CHRONIC HYPOXIA DUE TO HIGH ALTITUDE AT THE EARLY STAGE OF INCUBATION INFLUENCED PRE- AND POSTNATAL PARAMETERS RELATED TO ASCITES SYNDROME IN BROILER CHICKENS

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Abstract

In order to investigate the influence of hypoxia during the early stage of incubation on embryonic and hatching events and consequently on incidence of ascites in broiler chickens, one thousand eggs were incubated in two commercial incubators. Half of eggs were incubated in a low altitude incubator until hatched. The second half were incubated at high altitude until day 10 and then transferred to low altitude incubator. After hatching, day-old chicks from each group were housed and reared at a high altitude farm. During the embryonic development and growing period parameters related to ascites were determined. plasma corticosterone, T_3 and T_4 levels were significantly higher in embryos incubated at high altitude compared to low altitude embryos, at day 10 and 19 of incubation. Consequently chicks from high altitude incubator hatched earlier than their counterparts in low altitude. During the six weeks, high altitude hatched chickens showed numerically lower right ventricular hypertrophy and ascites mortality than to low altitude incubated chickens. These results indicated that early prenatal hypoxia due to high altitude changed the endocrine functions of embryos, enhanced embryo growth, shorten the hatching process of chickens, and consequently decreased the incidence of ascites incidence in broiler chickens.

Key words: Hypoxia, Incubation, Ascites, Thyroid hormones, Chickens

Introduction

Ascites syndrome is multifactorial and mainly caused by exogenous and/or endogenous factors (Decuypere *et al*., 2000). Changes induced by environmental conditions such as hypoxia and hypercapnia may play a role in the genotype and environment interaction in ascites susceptibility (Decuypere, 2002; Hassanzadeh *et al*., 2004, 2008). Oxygen and carbon dioxoide exchanges are of fundamental importance for embryonic development during incubation, together with a number of other physical factors that have to be controlled in the incubator. They may not only affect liability of the embryo, but also affect embryonic development, hatchability, pipping and as well as later development and functioning (Decuypere *et al*., 2001). Since chick embryo consumes 60% more oxygen between the start of pulmonary breathing and hatching compared to earlier stages (Visschedijk, 1968), it is possible that a shortage of oxygen occurs during the interval between internal pipping and hatching. The reduction of the later prenatal and perinatal period might reduce this hypoxic situation. Additionally, glucocorticoids and thyroid hormones are involved in the preparation for pipping and hatching process in chick embryos (Decuypere *et al*., 1991) are important for regulating metabolic rate during the post-hatch period (Decuypere *et al*., 2000) and are basically linked with ascites susceptibility in broiler chickens (Hassanzadeh *et al*., 2004; De Smit *et al*., 2006; 2008). Base of these the aim of present study was to investigate the influence of hypoxia, during the early stage of embryonic development on parameters related to ascites syndrome in broiler chickens.

Materials and Methods

One thousand eggs from a commercial broiler line were incubated in two similar commercial incubators. All eggs were numerated and weighed individually. Half of the eggs were incubated in an incubator situated at low altitude (Lin) until hatched. The second half eggs were incubated in another incubator located at high altitude (Hin) until days 10 of incubation. At day 10, the Hin eggs were transferred to Lin incubator at low altitude. Early hatching at 482 h of incubation and final hatching at 508 h of incubation were recorded. The relative embryo weight was calculated per group at day 19 of incubation (Hassanzadeh *et al*., 2004) and newly hatched chicks from each

group were weighed. Blood samples were collected in heparinized tubes from embryos by cardiac puncture at 10th and 19th day of incubation and from newly hatched chicks for determination of plasma thyroid (T_3, T_4) and corticosterone hormone levels as described earlier (Hassanzadeh *et al*., 2000, 2004).

One hundred and twenty five day-old chicks from each group were randomly selected and housed under standard condition at a high altitude farm (2100 m above sea level). They were divided over 10 floor pens and reared until 6 weeks of age. Feed and water were provided *ad libitum*. The cold temperature was applied and regulated as described by Hassanzadeh *et al*. (2002). Body weights and feed intake were recorded every 2 weeks and daily mortality was examined for lesions of heart failure and ascites. At the end of the experiment 50 chickens from each group were randomly taken and slaughtered, for determination of right ventricle/total ventricle (RV/TV) ratios as reported (Hassanzadeh et al., 2000). Statistical analyses were performed using the "General linear model procedure" (SAS, 1998).

Results

The results of embryonic and hatching parameters of two group eggs are presented in Table 1. Earlier hatching at 482 h of incubation was numerically higher in Hin group eggs (41%) compared with the eggs of Lin group (30.6%). At the end of incubation (508 h), final hatchability was higher in Hin group (86 %) compared with the Lin group eggs (83 %). The absolute and the relative weights of embryos at day 19 of incubation were higher in group Hin but the differences were not significant (Table 1).

Hin embryos showed significantly higher plasma corticosterone, T_3 and T_4 concentrations compared to Lin embryos at days 10 and 19 of incubation (Figure 1). However, no significant differences were found between the mean plasma thyroid and corticosterone hormones levels of the Hin and Lin newly-hatched chicks.

During the 6 weeks of growing period, 23 (9.2%) of the 250 chickens died due to RVH and ascites (Table 2). Ascites mortality was markedly higher in Lin chickens (15 birds) compared to Hin ones (8 birds). The number of surviving birds that showed a RV/TV ratios over 0.25 and 0.29 was obviously higher in Lin group birds compared with the Hin group birds.

Mean body weight of newly-hatched chicks from Hin incubator were significantly ($p<0.01$) higher than those of chicks hatched in Lin incubator (Table 3). Hin chickens had a significantly ($p<0.01$) higher mean body weight compared to the Lin chickens at day 42 of age. FCR showed the same pattern of differences as for body weight, resulting only in significant differences in cumulative FCR.

Discussion

Decuypere, (2002) reported that a high carbon dioxide concentration in the air chamber is trigger for hatching. It has been shown that increased Co2 concentrations in the incubator (Buys *et al*., 1998; Hassanzadeh *et al*., 2002; De Smit *et al*., 2006; 2008) or hypoxia during the embryonic development at high altitude (Hassanzadeh *et al*., 2004, 2008), changed the developmental trajectories of the chick embryos, consequently lead to beneficial effect on hatching time, on post-hatch parameters and ascites incidence. The present work demonstrates that eggs incubated in a hypoxic environment at high altitude during the first 10 days of incubation compared to the incubated eggs in a normal atmospheric environment at sea levels, differed not only in the percentage of early hatching at 482 h, final hatchability at 508 h and body weight of newly hatched chickens, but also in plasma corticosterone, T_3, T_4 levels of embryos at days 10th and 19th of incubation. Thyroid hormones are known to be involved in the complex processes of transition from allantoic to pulmonary respiration and to play role in the length of the incubation process (Decuypere et al., 1991). This was confirmed by the results of Buys et al. (1998); Hassanzadeh et al., 2004 and De Smit et al. (2006; 2008) showing a concomitant higher activity of thyroid hormones, earlier pipping and hatching of high CO2 incubated embryos compared to the normal CO2 incubated ones. The higher corticosterone levels in high altitude incubated-eggs might have served to boost the shift in T4 to T3 converting (Hassanzadeh et al., 2004). Considered together, high altitude incubation seems to be favored on T3 functions. All acting together may favor early pipping and hatching as reported in an experimental study of non ventilated incubator (De Smit et al,. 2006; 2008).

In the present study there was a lower incidence of ascites as well as a reduced RV/TV ratio of chickens slaughtered at 6 weeks in high altitude compared to the low altitude incubated eggs. This could be partly related to the decreasion in the time duration that embryo experiences hypoxia during the final stages of incubation. It is hypothesized that developmental changes induced by environmental or incubation conditions may play a role in the genotype and environment interaction in ascites susceptibility (Decuypere, 2002; Hassanzadeh *et al*., 2004) consequently is led to developmental trajectories of cardiopulmonary parameters in postnatal chickens

(Hassanzadeh *et al*., 2005, 2008). These authors argued that hypoxic conditions occurred during the embryonic stage altered some endogenous parameters in prenatal and postnatal chicks. This important development made favorable to increase in gas exchange area in broiler chickens, therefore lower susceptibility to pulmonary hypertension and ascites (Decuypere, 2002; Hassanzadeh et al., 2004, 2008; De Smit *et al,*. 2006).

Day-old chicks of the Hin group had a significantly higher body weight than Lin chickens. This could be related to the changes of endocrine functions such as plasma corticosterone and thyroid hormones levels in prenatal period which might lead to epigenetic adaptation in hypoxic condition. Such adaptation phenomenon that enhanced early growth, were previously observed in birds that underwent cold conditioning (Shinder *et al*., 2002) and also in chickens hatched experimentally in hypoxic or hypercapnic conditions (De Smit *et al*. 2006; 2008).

Overall, chicks of the Hin group had a different growth pattern during their post-hatch growing period, reaching their maximum growth at week 6 of age. Partly, explanation for the differences of final growth curves between Hin and Lin group chickens might be related to the numerically higher incidence of ascites and right ventricular hypertrophy in Lin chickens compared with the Hin counterparts, because ascites causes a significant deterioration in the growth performance of broiler chickens (Julian, 2000; Hassanzadeh *et al*., 2002, 2004).

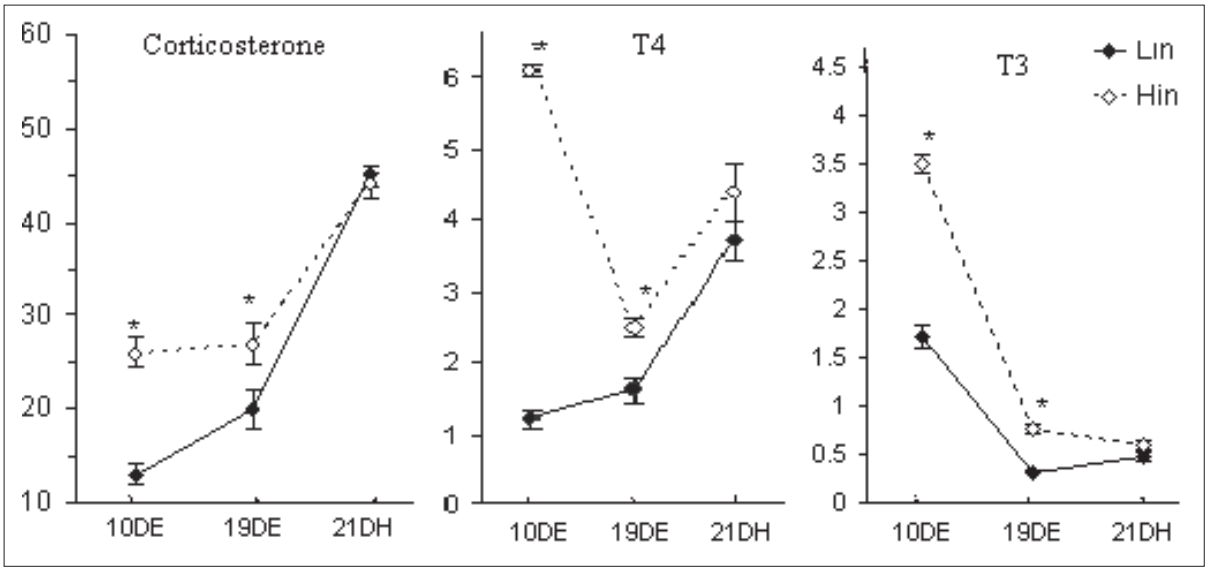
In conclusion our results confirm again the fundamental role of incubation condition in etiology of ascites syndrome by altering the developmental trajectories of some endogenous parameters in prenatal and postnatal chicks. The development of these important parameters is favorable to results to lower susceptibility of birds to pulmonary hypertension.

Table 1. Hatching parameters of commercial broiler eggs incubated in two different altitude schedules

Parameter/groups	Hin	Lin
Earlier hatching at 482 h of incubation	205/500 (41%)	153/500 (30.6%)
Final hatching at 508 h of incubation	428/500 (86%)	413/500 (83%)
Absolute embryo weight at ED19	38.97 ± 0.62	37.26 ± 1.04
Relative embryo weight at ED19 *	76.42 ± 1.14	73.49 ± 1.45

* Embryo weight / egg weight ratio x100

Figure 1. Plasma corticosterone, T_3 and T_4 (ng/ml) concentrations in embryos at days 10 and 19 of incubation and in newly hatched chicks of commercial broiler eggs incubated in two different altitude schedules. Values are means ± SEM. (n= 60)



Within age of measurement asterisks indicate significant difference ($p<0.05$)

Table 2. Weekly ascites mortality, RV/TV ratios in broiler chickens slaughtered at 6 weeks that were incubated in two different altitude schedules

Groups	Ascites mortality					RV/TV	
	Week 3	Week 4	Week 5	Week 6	Total	0.25 - 0.29	≥ 0.29
Hin	-	1	4	3	8	11/50	13/50
Lin	1	2	5	7	15	16/50	21/50

RV/TV= Right ventricle to total ventricular weight ratio of 50 broiler chickens that slaughtered at day 42

Table 3. Mean body weight, feed intake and feed conversion ratios in commercial broiler chickens that were incubated in two different altitude schedules (Values are means ± SEM).

Parameters	Hin	Lin	P- Value
Body weight (g/chicken)			
Day 1	40 ± 0.4	38 ± 0.4	0.005
Day 14	314 ± 2	309± 8	Ns[1]
Day 28	1055 ± 12	977 ± 23	Ns
Day 42	2072 ± 24	1911 ± 72	0.01
Feed intake (g/chicken)			
Day 1	318 ± 20	304 ± 5	Ns
Day 14	1189 ± 39	1207 ± 37	Ns
Day 28	2151 ± 59	2079 ± 123	Ns
Day 42	3636 ± 42	3593 ± 45	Ns
Feed conversion ratio			
Day 1-14	1.08 ± 0.02	1.13 ± 0.02	Ns
Day 14-28	1. 6 ± 0.04	1.72 ± 0.04	Ns
Day 28-42	2.11 ± 0.21	2.32 ± 0.05	Ns
Cumulative	1.79 ± 0.01	1.92 ± 0.02	0.01

[1] Non significant; a,b: Significant difference between two groups ($p<0.05$)

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