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EFFECTS OF ASCITES (PULMONARY HYPERTENSION SYNDROME) ON BLOOD GAS, BLOOD OXIMETRY PARAMETERS AND HEART SECTIONS OF BROILERS GROWN AT HIGH ALTITUDE

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ABSTRACT

The aim of this study was to determine the effects of ascites syndrome on some blood parameters and heart tissue of 300 broilers grown at high altitude (1727m) in Van Province. Ascites is a metabolic disorder in which excess amounts of ascitic fluids accumulate in the body cavity due to the cardiovascular problems resulted by inadequate supply of oxygen. Significant ($P<0.05$) differences were detected in blood oximetry parameters of SO_2, ctHb, FO_2Hb and Hct_c (Hematocrit) between ascitic and healthy chicks. SO_2 and FO_2Hb concentrations were higher in healthy broiles while blood hemoglobin and hematocrit values were higher ($P<0.05$) in ascitic broilers. Similarly, right ventricular diameter significantly ($P<0.05$) increased in ascitic broilers. While right ventricular diameter of healthy broilers was 1.60 mm, right ventricular diameter of ascitic broilers increased to 4.92 mm. These findings indicate that blood oximetry parameters and heart sections can be used as indicators of ascites susceptibility in broilers grown at high altitudes. The results of the current study revealed that rearing of broilers at high altitude usually leads to ascites exhibited by decreased SO2 and FO2Hb oxygen saturation and increased ctHb and Hctc levels in blood and significantly enlarged right ventricular diameter.

Key words: Ascites, broiler, high altitude, blood gas, heart.

INTRODUCTION

Ascites orPulmonary Hypertension Syndrome (PHS) is one of the major causes of mortality and morbidity in modern broiler production. Ascites is a complex problem caused by both genetics and environment. Since broilers are fast growing chickens with high metabolic rate, their oxygen demand increases during intake of nutrient-rich rations and exposure to cold stress conditions. In such situations, the proportionately underdeveloped cardio-respiratory system of modern broilers fails to fulfill the required oxygen demand, resulting in hypoxemia (Balog *et al*., 2003; Aftab and Khan, 2005).Poor ambient air quality (dust, CO, CO_2 and ammonia etc.) in the poultry house will trigger ascites due to lung irritation and reduced oxygen intake (Bootje *et al*., 1998; McGovern *et al*., 1998).The percentage of oxygen in the air changes with altitude from sea level. At high altitude, atmospheric pressure, hence oxygen pressure, is lower than that at sea level. Due to this fact, altitude has been reported to be an important factor in ascites development(Ocak, 2006; Druyan, 2012; Franciosini *et al*., 2012). 13%, 27% and 80% of the broilers at 1980, 2438 and 2896m altitudes respectively developed ascites (Koç, 2007). Although ascites symptoms can be displayed even at lower altitudes during winter months, the effects of the syndrome become more dramatical at altitudes above 1200 meter (4000 ft.). Ascites is a major concern in countries like Mexico, Colombia, Ecuador, Peru and Boliviawith altitudes of 2000-2800 meters (6600-9200 ft.) (Ranson, 2005).

There are diverse clinical manifestations of ascites incidence in broilers. These are, pulmonary hypertension, right ventricular hypertrophy, central and portal venous congestion, liver damage and accumulation of 40-260 ml yellow fluid with fibrin clots in abdominal cavity (Tafti and Karima, 2000; Balog *et al*., 2003). Additionally, ascites produces increased systemic venous pressure, valvular insufficieny and right ventricular hypertrophy (Franciosini *et al*., 2012). Ascites is associated with a typical mortality rate of about 8-12%, which increases up to 25% in severe cases (Ranson, 2005). Ascites syndrome is still a major problem for poultry industry in various parts of the world. Despite the intensive investigations of this syndrome for many years, its pathogenesis remains unclear(Wang *et al*., 2012). The aim of the present study was to determine the effects of ascites syndrome on blood gas and morphology of broilers grown at high altitude (1727m) in Van Province.

MATERIALS AND METHODS

Three hundred (300) Ross (308) broiler chicks were used in this trial. All chicks were grown at individual plots on the ground. After coating the plot bases with lime, wood shaving was used as litter material.

Rations were prepared according to the National Research Council (1994) standarts. Corn and soybean meal based rations were used in the trial containing 23.82% CP and 3.048 kcal/kg/ME between days 0-11; 22.70 %CP and 3.200 kcal/kg ME between days 11-22; and 20.68% CP and 3.316 kcal/kg ME between days 22-42. Trial units were heated with electrical heaters. An initial temperature of 33^0 C was maintained which was reduced by3 ^{0}C per week and then finally maintained at 24 ^{0}C starting from 4th weeks onwards. Throughout the trial, broiler chicks were fed *ad libitum* under continuous illumination (24-h). Blood and heart samples of 42 day-old 10 male broilers each from acitic and healthy groups were analyzed.

Blood Gas, Oximetry, Electrolyte and Metabolite Analyses: At 42nd day of trial, blood samples taken from the wing wein of chicks were collected in heparin containing syringes to avoid clot formation. The analyzers used in this study employ the following measurement principles: Potentiometric techniques are applied to the sensors of pH, pCO_2, Ca^{+2}, K^+, Na^+, and Cl^- ; the optic system to the sensors of pO_2; amperometric techniques to the sensors of Glucose, and spectrophotometric techniques to measure Hb, COHb, MetHb and calculated $CHCO_3$, SO_2, hematocrit, $mOsm_c$, cBasee (Radiometer ABL 700, Radiometer Medical ApS, Brønshøj, Denmark).

Heart Histological Analysis: The broilers were slaughtered at 42nd day of the trial. Heart tissues from 10 broilers each of ascitic and healthy groups were fixed in 10% formaldehyde solution and transferred to Van Regional Training and Research Hospital, Pathology Laboratory for analyses. Horizontal sections were taken from the heart including right and left ventricle and casetted. Tissues in cassettes were processed with an autotechnic tissue processing equipment (Leica ASP 300). Tissue samples were vertically embedded in paraffin block.4 micron thick sections were taken from the paraffin blocks with fully motorized Thermo Shandon Microtome. Sections were heated in dry oven at 70 ºC for 40 mins for deparaffinization. The sections were transferred to xylen and alcohol, then washed and stained with Haematoxylin and eosin and covered with lamella. Histomorphological examinations of the prepared sections were performed under light microscope and the widest diameters were labeled with slide pen and measured by compass meticulously.

Statistical Analysis: Evaluation of the average differences between two independent groups (ascitic and healthy chicks) was indipendent t test. Statistical analyzes were peformed in SAS package programs (Version 9.2). (SAS, 2010).

RESULTS AND DISCUSSION

In the trial, ascites symptoms were noticeable from 3 weeks onward. Rapid breathing rate was observed due to physical restriction of large abdominal air sacs. These were followed by abdominal enlargement and cyanosis development in ascitic chicks in the following weeks. A dark yellow and clear fluid like blood plasm was observed in the abdominal cavity. Heart was enlarged and pericardium was distended by fluid. In the present study, ascites-induced mortality rate was 17.3%.

Blood gas, blood oximetry, blood electrolyte, blood metabolite and blood acid-base concentrations of healthy and ascitic chicks are given in Table 1. There were no significant ($P>0.05$) differences in blood pH, pCO_2, pO_2, $cHCO_3$, FHHb, FMetHb, cK^+, cNa^+, cCa^{2+}, cCl^-, cGlukoz, $mOsm_c$, cBasee parameters between healthy and ascitic chicks. The findings of the current study on blood gas parameters are supported by some research which also found no significant differences between healthy and ascitic chicks in pCO_2, pO_2, HCO_3 (Ladmakhi *et al*., 1997); in pH, bicarbonate

(HCO_3) and pCO_2 levels (Daneshyar *et al*., 2007); in blood pH (Van As *et al*., 2010) and in pCO_2, pO_2 and HCO_3 levels (Hafshejani *et al*., 2012). Contrary to the findings of this study, Guo *et al*. (2007)

have reported significantly higher K^+ ion concentration in ascitic broilers. Daneshyar *et al*. (2009) detected a significant increase in fasting blood sugar levels of ascitic chicks under cold stress. Wang *et al*. (2009) observed significantly increased serum glucose levels in ascitic broilers. Similarly, Closter *et al*. (2009) suggested that blood gas parameters can be used as indicators of ascites susceptibility in chicks. They measured bicarbonate (HCO_3) concentration as 26.88mmol/L and pH value as 7.38 in venous blood of ascitic chicks under cold stress. Moayyedian *et al*. (2011) found significantly increased pCO_2 and significantly decreased pO_2 in cold-stress induced ascitic chicks. Hafshejani *et al*. (2012) reported significantly decreased pH levels in ascitic chicks.

Differences in blood oximetry parameters of SO_2, ctHb, FO_2Hband Hct_c (Hematocrit) were found to be statistically ($P<0.05$) significant between groups. While $SO_2$and FO_2Hb concentrations were higher in healthy birds, higher ($P<0.05$) hemoglobin and hematocrit values were detected in ascitic chicks. In the current study, blood hematocrit value of healthy chicks was 31.00% which increased up to 41.31% in ascitic chicks. The similar findings have also been reported by Zerehdaran *et al.* (2006) who reported blood hematocrit values in healthy and ascitic chicks as 28.28 and 35.40%, respectively. Huchzermeyer (2012) also observed an ascites-induced increase in blood hematocrit value which was measured as 18-30 and 35-60% in normal and ascitic chicks. The increase in hematocrit values of ascitic chicks is explained by increased oxygen demand of tissues due to enhanced metabolic rate and decreased oxygen saturation. Increased hematocrit value results increased red blood cell production (erythropoiesis) and decline in arterial blood oxygen saturation. Enhanced erythropoiesis accounts for increased blood viscosity and blood pressure which contribute enhanced cardiac work load in ascitic chicks (Druyan, 2012).

The findings of the current study on blood oximetry parameters are in accordance with the findings of Balog (2003), Luger *et al*. (2003) İpek and Şahan (2006), Özkan *et al*. (2006), Gou *et al*. (2007) and Wang *et al*. (2009). Similarly, Daneshyar *et al*. (2007) found that ascites incidence lead to a significant decline in oxygen saturation. Van As *et al*. (2010) observed a significant increase in $pvCO_2$ and hematocrit values and a significant decrease in pvO_2 value due to ascites incidence. Similarly, Hafshejani *et al*. (2012) detected significantly higher blood hematocrit values in ascitic chicks compared to those of healthy chicks. However, the findings of Hafshejani *et al*. (2012) have indicated no significant change in hemoglobin levels which differ from the findings of the present study. Such differences in blood oximetry parameters can be attributed to poor response of systemic circulation to changes in environmental conditions and increased metabolic rate (Druyan, 2012). The increase in hematocrit values of ascitic chicks could be explained by increased oxygen demand of tissues due to enhanced metabolic rate and decreased oxygen saturation.

Table 1: Comparison of some blood parameters of ascitic and healthy broilers

Paremeters	Groups			
	Healthy broilers	Sick (Ascitic) broilers	SEM	P
Blood Gas Parameters				
pH	7.29	7.28	0.0237	0.9012
pCO_2, mmHg	61.53	69.24	2.8436	0.2168
pO_2, mmHg	1.92	2.40	0.1960	0.2254
$cHCO_3$, mmol/L	26.03	25.07	0.8613	0.5573
Blood Qximetry Parameters				
SO_2, %	1.65a	1.33b	0.0721	0.0343
ctHb, g/dL	10.02b	13.46a	0.4821	0.0026
FO_2Hb, %	1.65a	1.31b	0.0718	0.0278
Hct_c (Hematocrit), %	31.00b	41.31a	1.4534	0.0027
FHHb, %	95.65	96.43	0.3269	0.2232
FMetHb, %	3.07	2.92	0.2050	0.6970
Elektrolyte Parameters				
cK^+, mmol/L	4.37	5.84	0.5677	0.1864
cNa^+, mmol/L	144.33	141.29	1.4567	0.2810
cCa^{2+}, mmol/L	1.25	1.03	0.0706	0.1165
cCl^-, mmol/L	106.67	104.57	1.5132	0.4687
MetaboliteParameters				
cGlucose, mg/dL	244.17	237.14	13.8849	0.7891
$mOsm_c$, mmol/kg	302.75	295.64	2.6811	0.1770
Acid-Base Balance				
cBasee, mmol/L	5.24	5.21	0.9440	0.9886

a, b: Differences between group averages for the same parameter denoted by different letters in the same row are statistically significant ($P<0.05$). SEM: Standart error of difference between means.

The effects of high altitude on right and left ventricle of heart in healthy and ascitic chicks are shown in Table 2. No significant ($P>0.05$) differences were recorded in left ventricle sections between groups, whereas significant ($P<0.05$) increase was detected in right ventricular diameter of ascitic chicks. While right ventricular diameter of healthy chickswas measured as 1.60 mm, it increased up to 4.92 mm in ascitic chicks. Heart sections of healthy and ascitic chicks are depicted in Figure 1.The findings of the current study are supported by those of Tafti and Karima (2000) who also reported right ventricular distension and enlargment in ascitic broilers. Similarly, Olkowski *et al*. (2001) observed that ascites development in fast growing broilers which were fed *ad libitum* and raised at low altitude (350m)resulted in morphological and pathological changes in the heart and significant enlargement in right ventricle. Ocak (2006) reported that broilers at high altitude were more susceptible to right ventricular failure. Druyan (2012) observed right ventricular hypertrophy in ascites incidence. This is attributed to increased hematocrit concentration, increased red blood cell production (erythropoiesis) and decline in arterial blood oxygen saturation since increased blood viscosity contributes enhanced cardiac work load. In ascitic chicks, the heart tries to pump more blood through the lungs to meet the body's oxygen requirement and right ventricle significantly enlarges in response to increased work load.

There is an index (right ventricle: total ventricle ratio) which shows the change in heart tissue of ascitic chicks. Balog (2003) reported that right ventricle: total ventricle ratio above 0.27 strongly indicates the start of ascites development. Similarly, Luger *et al*. (2003), İpek and Şahan (2006), Özkan *et al*. (2006), Zerehdaran *et al* (2006), Daneshyar *et al*. (2007), Huchzermeyer (2012) have reported that right ventricular diameter and right ventricle: total ventricle ratio were higher in ascitic chicks. The findings of these researchers support our findings which indicate significant enlargment in right ventricule in ascites incidence. High hematocrit values increase blood pressure and lead to significant right ventricular over burden (Huchzermeyer, 2012). At higher altitudes, increase in oxygen demand increases heart beat rate and amount of blood pumped to lungs. This might have increased workload on right ventricle leading to distension and enlargement of right ventricle.

Due to the improvements in genetics, feeding and management, broiler chicks grow faster and attain quicker weight quicker. However, their pulmonary and cardiac capacities do not develop at the same pace. The proportionately underdeveloped cardio pulmonary system fails to fulfill the required oxygen demand because of high altitude(1727m). In broilers raised at high altitudes, heart contraction rate increases to cope with high oxygen deficiency and high venous blood transformation. This leads to fatigue, depression and heart failure in animals. These symtopms are followed by fluid accumulation in body cavities in later stages due to the stress of increased venous blood pressure on the liver.

Table 2. Effects of high altitude on right and left ventricle in healthy and ascitic broilers

Groups	Heart Sections (mm)	
	Left ventricle	Right ventricle
Healthy broilers	3.06	1.60b
Ascitic broilers	2.38	4.92a
SEM	0.3721	0.3299
P	0.3515	<.0001

a, b: Differences between group averages for the same parameter denoted by different letters in the same column are statistically significant (P<0.0001).
SEM: Standart error of difference between means

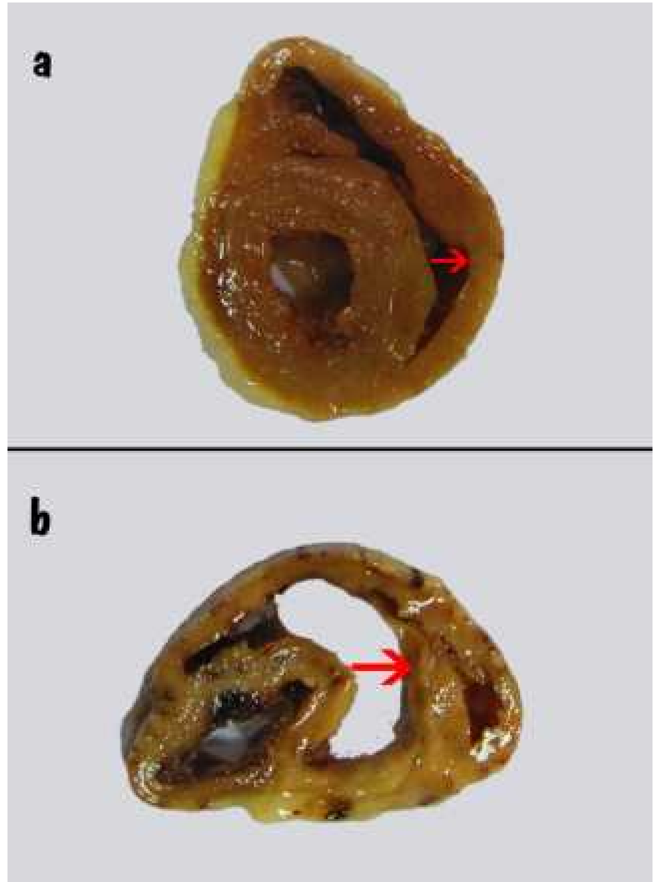

Figure 1 a). Healthy heart section b) Ascitic heart section

Conclusıons: The findings of this study indicate that blood oximetry parameters and heart sections can be used as indicators of ascites susceptibility in broilers raised at high altitudes. It was observed that high altitude by leading to decline in SO_2, and FO_2Hb oxygen saturation and increase in ctHb ve Hctc (Hematocrit) induces ascites development and significant enlargement in right ventriclular diameter. Since the effect of high altitude can not be eliminated, more comprehensive studies will be required to explore mitigation possibilities through improvements in nutritional and management conditions.

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