

Anatomical parameters of cardiopulmonary system, venous blood pCO₂ and pO₂ tensions and the development of ascites syndrome in two genetic line chickens that are differing in their growth rate

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Summary

The present study was designed to examine the anatomical parameters of the cardiopulmonary system, the function of venous blood gas parameters and the development of ascites incidence in two genetic line chickens. Three hundred forty day-old chickens from two pure broiler breeder lines, which were different in their growth rate and susceptibility to ascites syndrome were obtained. The relative heart and lung weights, the volumes of the heart, lung and thorax cavity, the incidence of ascites, and the venous blood gas parameters in these two genetic line chickens were followed. In the present study, the incidence of ascites and right ventricular hypertrophy was markedly higher in the fast-growing broiler chickens compared to the slow-growing chickens, as two genetic line chickens exhibited significant differences in their growth performance traits. The volumes of the thorax cavity, before and after removing the heart and lung tissues, were lower in fast-growing broiler chickens compared to the slow-growing chickens. The relative lung volume was significantly lower in the fast growth chickens than the slow growth chickens at the earlier age, but it did not differ at the later age. Additionally, a rise in carbon dioxide tension and a decline in oxygen pressure in the venous blood of rapid growth compared to the slow growth broiler chickens were observed. It could be concluded that there is an association between the insufficiencies of the cardiopulmonary system with the function of the venous blood gas parameters and the development of ascites syndrome in fast growing broiler chickens.

Key words: Ascites, pCO₂, Broiler chickens, Anatomy, Cardiopulmonary system

Introduction

Ascites syndrome is no longer restricted to birds kept at high altitude, as initially reported by Cueva *et al.* (1974), but it is now also found in flocks at sea level (Albers and Frankenhuis, 1990; Hassanzadeh *et al.*, 2000, 2001). The increasing susceptibility of broiler chickens to ascites coincided with a continuing genetic and nutritional improvement in their food efficiency and growth rate (Julian, 1993). A study done by Scheele *et al.* (1992) illustrated that broilers from stocks differing in growth rate and feed efficiency differ in their susceptibility to ascites. In the study of Buys *et al.* (1999) the highest incidence of ascites was found in broiler stocks that combined high feed efficiency with fast growth rate, whereas in broilers from stocks with either slower

growth or low feed efficiency the incidence of ascites was much lower.

Oxygen is a critical component in energy metabolism. Broilers use energy for thermoregulation, activity and growth, and any form of energy utilization creates a demand for oxygen. Differences in the oxygen requirements between fast-growing and slow-growing lines are associated with the more pronounced occurrence of right ventricular hypertrophy (RVH) and ascites in the fast growing line (Peacock *et al.*, 1990). Buys *et al.* (1995) reported that chickens of an ascites sensitive line consumed more oxygen due to high metabolic activity, hence showing significantly higher carbon dioxide tension (pCO₂) and lower oxygen tension (pO₂) in venous blood, compared to an ascites resistant line of birds. Scheele *et al.* (2005)

showed that high carbon dioxide tensions in the venous blood of juvenile domestic chickens are a predisposing factor for the development of RVH and ascites.

There is evidence of a genetic susceptibility to the pulmonary hypertension syndrome, which is hypothesised to be related to the rate of growth and vascular lung capacity (Wideman and French, 2000; Hassanzadeh *et al.*, 2003, 2005a, b). The anatomy and physiology of the avian respiratory and cardiovascular systems are important in the susceptibility to pulmonary hypertension syndrome and ascites (Decuypere *et al.*, 2000; Hassanzadeh *et al.*, 2005b, 2008). Decuypere *et al.* (2000) indicated that alteration in the proportional growth as a result of indirect selection for greater muscularity may have had the effect of producing birds with a relatively small respiratory and cardiovascular system. Authors argued that if the development of ascites syndrome is a consequence of structural or morphological/histological alterations, this must result in observable physiological changes. Julian (1993) described that fast-growing broiler chickens have increased blood flow as bodily oxygen requirements increase, and they may not be full oxygenating the haemoglobin, while the breast muscle development hinders respiration, as does intra-abdominal pressure from the internal organs or body fat (Jones, 1995).

Our recent studies indicated that cardiopulmonary parameters are extremely unfavorable in broiler chickens compared to layer and native chickens and suggested a reduction in the gas exchange area in broilers and therefore a higher susceptibility to pulmonary hypertension and ascites (Hassanzadeh *et al.*, 2005b, 2008). Experimental results obtained in other studies pointed out that, at a low ambient temperature, high pCO₂ tension in the venous blood of young broiler chickens was a reliable predictor for ascites susceptibility at later age (Scheele *et al.*, 2003, 2005; Hassanzadeh *et al.*, 2010). In the present study, we examined anatomical parameters of the cardiopulmonary system and the function of blood gas parameters in two broiler lines that were differing in their growth rate and susceptibility to ascites

syndrome.

Materials and Methods

Three hundred forty day-old male chickens were obtained from two broiler lines (GGP stock of Pure Broiler Line Co., Babolkenar, Iran) which were expected to be different in the susceptibility to ascites syndrome. Pure sire line was primarily selected for a higher growth (Hg) rate in combination with a low feed conversion ratio (FCR), whereas the chickens of the second line were obtained from a broiler breeder stock with a slower growth (Lg) rate combined with a higher FCR. Two group chickens were wing-banded for individual identification and placed in 8 floor pens (4 pens each line) under a nearly continuous lighting programme (23 h light and 1 h dark) until six weeks of age. Birds had *ad libitum* access to commercial broiler crumble (starter 22% CP and 2921 Kcal/kg ME) and pelleted (grower 19% CP and 3200 Kcal/kg ME) food, respectively. In order to achieve the development of the ascites incidence, the experiment was performed in the middle of January until the end of February, in a farm located at low altitude which had regular ascites symptoms during the winter.

At 12, 21 and 28 days of age from 40 randomly selected birds per group (10 birds per pen), venous blood samples were taken and collected in heparinized tubes on ice for the determination of pCO₂, pO₂ and pH values by a blood gas analyzer (ABL 5; Radiometer system, Copenhagen, Denmark).

To measure the anatomical parameters, at days 7, 14, 21, 28 and 42, five chickens from each genetic line (at least 1 bird per pen in each sampling time) were randomly selected and wing-band numbers recorded. The chickens were fasted for 2 h before euthanasia. The chickens were weighed, euthanized with chloroform and placed in dorsal recumbency. The mouth and esophagus were cut open and the trachea was cut free from underlying tissue as described by Hassanzadeh *et al.* (2005b, 2008). A cannula was inserted through the larynx into the trachea and tied in place. The lungs were fixed by intratracheal infusion of 10% phosphate-buffered formalin at 25 cm above the table on which the chicken was

lying. The formalin was moved back and forth through the lung by squeezing the abdominal air sacs, which also filled with fixative. When the fixative stopped flowing, with the funnel filled to the 25 cm level, the trachea was ligated and the cannula was removed. The birds were held overnight at 4° to allow total fixation. After approximately 48 h, the abdomen was opened and chickens were again placed in 10% phosphate-buffered formalin for a minimum of 4 weeks. Then, the digestive system and liver were removed. The body was cut transversally through the muscles of the abdominal wall, behind the sternal cartilage, following the caudal border of the last ribs. The vertebral column was cut between the moveable thoracic vertebra and the synsacrum. The body of the chickens was kept vertically by a holder. Thorax cavity was filled with the water and water volume measured in cm³ before removing the heart, pericardium and lungs (TV1), after removal of the heart and pericardium (TV2) and after removal of the heart, pericardium and lungs (TV3). The volumes of the heart, the total lung and thorax cavity were calculated using the formula:

TV1 = Volume of the thorax cavity before removal of the heart, pericardium and lungs

TV2 = Volume of the thorax cavity after removal of the heart, pericardium and before removing the lungs

TV3 = Volume of the thorax cavity after removing the heart, pericardium and lungs

TV2-TV1 = Heart volume

TV3-TV2 = Lung volume

To record the weights of the lung and heart in the individual birds, large blood vessels, gross fat, clotted blood, bronchia and connective tissue were removed and the surface of each lung was blotted dry. All parameters were computed as a percentage of body weight.

Body weight, feed intake and FCR were

recorded every 2 weeks. Mortality was recorded daily and the birds were autopsied for lesions of heart failure and ascites. At the end of the experiment, 100 surviving chickens from each group were randomly chosen, slaughtered and examined for lesions of ascites (Julian, 1993; Hassanzadeh *et al.*, 2002). For growth performance and blood parameters statistical analysis was performed using the “General linear model procedure” (SAS, 2002). If a significant overall effect ($P < 0.05$) was found, treatment means were compared by using the Scheffe test. For anatomical parameters, statistical analyses used the t-test procedure. The rate of ascites incidence and RV/TV ratios were analyzed using Chi-square (X^2) test.

Results

The number of birds that developed RVH or ascites and the RV/TV ratios of the surviving birds that were slaughtered and autopsied at the end are presented in Table 1. As was expected, 14 of the 170 birds (8.2%) from the Hg line chickens died due to RVH and ascites. In contrast, the chickens of the Lg line, which showed a slower growth rate, had a significantly ($P < 0.01$) lower percentage mortality (4 birds, 2.3%) due to RVH and ascites. Among the 100 survived chickens from the Hg line that were slaughtered and examined at the end of week six, 5 birds had RV/TV ratios between 0.25 to 0.29 and 15 birds showed a RV/TV equal to or over the 0.29, while in the Lg group, from a similar 100 birds, 3 birds had RV/TV ratios from 0.25 to 0.29 and 5 birds ($P < 0.05$) had a RV/TV equal to or over the 0.29 (Table 1).

Table 2 shows the average values of the venous blood pCO₂ and pO₂ tensions as well as the pH levels according to the line. The level of pCO₂ was significantly higher in

Table 1: Weekly ascites mortality and right ventricular/total ventricular (RV/TV) ratios in two line broiler chickens that were slaughtered at the end of the experiment

Groups	Weekly ascites mortality					RV/TV of survived birds		
	wk 2	wk 3	wk 4	wk 5	wk 6	Total	25≤ to >29	≥29
Age								
Hg	-	-	1	4	9	14 (8.2%)**	5/100	15/100*
Lg	-	-	1	-	3	4 (2.3%)	3/100	5/100

Values followed by asterisks indicate significant differences between the two line chickens (* $P < 0.05$; ** $P < 0.01$)

the venous blood of Hg than to the Lg chickens at 12, 21 and 28 days of age. Except at day 12 when Hg chickens showed a significantly lower pO₂ tension than the Lg chickens, no differences were found between the pO₂ values of the two line chickens at the later age. The average blood pH levels were similar for the two line chickens at all ages.

The results of the anatomical parameters are presented in Figs. 1 to 3. In both genetic lines, the relative heart and lung weights as a percentage of body weight decreased significantly with the increasing of age (Fig. 1). Throughout the experiment there was no significant difference between the relative heart and lung weights of the two line chickens when expressed as a function of body weight.

Table 2: The average partial pressure of carbon dioxide (pCO₂) and oxygen (pO₂) tensions and the pH levels in venous blood of two line broiler chickens. Values are means ± SEM

Age	groups		P-values
	Hg	Lg	
PcO₂ (mm Hg)			
day 12	53±0.7	43±0.6	0.001
day 21	51±0.7	43±0.5	0.001
day 28	56±1.0	48±0.6	0.01
PO₂ (mm Hg)			
day 12	55±2	70±2	0.0001
day 28	70±1	68±2	NS
day 42	77±2	80±3	NS
PH levels			
day 12	7.31±0.008	7.32±0.005	NS
day 28	7.33±0.001	7.32±0.005	NS
day 42	7.33±0.001	7.33±0.001	NS

NS = not significant

Heart volume, as a percentage of body weight, decreased significantly with increasing of age in both groups of chickens (Fig. 2). Relative heart volume was only significantly higher in Hg chickens than in Lg chickens at 42 days of age. Although relative lung volume to body weight of the 7-day-old chickens was significantly lower in the Hg line compared with the Lg line chickens, no significant differences were found on later ages (Fig. 2).

The volumes of the thorax cavity as a percentage of body weight, before and after removal of the heart and lung tissues,

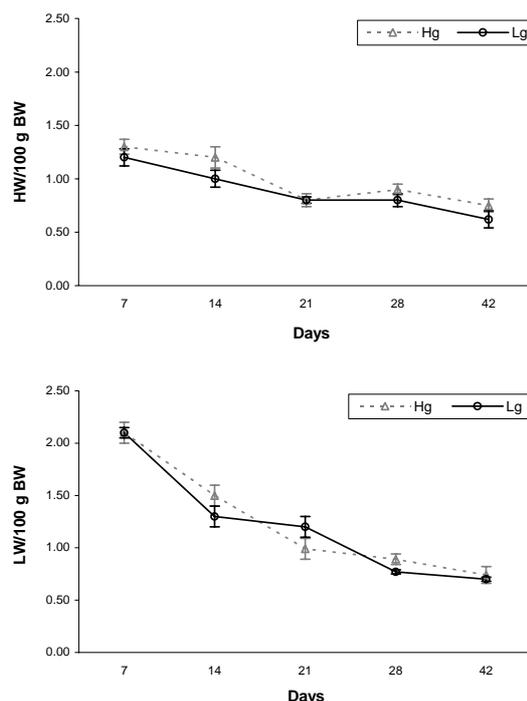


Fig. 1: Heart weight (HW) and lung weight (LW) as a percentage of body weight (BW) during the 6 weeks of age in higher growth (Hg) and slower growth (Lg) rate broiler chickens

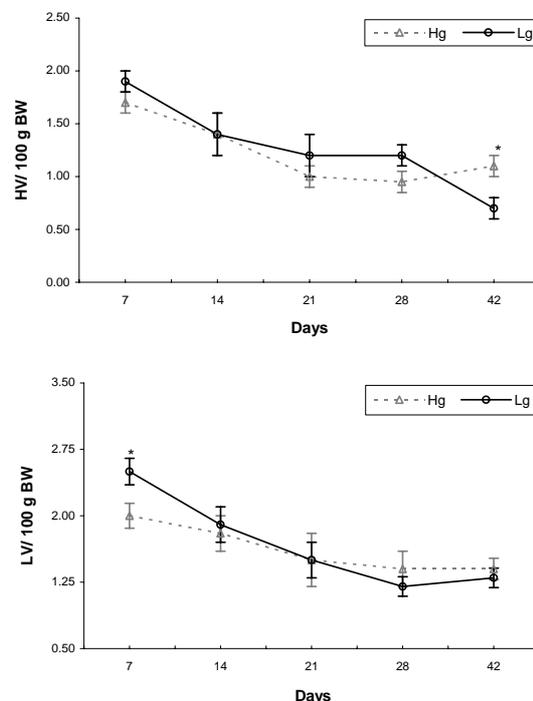


Fig. 2: Heart volume (HV) and lung volume (LV) as a percentage of body weight during the 6 weeks of age in higher growth (Hg) and slower growth (Lg) rate broiler chickens. * with in same age, values having different stars are significantly different (P<0.05)

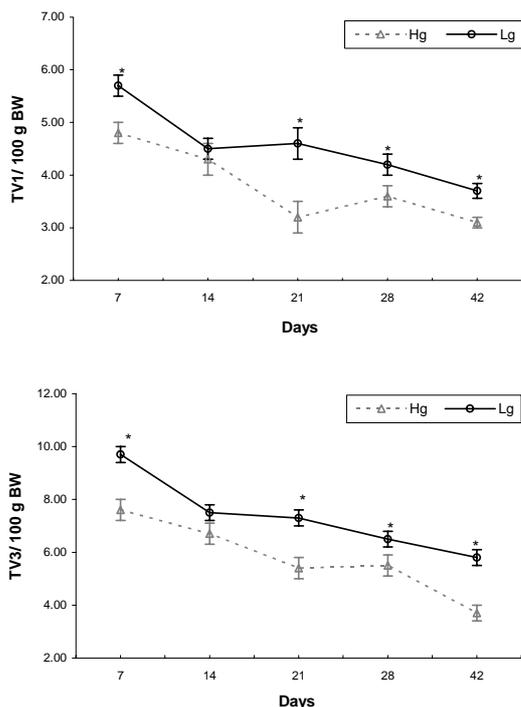


Fig. 3: Thorax volume before removal of heart and lungs (TV₁) and after removal of heart and lungs (TV₃) as a percentage of body weight during the 6 weeks of age in higher growth (Hg) and slower growth (Lg) rate broiler chickens. * with in same age, values having different stars are significantly different (P<0.05)

decreased with increasing of age in both groups of chickens (Fig. 3). The volume of thorax cavity before removal of the heart and lung tissues (TV₁) was significantly lower in the Hg broiler chickens than that of the Lg chickens at 7, 21, 28 and 42 days of age, when expressed as a function of body weight. The same phenomenon was observed for the relative volume of the thorax cavity after removal of the heart and lung tissues (TV₃) during the 6 weeks of the experiment.

As was expected, the mean body weight of the Hg line chickens was significantly higher during the experimental period (Table 3). Generally, the food consumption of the Hg chickens was higher, but there were significant differences only during the first 4 weeks of the growing period. The Hg chickens showed significantly lower FCR compared to the Lg line chickens from 1 to 28 days of age. Therefore, the Hg line chickens showed a significantly higher cumulative feed intake and a lower final

FCR compared to the Lg chickens (Table 3).

The ratios of abdominal fat and liver weights as a percentage of body weight did not differ between the two genetic lines (Table 3).

Table 3: Mean body weight, feed intake, feed conversion ratios (FCR), relative liver and abdominal fat weights at slaughtered age in two broiler line chickens. Values are means ± SEM

Age	groups		P-values
	Hg	Lg	
Body weight (g/chicken)			
day 1	50±0.5	48±0.4	0.05
day 14	449±8	357±10	0.0001
day 28	1401±40	1128±32	0.0001
day 42	2569±70	2266±58	0.0001
Feed intake (g/chicken)			
day 1-14	512±14	477±10	0.05
day 14-28	1587±49	1418±41	0.05
day 28-42	2328±95	2291±46	NS
day 1-42	4427±98	4186±55	0.001
Feed conversion ratio			
day 1-14	1.27±0.03	1.50±0.03	0.001
day 14-28	1.67±0.06	1.84±0.04	0.01
day 28-42	1.95±0.07	2.00±0.05	NS
day 1-42	1.75±0.03	1.90±0.02	0.01
Liver (g /100 g BW)			
day 42	1.51±0.19	1.38±0.12	NS
Fat (g)/100 g BW			
day 42	1.95±0.08	1.88±0.06	NS

NS = not significant

Discussion

Until now there has been no consensus about the origin of the ascites syndrome in broiler chickens at sea level. At high altitudes, chronic hypoxia induces a marked increase in pulmonary arterial pressure and right ventricular hypertrophy in chickens followed by ascites (Sillau *et al.*, 1980; Julian, 1993; Hassanzadeh *et al.*, 2002, 2003, 2004). Powel (2000) suggested that the increased growth rate in modern fast growing broiler chickens at sea level requires a higher metabolic rate, and thus an increase in cardiac output, causing pulmonary hypertension and ascites. Experimental results obtained in other studies pointed to the important role of hypercapnia (high pCO₂ in the blood) in activating vasoconstriction and high blood pressure at sea level, as an evident feature in ascites susceptible broiler population (Buys *et al.*, 1999; Scheele *et al.*, 2003, 2005).

Ascites mortality was achieved by

supplying a high quality pelleted diet in a poultry farm that always showed the incidence of ascites during the cold season. As increased susceptibility of broiler chickens to ascites has previously been linked with high growth rate and feed efficiency (Scheele *et al.*, 1992; Decuyper *et al.*, 2005; Julian, 2005; Hassanzadeh *et al.*, 2010), in the present study susceptibility to ascites significantly ($P < 0.01$) differed among the two line chickens. Both stocks exhibited marked differences in performance traits (growth rate and FCR) and susceptibility for ascites.

A strong relationship between the partial pressure of carbon dioxide and ascites susceptibility was investigated previously (Hassanzadeh *et al.*, 1997; Buys *et al.*, 1998; Scheele *et al.*, 2003, 2005; Hassanzadeh *et al.*, 2010). The study by Buys *et al.* (1998) showed that the high pCO_2 values in the venous blood of the broiler chickens can be determined by genetic background. The present study revealed that the Hg broiler chickens-being extremely prone to right ventricular hypertrophy and ascites, were also characterized by the higher pCO_2 values. However, in the Lg line, relatively slow-growing broiler chickens have a correspondingly lower demand for oxygen and hence exhibited lower venous blood pCO_2 levels, as well as fewer ascites, confirming earlier studies (Scheele *et al.*, 2003, 2005; Hassanzadeh *et al.*, 2010). However, the differences on mean pCO_2 values observed between this study and previous studies might be due to the different experimental designs and could be an interaction between environmental factors such as altitude and the genetic line of birds.

Hassanzadeh *et al.* (2005b, 2008) reported a causal relationship between the anatomical parameters of cardiopulmonary systems with the susceptibility to ascites in broiler chickens. As was shown before (Wideman *et al.*, 1995; Julian, 1989), the relative lung volume decreased with age in the broiler chickens when this parameter is expressed as a percentage of body weight. In our findings, relative lung volume decreased with age in the line group chickens, hence an age effect related to age-related differences in proportional growth might be suggested,

as was observed in the previous report (Hassanzadeh *et al.*, 2005b).

Timmwood *et al.* (1987) reported that lung volume relative to body weight is lower in selected than unselected male turkeys. In the present study, relative lung volume as a percentage of body weight only significantly differed at day 7, between two genetic lines of chickens. Increasing the relative heart volume at 6 weeks of age, in the Hg chickens compared to the Lg chickens, might be related to the enlargement of the heart ventricles in the sampled-birds, as the number of chickens involved with heart failure and ascites were more apparent at that age (Table 1).

The significant differences between the volumes of thorax cavities when expressed as a function of body weight, before and after removing the heart and lungs in the Hg compared to the Lg chickens is probably caused by the much larger muscle mass in heavy broiler chickens than the slower growing chickens as was argued earlier (Julian, 1989; Jones, 1995; Decuyper *et al.*, 2000; Hassanzadeh *et al.*, 2005b, 2008). However, the pressure from the abdominal contents to the thorax cavity could be the causal factor for the reduction of the gas exchange area (Julian, 1993) but it might not be the main causal factor here, because the relative liver and abdominal fat weights to body weight did not differ between the two genetic line chickens (Table 3).

The data presented here showed significant differences between the anatomical parameters of the cardiopulmonary system and the levels of venous blood gas parameters in two genetic line chickens that differed in growth rate and ascites susceptibility. Such differences between blood gas parameters could be due to an extremely high metabolic demand by the tissues, as part of the events during the development of the syndrome (Buys *et al.*, 1995). In addition, insufficient development of the respiratory and cardiovascular systems might potentiate and act in an additive or even in a synergistic way for developing ascites syndrome. The connection between the insufficiencies of the cardiopulmonary system and functional changes in the venous blood parameters involved in the development of ascites needs

further investigation.

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