Effect of chronic hypoxia during the early stage of incubation on prenatal and postnatal parameters related to ascites syndrome in broiler chickens

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(Received 18 Feb 2009; revised version 11 Oct 2009; accepted 24 Nov 2009)

Summary

To investigate the influence of hypoxia during the early stage of incubation on embryonic development and hatching events, and consequently on incidence of ascites in broiler chickens, one thousand fertile eggs were incubated in two commercial incubators. Half the eggs were incubated in a low altitude incubator until hatched. The second half were incubated in a high altitude incubator until day 10 and then transferred to a low altitude incubator. Day-old chicks from each group were housed and reared at a high altitude farm. Chicks from the high altitude incubator hatched earlier and showed significantly higher body weights than their counterparts in the lower altitude. High altitude embryos indicated significantly ($P<0.001$) higher plasma corticosterone, T3 and T4 levels at day 10 and 19 of incubation. During the growing period, high altitude hatched chickens indicated lower right ventricular hypertrophy and ascites mortality than the low altitude hatched chickens. These results indicated that early prenatal hypoxia due to high altitude may change the endocrine functions of embryos, enhanced embryo growth, shorten the hatching process of chickens and consequently decrease the incidence of ascites incidence in broiler chickens.

Key words: Hypoxia, Incubation, Ascites, Thyroid, Broiler chickens

Introduction

Ascites syndrome is multifactorial and mainly caused by exogenous and/or endogenous factors (Decuypere *et al*., 2000). The peak of ascites incidence occurs during weeks 5 to 6 of the growing period, but it is thought that the etiology of the syndrome is initiated much earlier, even during the embryonic stage (Coleman and Coleman, 1991). Epigenetic adaptation is an adaptation to an expected environment, innate, but not genetically fixed, and is mainly caused by changes in gene expression (Nichelmann *et al*., 1999; Tzschentke *et al*., 2001). In the course of the prenatal or early postnatal ontogeny, when epigenetic adaptation occurs, environmental effects may have a strong influence on the determination of the set-point of physiological control systems (Dörner, 1974). It is hypothesized that developmental 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 hatching events as well as later development and functioning (Decuypere *et al*., 2001; Tona *et al*., 2005). Since the 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. A reduction of the later prenatal and perinatal period might reduce this hypoxic situation. Hassanzadeh *et al*. (2002) showed that eggs incubated in an environment with a relatively high concentration of carbon dioxide hatched earlier than in an environment with normal amounts and showed a lower incidence of ascites during the growing period. Glucocorticoids and thyroid hormones involved in the preparation for the pipping and hatching process in chick embryos (Decuypere *et al*., 1991) are important for regulating the 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). This becomes even more apparent under adverse environmental conditions; such as low ambient temperature (Scheele *et al*., 1992) and high altitude (Hassanzadeh *et al*., 1999, 2004).

The partial pressure of oxygen becomes lower with increasing altitude. At sea level, oxygen makes up 20.9% of the atmosphere and the equivalent percentage of oxygen drops approximately 1% for every 500 m rise in altitude (Julian, 2000). The aim of this experiment was to investigate the influence of hypoxia, due to high altitude, at the early stage of embryonic development on hatching parameters and consequently on the incidence of ascites syndrome in broiler chickens.

Materials and Methods

Incubation period

One thousand eggs from a commercial broiler line (Ross) were incubated under standard conditions in two similar commercial incubators. Both incubation parameters were as follows: temperature 37.8°C and humidity 62%. All eggs were numerated and weighed individually. Half of the eggs were incubated in an incubator situated at low altitude (Lin), at sea level, in the north of Iran (Gillan province) until hatched. The second half of the eggs were incubated in another incubator located at high altitude (Hin), 1800 m above sea level, in the north-west of Iran (Eastern Azarbajan province), until day 10 of incubation. At day 10, the Hin eggs were transferred to the Lin incubator at low altitude by a special car that was already prepared to give minimum stress to embryos. Early hatching at 482 h of incubation and final hatching at 508 h of incubation were recorded. The relative embryo weight was calculated in 20 eggs per group as the ratio of embryo weight to egg weight at day 19 of incubation (Dewil *et al*., 1996; Hassanzadeh *et al*., 2004). At the end of incubation (508 h) 50 hatched chicks from each group were selected randomly and weighed. Blood samples were collected in heparinized tubes from 20 embryos per incubator by cardiac puncture at the 10th and 19th day of incubation and from newly hatched chicks for determination of plasma thyroid (T3, T4) and corticosterone hormone levels as described earlier (Decuypere *et al*., 1983; Meeuwis *et al*., 1989; Hassanzadeh *et al*., 2000, 2004).

Growing period

One hundred and twenty five day old chicks from each group were randomly selected and housed under standard condition at an experimental farm, located at high altitude (2100 m above sea level) in Shahrekord University. They were divided over 10 floor pens (5 replications of 25 chicks per group) and were reared under a continuous lighting programme until 6 weeks of age, and feed and water were provided *ad libitum*. Feed was formulated according to the specifications of the National Research Council (1994). The temperature was regulated as described by Hassanzadeh *et al*. (2002). Briefly, it was set initially at 33°C and gradually reduced by 1°C every 2 days. During the period of 14 to 28 days the electrical heating system was turned off during the night while the minimum environmental temperature did not descend below 15°C.

At days 7, 14, 28 and 42, blood samples were taken from the brachial vein of 10 chickens per group for determination of haematocrit levels and plasma corticosterone, T3 and T4 concentrations. Haematocrit was measured immediately while plasma was stored at -20°C until hormonal analysis. 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 (day 42) 50 chickens from each group were randomly taken and slaughtered, the heart was removed and the atria, major vessels and fat were trimmed off. The right ventricle/total ventricle (RV/TV) ratio and total ventricle/body weight (TV/BW) ratio were determined and classified as reported earlier (Julian, 1987).

Values are expressed as means ± SEM. Statistical analyses were performed using the "General Linear Model procedure". If significant overall effects (P<0.05) were found, treatment means were compared using the Scheffe's test.

Results

Incubation period

The results of embryonic and hatching parameters of the eggs in two groups are presented in Table 1. Earlier hatching at 482 h of incubation was numerically higher in the Hin group eggs (41%) compared with the eggs of the Lin group (30.6%). At the end of incubation (508 h), final hatchability was higher in the 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, T3 and T4 concentrations compared to Lin embryos at days 10 and 19 of incubation (Fig. 1). However, no significant differences were found between the mean plasma thyroid and corticosterone hormone levels of the Hin and Lin newly-hatched chicks.

Ascites mortality and RVH

The number of broiler chickens that developed right ventricular hypertrophy and ascites at different ages, and also the RV/TV and TV/BW ratios of surviving chickens that were randomly selected and slaughtered at 6 weeks of age are shown in Table 2. The first incidence of ascites mortality occurred at

Table 1: Hatching parameters of commercial broiler eggs incubated at high (Hin) and low (Lin) altitudes

Parameter	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 DE19*	39 ± 0.62	37 ± 1.04
Relative embryo weight at DE19**	76.4 ± 1.14	73.5 ± 1.45

*DE: Day of embryo, and **Embryo weight/egg weight ratio ×100

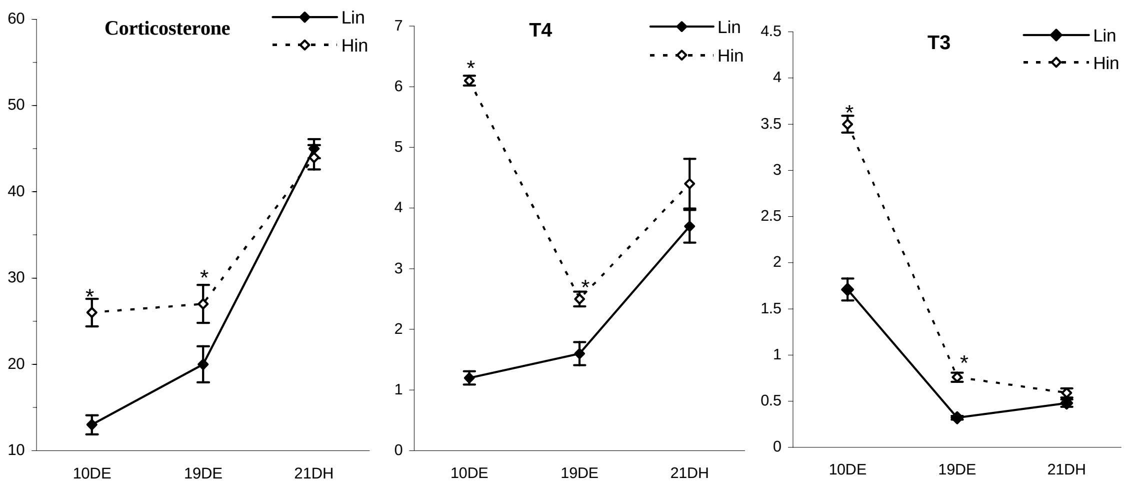

Fig. 1: Plasma corticosterone, T_3 and T_4 (ng/ml) concentrations in embryos at days 10 and 19 of incubation and in newly hatched chicks of commercial broiler eggs incubated at high (Hin) and low (Lin) altitudes. Values are means ± SEM, (n = 60). *Within age asterisks indicate significant difference between two groups (P<0.05). DE: Day of embryo, and DH: Day hatched

Table 2: Weekly ascites mortality, RV/TV and TV/BW ratios of two group broiler chickens that were slaughtered at 6 weeks

Groups	Ascites mortality					RV/TV		TV/BW (%)
	Wk 3	Wk 4	Wk 5	Wk 6	Total	0.25-0.29	≥0.29	
Hin	-	1	4	3	8	11/50	13/50	0.38 ± 0.01*
Lin	1	2	5	7	15	16/50	21/50	0.42 ± 0.01

RV/TV = Right ventricle to total ventricular weight ratio of 50 broiler chickens slaughtered at day 42. TV/BW = Total ventricle to body weight ratio of 50 broiler chickens slaughtered at day 42. *Significant difference between two groups (P<0.05)

Table 3: Mean plasma corticosterone, T4, T3 levels and haematocrit values of commercial broiler chickens that were hatched at high (Hin) and low (Lin) altitudes (values are means ± SEM)

Parameters	Hin	Lin	P-value
Corticosterone (ng/ml)			
Day 7	43 ± 1.1	41 ± 2.2	NS
Day 14	31 ± 2.6	29 ± 2.5	NS
Day 28	21 ± 2.7	23 ± 2.7	NS
Day 42	22 ± 2.6	20 ± 2.1	NS
T4 (ng/ml)			
Day 7	3.7 ± 0.43	3.4 ± 0.74	NS
Day 14	3.2 ± 0.46	2.6 ± 0.36	NS
Day 28	3.1 ± 0.37	2.9 ± 0.55	NS
Day 42	3.2 ± 0.38	3.9 ± 0.47	NS
T3 (ng/ml)			
Day 7	2.86 ± 0.16	2.70 ± 0.22	NS
Day 14	2.13 ± 0.17	2.53 ± 0.12	NS
Day 28	3.59 ± 0.31	3.47 ± 0.21	NS
Day 42	2.06 ± 0.21	2.18 ± 0.47	NS
Haematocrit (%)			
Day 7	36 ± 2*	43 ± 3	0.01
Day 14	39 ± 2	38 ± 3	NS
Day 28	44 ± 3	43 ± 4	NS
Day 42	40 ± 3	41 ± 2	NS

* Indicate significant difference between two groups (P<0.05). NS: Not significant

day 19 of age in Lin and at day 24 in Hin broiler chickens. Ascites mortality was markedly higher in Lin chickens (15 birds) compared to the Hin (8 birds). The number of surviving birds that showed a RV/TV ratio over 0.25 and 0.29 was obviously higher in the Lin group birds compared with the Hin group birds, furthermore, TV/BW ratio was significantly (P<0.01) higher in the Lin chickens compared to the Hin birds.

Post-hatched endocrinological and haematological parameters

Neither the plasma corticosterone concentration nor plasma T3 and T4 levels were significantly affected by the two different altitudes during the growing period (Table 3). Lin chickens showed significantly (P<0.05) higher haematocrit values than Hin chickens only at day 7 and no significant difference was observed at the later ages.

Growth performance

Mean body weight, feed intake and feed conversion ratio of the two groups and the results of the statistical analyses are summarized in Table 4. Mean body weight of newly-hatched chicks from the Hin incubator were significantly (P<0.01) higher than those of chicks hatched in the Lin incubator. Throughout the experiment, there were no significant differences between the feed intakes of the two groups. In contrast, Hin chickens had a significantly (P<0.01) higher mean body weight compared to the Lin chickens at day 42. FCR showed the

Table 4: Mean body weight, feed intake and feed conversion ratios in commercial broiler chickens that were hatched at high (Hin) and low (Lin) altitudes (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
Day 28	1055 ± 12	977 ± 23	NS
Day 42	2072 ± 24*	1911 ± 72	0.01
Feed intake (g/chicken)			
Day 1-14	318 ± 20	304 ± 10	NS
Day 14-28	1189 ± 39	1207 ± 37	NS
Day 28-42	2151 ± 59	2079 ± 123	NS
Day 1-42	3658 ± 62	3590 ± 45	NS
Feed conversion ratio			
Day 1-14	1.05 ± 0.02	1.12 ± 0.02	NS
Day 14-28	1.62 ± 0.04	1.79 ± 0.04	NS
Day 28-42	2.11 ± 0.21	2.22 ± 0.05	NS
Cumulative	1.80 ± 0.01*	1.94 ± 0.02	0.01

* Indicate significant difference between two groups ($P<0.05$). NS: Not significant

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 a trigger for hatching. Recent studies have been focused on the CO_2 concentration in the incubator. In these studies, it has been shown that increased CO_2 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 leading to a beneficial effect on hatching time, on post-hatch parameters and on 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 level, 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, T3, and T4 levels of embryos at days 10 and 19 of incubation. Thyroid hormones are known to be involved in the complex processes of transition from allantoic to pulmonary respiration and to play a role in the length of the incubation process (Decuypere *et al*., 1991; Dewil *et al*., 1996). This was confirmed by the results of Buys *et al*. (1998); Hassanzadeh *et al*. (2003) and De Smit *et al*. (2006, 2008) showing a concomitant higher activity of thyroid hormones, earlier pipping and hatching of high CO_2 incubated embryos compared to the normal CO_2 incubated ones. On the other hand, Decuypere *et al*. (1983) and Meeuwis *et al*. (1989) demonstrated that corticosterone is required for peripheral conversion of T4 to T3 during prenatal life. Thus, the higher corticosterone levels in the high altitude incubated-eggs might have served to boost the shift in T4 to T3 conversion (Hassanzadeh *et al*., 2004). Considered together, high altitude incubation seems to be favored on T3 functions. Both acting together may favour early pipping and hatching as confirmed by Tona *et al*. (2003) and Hassanzadeh *et al*. (2004). It can be concluded here that stimulated earlier hatching is also reflected in an earlier increase in T3 and T4 levels of these embryos at high altitude incubation, as was reported in the non ventilated incubator (De Smit *et al*., 2006, 2008). Additionally, Decuypere (2002) reported that severity of embryonic hypoxia may be related to the porosity and structure of the egg shell and hence, to the partial pressures of oxygen and carbon dioxide in the egg and air chamber, especially during the last days of incubation.

Sadler *et al*. (1954) argued that the beneficial effects of carbon dioxide were the result of the reduction of pH of albumen which might have retarded the apparent breakdown of the chalaziferous membrane and to the thick layer of albumen. Such phenomenon might have happened here, consequently these events led to a reduction in the length of incubation.

The observed clinical signs and the occurrence of right ventricular failure of ascitic birds correspond with those reported at high altitude and low environmental temperature (Sillau *et al*., 1980; Hassanzadeh *et al*., 2003, 2004). Structural and endocrine changes often linked with ascites susceptibility may be influenced in early stages of development, even during embryogenesis. 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 at high altitude compared to the low altitude incubated eggs. This could be partly related to the decrease 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; Hassanzadeh, 2009) and consequently has led to developmental trajectories of cardiopulmonary parameters in postnatal chickens (Hassanzadeh *et al*., 2005, 2008). In these studies, hypoxic conditions that occurred during the embryonic stage altered some endogenous parameters in prenatal chicks.

This important development makes an increase in the gas exchange area in broiler chickens favorable, therefore lower susceptibility to pulmonary hypertension and ascites (Buys *et al*., 1998; Decuypere, 2002; Hassanzadeh *et al*., 2004, 2008; De Smit *et al*., 2006, 2008). Moreover, the evidence for the role of hypoxia during the chick embryonic development in the control of pulmonary surfactant was provided by Blacker *et al*. (2004). The authors argued that corticosterone and thyroid hormones also have an important role in the development of avian pulmonary surfactant. Therefore, one possible explanation is an interaction between environmental and endogenous physiological factors during the critical development of chick embryos (Decuypere, 2002; Blacker *et al*., 2004; Hassanzadeh *et al*., 2004), results in a change in the physiological heterokairy of the surfactant system by altering both the rate and onset of surfactant lipids development and earlier commencement of air breathing.

Except for the significant difference between haematocrit values of the two groups at day 7, no significant differences were observed at later ages. This finding is in agreement with previous reports in which there is not always an association between the ascites syndrome and haematocrit values (Shlosberg *et al*., 1998; Hassanzadeh *et al*., 2000; Scheele *et al*., 2005). However the average haematocrit values of both groups chickens were higher than the normal values. Such high haematocrit levels could be related to rearing of chickens at high altitude (2100 m above sea level) and cold challenge as reported previously (Wideman *et al*., 1998; Luger *et al*., 2001).

In the present study, day-old chicks of the Hin group had a significantly higher body weight than the Lin chickens. This could be related to the changes of endocrine functions such as plasma corticosterone and thyroid hormone levels in the 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, the Hin group chicks had a different growth pattern during their post-hatch growing period, reaching their maximum growth at 6 weeks of age. Explanation for the differences of the final growth curves between the 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 cause a significant deterioration in the growth performance of broiler chickens (Julian, 1993; Hassanzadeh *et al*., 2002, 2004).

In conclusion, our results again confirm 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 favourable to increase the gas exchange area and results in lower susceptibility of birds to pulmonary hypertension. However, the best time for induction of hypoxia and also the duration and intensity of hypoxia that resulted in the optimal results needs more experiments to be performed.

Acknowledgements

This research was funded by the research committee University of Tehran, Faculty of Veterinary Medicine. G. Nackaerts from K. U. Leuven, Belgium is acknowledged for hormonal analyzing.

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