV/TV _{AICs}	327	0.23 (0.14)
	Ascites _{AICs}	464	0.42 (0.09)
	RV/LV _{SBCs}	468	0.59 (0.12)
	RV/TV _{SBCs}	468	0.62 (0.09)
Second	RV/LV _{AICs}	457	0.22 (0.00)
	RV/TV _{AICs}	422	0.30 (0.09)
	Ascites _{AICs}	668	0.51 (0.11)

RV/LV: right ventricular/left ventricular weight ratio; RV/TV: right ventricular/total ventricular weight ratio and Ascites: water belly. SE: standard error and PSD: posterior standard deviation.

Tables 8 and 9 report the genetic correlation between AITs and GTs. The results of the correlational analysis indicate significant negative genetic correlations between AITs including RV/LV and RV/TV and GTs under AICs after 21 days of the rearing period because ascitic affected birds showed lower growth related parameters as compared with healthy birds under AICs. Because ascitic birds under AICs conditions have a greater growth retardation than healthy birds, this amount of negative genetic correlation seems reasonable.

Generation	Trait ¹	RV/LV _{SBCs}	RV/TV _{SBCs}	RV/LV _{AICs}	RV/TV _{AICs}	Ascites _{AICs}
	BW_{28}	-0.04	0.01	-0.19	-0.19	0.13
	BW_{35}	-0.14	-0.09	-0.20	-0.24	0.18
	BW_{42}	-0.29	-0.19	-0.18	-0.27	-0.02
First	GR ₂₁₋₂₈	0.11	0.16	-0.10	-0.08	-0.11
	GR ₂₈₋₃₅	-0.55	-0.47	-0.54	-0.70	-0.03
	GR ₃₅₋₄₂	NC^2	NC	-0.18	-0.45	-0.74
	GR ₂₁₋₄₂	-0.46	-0.33	-0.17	-0.28	-0.37
	BW_1	NC	NC	NC	NC	NC
	BW_7	0.02	0.00	0.09	0.01	0.29
	BW_{14}	-0.19	-0.29	-0.28	-0.37	0.23
	BW_{21}	-0.11	-0.17	-0.19	-0.21	0.19
	BW_{28}	-0.21	-0.19	-0.23	-0.22	0.17
	BW35	-0.15	-0.21	-0.24	-0.25	0.21
Second	BW_{42}	-0.16	-0.22	-0.21	-0.34	-0.17
	GR ₁₋₇	-0.03	-0.04	0.00	-0.08	0.17
	GR ₇₋₁₄	-0.24	-0.37	-0.38	-0.42	0.15
	GR ₁₄₋₂₁	-0.26	-0.28	-0.30	-0.25	0.18
	GR ₁₋₂₁	-0.20	-0.43	-0.28	-0.52	-0.60
	GR ₂₁₋₂₈	-0.29	-0.30	-0.30	-0.36	0.18
	GR ₂₈₋₃₅	-0.09	-0.11	-0.14	-0.10	0.00
	GR ₃₅₋₄₂	-0.47	-0.58	-0.59	-0.73	-0.11
	GR ₂₁₋₄₂	-0.18	-0.27	-0.24	-0.36	-0.31
	GR ₁₋₄₂	-0.22	-0.24	-0.26	-0.30	-0.39

 Table 8
 Genetic correlation between indicator traits (AITs) measured under ascites inducing conditions (AICs) and standard breeding conditions (SBCs) with growth traits (GTs) measured under AICs*

 BW_1 to BW_{42} : body weight traits between the ages of one and 42 days; GR_{1-7} to $GR_{1.42}$: growth rate traits at these intervals; RV/LV: right ventricular/left ventricular weight ratio; RV/TV: right ventricular/left ventricular weight ratio and Ascites: water belly.

NC: gibbs samples or convergence problems.

* Standard error for RV/TV in the range of 0.25 to 0.60 and for the RV/LV in range of 0.26 to 0.61 and posterior standard deviation for the water belly were in the range of 0.09 to 0.61.

However, a possible explanation for this negative correlation is a lack of genetic association between fast growth and the incidence of the ascites syndrome.

A significant negative correlation was found between AITs (RV/TV and RV/LV) and GTs under SBCs condition although no significant correlation was found between GTs and the incidence of ascites. Closter *et al.* (2009) reported the genetic correlation between RV/TV and body weight at 14 and 35 days of age as being 0.19 and -0.18, respectively.

Also, the authors demonstrated a positive genetic correlation between RV/TV and body weight in both males and females at 2, 5 and 7 weeks of age but become a negative trend at older ages. This outcome is consistent with what has been reported by Pakdel *et al.* (2005a) and Pakdel *et al.* (2005b).

As mentioned in the literature review, the history of genetic selection of commercial birds has caused a variable incidence of ascites in commercial chicken flocks. Our data showed a high to moderate degree of positive correlation between water belly and GTs measured under AICs before 28 days of age indicating that fast-growing birds are more susceptible to ascites, presumably due to need for more oxygen at an early age.

No statistical association between fast growth rate and ascites was found in the present study which is in accordance with findings reported by Druyan and Cahaner (2007) and Druyan et al. (2008) on the incidence of ascites. We speculate that this might be due to the genetic architecture of threshold nature of resistance and susceptibility and possible contribution of one or more major genes for controlling genetic variation of phenotype in this investigated trait. A similar conclusion was reached by Hasanpur et al. (2015) who highlighted that the pattern of the growth curve in healthy males and females is significantly higher than that for ascitic male and female birds. It is by now generally accepted that fast growing birds carry high susceptibility to ascites although this was not supported by our data. Naghous et al. (2012a) showed no significant difference between chickens raised in the two ascitic and control treatments. In general, with an increasing growth rate in broilers, the amount of oxygen demand in different tissues and organs increases. This will increase ascites mortality in fast-growing chickens under stressful conditions (Julian, 1993). Accordingly, some studies have suggested that genes associated with increased growth rate in broilers also increase the prevalence of ascites.

Generation	Trait ¹	RV/LV _{SBCs}	RV/TV _{SBCs}	RV/LV _{AICs}	RV/TV _{AICs}	Ascites _{AICs}
	BW_1	NC^{2}	NC	NC	NC	NC
	BW_7	0.01	0.02	0.13	0.06	0.22
	BW_{14}	-0.04	-0.04	-0.27	-0.32	NC
	BW_{21}	0.03	0.04	-0.18	-0.18	0.17
	BW_{28}	0.07	0.03	-0.32	-0.26	0.21
	BW35	0.05	0.02	-0.47	-0.40	-0.12
	BW_{42}	0.02	0.02	-0.51	-0.45	-0.06
First	GR1-7	-0.06	0.05-	0.01	0.10-	0.10
	GR ₇₋₁₄	-0.08	0.08-	-0.35	-0.39	0.12
	GR ₁₄₋₂₁	-0.08	0.08-	-0.26	-0.19	0.17
	GR ₁₋₂₁	-0.12	-0.10	-0.27	-0.28	0.13
	GR ₂₁₋₂₈	0.19	0.14	-0.03	0.06	0.41
	GR ₂₈₋₃₅	-0.09	0.11-	-0.71	-0.61	-0.37
	GR ₃₅₋₄₂	-0.29	0.25-	-0.42	-0.38	-0.65
	GR ₂₁₋₄₂	-0.08	-0.10	-0.48	-0.38	-0.32
	GR ₁₋₄₂	-0.11	-0.11	-0.44	-0.43	-0.22
	BW_1	NC	NC	NC	NC	NC
	BW_7	0.05	0.09	0.15	0.08	0.18
	BW_{14}	0.03	-0.02	-0.30	-0.31	0.11
	BW_{21}	0.03	0.07	-0.21	-0.19	0.13
	BW_{28}	0.10	0.06	-0.36	-0.29	0.17
	BW ₃₅	0.03	0.03	-0.50	-0.25	-0.21
	BW_{42}	0.00	0.08	-0.55	-0.50	-0.19
Second	GR1-7	-0.10	-0.02	0.03	0.14	0.13
	GR ₇₋₁₄	-0.12	-0.04	-0.38	-0.40	0.16
	GR ₁₄₋₂₁	-0.09	-0.06	-0.31	-0.23	0.16
	GR ₁₋₂₁	-0.26	-0.24	-0.46	-0.40	-0.48
	GR ₂₁₋₂₈	-0.14	-0.09	-0.29	-0.31	0.17
	GR ₂₈₋₃₅	0.16	0.18	-0.01	-0.07	-0.30
	GR ₃₅₋₄₂	-0.07	-0.19	-0.75	-0.66	-0.28
	GR ₂₁₋₄₂	-0.12	-0.19	-0.52	-0.45	0.19
	GR ₁₋₄₂	-0.09	-0.22	-0.50	-0.48	0.17

Table 9 Genetic correlation between indicator traits (AITs) measured under ascites inducing conditions (AICs) and SBCs with growth traits (GTs) measured under andard breeding conditions (SBCs)

 BW_1 to BW_{42} : body weight traits between the ages of one and 42 days; $GR_{1.7}$ to $GR_{1.42}$: growth rate traits at these intervals; RV/LV: right ventricular/left ventricular weight ratio; RV/TV: right ventricular/total ventricular weight ratio and Ascites: water belly.

NC: gibbs samples or convergence problems

* Standard error for RV/TV in the range of 0.20 to 0.49 and for the RV/LV in range of 0.11 to 0.61 and posterior standard deviation for the water belly were in the range of 0.11 to 0.61.

In other words, families with higher genetic potential for growth also have higher mortality due to ascites (Druyan *et al.* 2007a; Arce-Menocal *et al.* 2009). Future investigations are necessary to validate the kinds of conclusions that can be drawn from this study.

CONCLUSION

Association between GTs and ascites is an important indicator for understanding the genomics of resistance and susceptibility of commercial poultry strains to ascites. The present findings confirm that genetically fast growing birds have a tendency to develop symptoms of ascites. Selection for AITs may genetically improve resistance to ascites in the investigated chicken line. A further novel finding is that there were no consistently direct genetic correlations between AITs and GTs. This analysis provides the opportunity to grow birds to heavier live body weight as body weight does not appear to be the main reason for susceptibility to ascites syndrome.

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